EFFECT OF VEGETABLE OILS ON GROWTH PERFORMANCE AND QUALITY OF RED SEA BREAM *Pagrus major*

（マダイの成長と品質に及ぼす飼料中植物油の影響）

CONNIE FAY KOMILUS

2008

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CONNIE FAY KOMILUS

A thesis submitted in the partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Major Subject: Aquatic Animal Nutrition
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Dedications

This dissertation is dedicated with love to

my dearest father, Mr. Sylvester Jitimin Komilus and mother, Mdm Lucy Togium Langat for their everlasting love and for bringing me into this world;

my dearest brothers (Teddy and Reno) and sister (Patricia); no matter what, water will always run deep;

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List of publications/abstract/poster presentation

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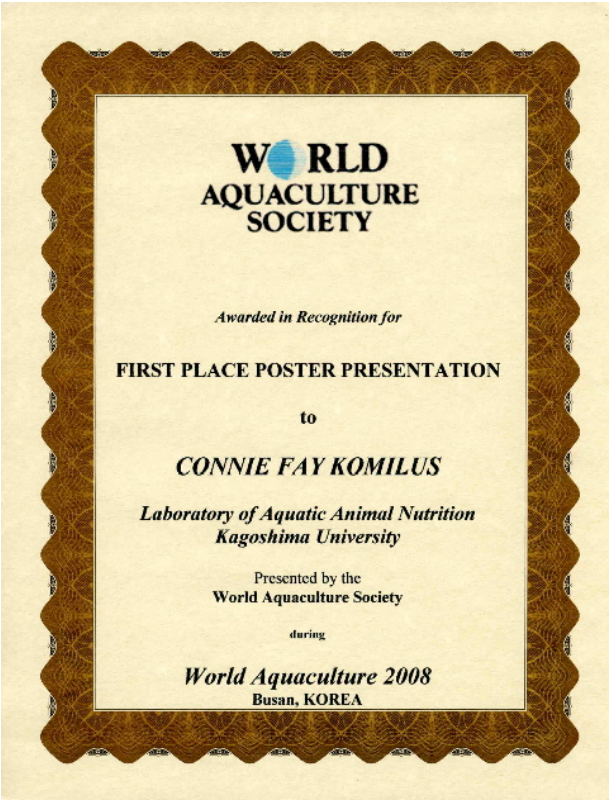
TEXTURE RHEOLOGY AND FRESHNESS QUALITY OF WILD AND CULTURED RED SEA BREAM *Pagrus major*

Komilus, C.F., N. Shichi., F. R. Michael., M. A. Kader., R. F. Traifalgar., J. Gao., S.Yokoyama., M. Ishikawa & S. Koshio



Abstract

Dietary lipid source is an important factor for growth, physiology and muscle quality of fish. However, efficacy of vegetable oil such as soybean or palm oil as replacement of fish oil is unclear. The present study demonstrated the effect of vegetable oils on growth performance, physiology and muscle quality of red sea bream *Pagrus major* by the following experiments.

1. Changes of physico-chemical properties of muscle during storage in wild and cultured red sea bream were compared for 48 hr. K-value at 48 hr was significantly higher in cultured than that in wild fish. Deterioration rate of muscle was faster in cultured than wild fish in terms of muscle pH. Rheological analysis indicated textures of wild fish were harder than those of cultured fish. Sensory scores of dorsal muscle were similar between wild and cultured fish. These results suggested quality changes in wild and cultured fish were similar within 48 hr after slaughter.

2. Effects of dietary palm oil (P) and soya oil (S) as replacement of fish oil on growth performance were investigated. Test diets with different ratios of fish oil (F) and palm oil or soya oil (ratio of F: P or S= 10:0, 8:2, 6:4 and 4:6) were fed to juvenile red sea bream for 50 days. Growth performance and fatty acid compositions of test fish were determined after feeding trial. Results suggested that both palm oil and soya oil could be replaced at 40% of fish oil in diet.

3. A 50-days feeding trial was conducted to evaluate the effects of combination of palm oil (P) with oxidized fish oil (Ox) on growth and oxidative stress status, with different ratios of dietary P and OF. Growth performances were not different in fish fed diet with Ox6F4P (60% oxidized fish oil to 40% palm oil), and suggested palm oil could be replaced 40% of oxidized

fish oil.

4. Further investigated effects of using suggested ratio of palm (40% replacement) and soya oil (40% replacement) for fish oil on physico-chemical properties and sensory analysis in fish. Results showed that there were significant differences among treatments on pH and k-value. However, sensory analysis showed that there was no significant differences among treatments and suggested that same muscle quality is held within 24h after slaughter both in palm and soya oil treatments.

These experiments clarified dietary vegetable lipid sources such as soybean or palm oil can be used for diet of red sea bream without quality loss of muscle, and suggested that replacement level for fish oil is around 40%.

要約 (Abstract in Japanese)

養殖魚の成長、生理状態および肉質は飼料脂質源に影響を受けるが、近年、魚油の代替源として注目されている植物性脂質源がマダイの成長、生理状態および品質に及ぼす影響については明らかにされていない。したがって、本研究では大豆油とパーム油がマダイの成長、生理状態および肉質に及ぼす効果を以下の実験により検討した。

1. 保存中の筋肉の物理化学的性状の変化を養殖および天然マダイについて経時的に調べた。48 時間一定の条件下で保存された筋肉の K 値は養殖が天然マダイよりも高い値を示した。また、筋肉 pH の変化より、養殖は天然マダイよりも品質の低下が早く起こると示唆されたが、背筋を用いた官能検査では養殖と天然の間に差は見られなかった。以上より、物理化学的性状の変化は養殖と天然マダイの間に捕殺後 48 時間以内では差はないと考えられた。

2. 飼料中魚油(F)の代替源として、大豆油(S)とパーム油(P)がマダイ稚魚の成長に及ぼす効果を検討した。飼料中の魚油をそれぞれの脂質源で異なる比率（F:S および P=10:0, 8:2, 6:4 および 4:6）で置き換えた飼料を用いて 50 日間の飼育試験を行った。成長指標および魚体の脂肪酸分析より、魚油の 40%はこれらの植物油で代替可能であると示唆された。

3. 飼料中の魚油はマダイへの必須脂肪酸供給源として重要であると同時に、容易に酸化され栄養価の低下を招く。一方、植物油は比較的高い抗酸化性やオレイン酸に富む等の特徴により、酸化魚油と混合すればその栄養価が改善されると考えられるが、詳細に検討された例は少ない。本実験において酸化魚油とパーム油を異なる比率で混合した飼料を用い、マダイ稚魚の成長、脂肪酸組成、酸化ストレスの度合いを調べた結果、酸化魚油の 40%をパーム油で置き換えた試験区は（未酸化）魚油区と同等の成長、酸化ストレス度を示したことから、パーム油は劣化した魚油の栄養価を改善すると示唆された。

4. 植物油がマダイの肉質に及ぼす影響を物理化学的性状および官能検査によって明らかにした。大豆およびパーム油（代替レベル：40%）添加飼料を 120 日間マダイ稚魚（100g）に与えた場合、K 値や pH の経時的変化は試験区間で異なっていたが、官能検査では捕殺後 24 時間以内での差は見られなかったため、植物油の代替による品質低下は無いと考えられた。

これらの実験により、マダイ飼料の魚油は植物油で代替可能であり、その代替率は大豆油、パーム油ともに魚油の約 40%であると推察された。

α	-	Alpha
ATP	-	Adenosine triphosphate
ADP	-	Adenosine triphosphate
AMP	-	Adenosine monophosphate
AOAC	-	Association of Official Analytical Chemists
ANOVA	-	Analysis of variance
β	-	Beta
BAP	-	Biological antioxidant potential
BF_3	-	Boron triflouride
BHT	-	2,6 Di-t-butyl-p-cresol
BUN	-	Blood urea nitrogen
BWG	-	Body weight gain
°C	-	celcius
Co, Ltd	-	Company limited
DHA	-	Docosahexaenoic acid
DM	-	Dry matter
d-ROMs	-	d-Reactive oxygen metabolites
EFA	-	Essential fatty acid
EPA	-	Eicosapentaenoic acid
E_o	-	Xanthine oxidase + nucleoside phosphorylase
F	-	Fish oil
FAO	-	Food and Agriculture Organization
FAME	-	Fatty acid methyl esters
FCR	-	Feed conversion ratio
$FeCl_3$	-	Ferric chloride
FI	-	Feed intake
Fig	-	Figure
FL	-	Fat loss
g	-	gram
Glu	-	Glucose
GOT	-	Glutamic oxaloacetic transaminase
GPT	-	Glutamic pyruvate transaminase

H	-	total storage period after slaughter
Hb	-	Hemoglobin
HCL	-	Hydrocloric acid
He	-	Helium
Hg	-	Hemaglobin
hr	-	Hour
hrs	-	Hours
HDPE	-	High density polyethelene
HUFA	-	Highly unsaturated fatty acids
HSI	-	Hepatosomatic index
Ht	-	Hematocrit
IMP	-	Inosine monophosphate
IU/l	-	International unit *per* liter
K	-	k-value of fillet
kg	-	kilogram
KOH	-	Potassium hydroxide
L	-	Liter
m	-	Meter
$meqkg^{-1}$	-	milliequivalent *per* liter
mg	-	milligram
min	-	minutes
ml	-	milliliter
mm	-	Millimeter
MONOs	-	Monoenes
N_2	-	Nitrogen
nm	-	nanometer
n-3	-	Omega 3
n-6	-	Omega 6
NS	-	Not significant
Ox	-	Oxidized
P	-	Palm oil
PBS	-	Phosphate buffer saline
ppt	-	Part per thousand
PUFA	-	Polyunsaturated fatty acid
QDR	-	Quality deterioration ratio

rpm	-	rate *per* minute
S	-	Soya oil
S1	-	Filtrate 1
S2	-	Filtrate 2
SDS	-	Sodium lauryl sulfate
SD	-	Standard division
S.E	-	Standard error
SGR	-	Specific growth rate
SPSS	-	Statistical Package for the Social Sciences
SR	-	Survival rate
TBA	-	4,6-Digydroxy-2 mercaptopyrimidine
TBARS	-	Thiobarbituric acid reactive substances
T-Bil	-	T-Bilirubin
TCA	-	Trichloroacetic acid
T-Cho	-	T-cholesterol
TG	-	Triglyceride
TSP	-	Total protein
TL	-	Total lipid
µl	-	microliter
V^1	-	Weight of filter paper (g)
V^2	-	Weight of filter paper containing aqueous fatty fraction (g)
V^3	-	Constant weight of filter paper containing fat (g)
WHC	-	Water holding capacity
WL	-	Water loss
W^s	-	Sample weight (g)
∑	-	Total

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Chapter 1

1. General introduction and objectives

1.1 Status of world aquaculture - trend and prospects

Aquatic animal includes all aquatic invertebrates consisting of fish, crustaceans, seaweeds, shellfish, jellyfishes, octopus and squids which are landed or cultured for commercial purposes worldwide is one the most important source of protein to human's diet today. The consumption of fish oil in particular provides utilization of proteins of high biological value, certain minerals and vitamins which scientifically proven in preventing coronary heart disease, decrease mild hypertension, prevents certain cardiac arrhythmias, sudden dead, lowers the incidence of diabetes and appears to alleviate symptoms of rheumatoid arthritis (Sidhu, 2003).

Globally, the annual per capita world seafood consumption rate is 16 kg per capita (Hardy 1999) and this trend will definitely continue to increase yearly to meet increasing demand of world's growing population. It is also a known fact that natural resource of aquatic animals is slowly depleting due to over-exploitation and emergence of aquaculture has taken place as the main provider of this protein-based source. According to FAO (2005), although this sector has begun to catch up scientifically with animal in the agriculture only in the last few decades, it currently represents the fastest growing segment of agriculture. Global aquaculture has grown at 11 percent a year over the past decade and is projected to continue increasing to realize the ever growing demand. In 1998, world aquaculture production of fish, crustaceans and mollusks has contributed about 30.9 million tonnes to the world market. Major producers for example Japan has accounted for 2.5 percent of total world aquaculture production by weight but by 6.5 percent by value due to production of high-value species. Such vast production capacity definitely requires high nutritional complete aquafeed to compliment the sustainability of this industry towards the future.

1.2 Quality related to fish consumption

1.2.1 Consumers' eating quality trend on aquatic animal

In parallel to the increasing demand, consumers too have developed an eating quality and become more health-conscious on their food intake including aquatic animals. The degrading factors of fish quality and degree of safety of either landed or cultured fish are therefore speculated and may caused likelihood of aquatic based-food declining intake in future. Although there is ongoing research to elucidate various problem in aquatic animals quality, additional control measures should be taken before results of such research are made known as concluded by Hays and Aylward (2003).

Evaluation of fish nutritive diets can be considered as a sound approach in ensuring cultured products are of high quality and meet the degree of food safety. This approach may also minimize variations in consumer's eating quality as suggested by Robb *et al* (2001). Therefore, this niche study attempts to provide a thorough information on enhancement of aquafeed diet using vegetable oils in efforts of optimizing fish quality which may satisfy consumers demand on eating quality.

1.2.2 Quality characteristics in aquatic animals' products

In general, terminology of "quality of aquatic animal" differs among consumers. Definition of quality depends on specific parameters desired by each consumer respectively thus making it difficult to define the term precisely and specifically. Nevertheless, most researchers on fish quality have used similar or same characteristics as parameters in determining fish quality. Lie (2001) defined characteristics of fish quality as color and appearance, smell and taste, texture, nutritional quality, shelf life and level of contaminants are

highly influenced by changes in parameters caused by fish nutrition. Arvanitoyannis *et al.,* (2005) added that sensory like sight, smell, taste, touch (by fingers and mouth) and hearing relates to some representative quality aspects of fish. Periago *et al* (2005) has also suggested that muscle cellularity is another parameter to a better understanding of flesh quality of seabass. In addition, Hamada-Sato *et al* (2005) has outlined three general parameters: time-temperature-tolerance as quality control for fresh fish. Therefore, the term "quality" with retrospect to aquatic animal can be classified using intrinsic parameters like appearance, flavor, texture, nutritional quality, muscle cellularity and health status of aquatic animals.

1.3 Aquafeeds and fish quality

1.3.1 Lipid requirements availability in aquatic animals' dietary

Aquafeeds constitute of protein, lipids, carbohydrates, vitamins and minerals. Different substance contains different chemical reaction and properties which are essential throughout the growth phase of aquatic animals. Relatively, source and concentration of lipid correlate to fish quality. Lipid source is normally used in fish diets due to its high content of n-3 high unsaturated fatty acid (HUFA). n-3 HUFA is an essential ingredient as it provides essential fatty acid (Watanabe and Vassallo-Agius 2003; Izquierdo *et al.,* 2003.; Tocher *et al.,* 2004) which is also important for the essential structure of living systems and regulation of life processes in fish (Rainuzzo *et al.,* 1997). In recent years, vegetable oils from soybean, palm and corn are also studied as alternatives that could be utilized in fish feed diets (Kumaraguruvasagam *et al.,* 2005; Merican and Shim 1994; Ng *et al*, 2004). Results from these researches indicated that besides fish oil, vegetable oil can replace basic properties available in fish oil for feed formulation.

1.3.2 Manipulation in aquafeed formulation

Fishes in particularly marine fish require fish oil from aquafeeds to obtain n-3 HUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These are essential fatty acids (EFA) essential for their normal growth (Watanabe, 1993). Fish oil is notably known as rich in EFA and is normally used as a lipid source in fish aquafeeds. Nevertheless, high dependency on marine fish oil as a major lipid source in aquafeeds has raised major concerns of sustainability for aquaculture in the future. This is due to limited supply of fish oil and expanding global nature of aquaculture products. Therefore, many efforts have been made by researchers to find alternative oils in replacing fish oil. One source which has some certain advantages to replace fish oil is vegetable oils.

As a dietary source of lipid, vegetable oils are cheaper, available in large quantities and less subject to oxidation than non-hydrogenated fish oil (Dosanjh *et al.,* 1984). In addition, fish flavor does not deteriorate when fed with partially oxidized herring oil mixed with canola oil as reported by Koshio *et al* (1994) on Atlantic salmon (*Salmo salar*). As vegetable oils like palm oil and soya oil are markedly rich in saturates and monoenes marine fish may have limited capacities in β-oxidation and catabolism metabolism (Robin and Skalli, 2006) of the fatty acids of vegetable oils in vivo. Nonetheless, the replacement of a fraction of the fish oils by vegetable oils rich in 18:3n-3 (linseed or canola) would probably decrease the feed costs, permit optimal growth and a feed conversion as efficient as the fish oils without significantly affecting the flesh's organoleptic qualities and maximize the accumulation of the total n-3 fatty acids (EPA + DHA + 18:3n-3) in the flesh of farmed fish as suggested by Guillou *et al* (1995) on salmonids. Therefore, EFA composition may be manipulated by replacing fish oil using vegetable oils to meet optimal growth.

1.3.3 Lipid importance to fish quality

Lipid has significant impacts on fish quality in which Chaiyapechara *et al* (2003) suggested that the difference in lipid's concentration (Shearer 1994) contributes to the implication of fish quality due to occurrence of lipid oxidation. Lipid oxidation is due to high proportion of PUFA content found in fish oil (Lim *et al.,* 2001) depending on type and amount of lipid in diets (Rainuzzo *et al.*, 1997). Although research on lipids interaction in aquatic animals particularly marine fishes were done earlier, there are still very limited information on relationship between lipid dietary in enhancing fish quality characteristics like appearance, aromatic compound, flavor, texture, nutritional quality, muscle cellularity and health status of aquatic animals prior to harvesting. Relationship between type and concentration of lipid in diets to meet standards of fish quality are also little known except for trout, seabass and channel catfish.

As farmed fishes usually contain relatively more lipid than their wild counterparts, there is possibility of high storage of lipids in cultured ones. Lipid content is also regarded as co-variant which is prerequisite in any model aiming at explaining sensory characteristics like flesh texture of cultured fish (Johansson *et al.,* 2000). Simultaneously, scientific information on physico-chemical composition between wild and cultured fish is also scarcely known (Periago *et al.,* 2005). As fish growth is usually associated with increase of fat deposition in fillet of the farmed trout, continuous deposition resulted a less firmer texture in the fillet including formation of less favorable fatty acid pattern; shrouding fish freshness with acidulous taste (Johansson and Kiessling, 1991**).** Johansson *et al* (2000) added that fish growth's positive correlation with age has significant effects on total odour intensity, juiciness, level on total taste and firmness. This result contradicts to Orban *et al* (1997) study in raw fish. They reported that raw fish intensively cultured has stronger seaweed odor but shows no differences in terms of

smell, flavor and appearance. Therefore, this niche study attempts to provide thorough information on enhancement of aquafeed diet in efforts of optimizing fish quality which may satisfy consumers demand on eating quality.

1.4 Properties of vegetable oils

Potential vegetable oils for fish oil replacement consist of palm oil, soya oil, canola oil and others. These oils are substances derived from plants. Vegetable oils come in several forms; some oils are liquid at room temperature while some are in solid fat. Substrates from vegetable oils are used in many way namely culinary uses, industrial uses, pet food additive and even fuel. In recent years, many researchers focused on fish oil replacement research related area using these oils and some produced encouraging results. Soybean oil, rapeseed oil, linseed oil and sunflower oil were reported to give comparative efficiency in growth performances and feed utilization to those fed with fish oil for several species like Atlantic salmon (Tortensen *et al*, 2000; Jordal *et al*, 2007; Ng *et al*, 2004), gilthead sea bream (Izquierdo *et al.*, 2005), European seabass (Mourente and Bell, 2006) and red sea bream (Glencross *et al.,* 2003).

Palm oil is another potential vegetable oil and has been marked as a novel dietary lipid source in aquaculture feeds (Ng, 2004). Palm oil potentials been accepted by researchers in recent years (Ng, 2006). Besides being rich in linoleic acids, and a component of essential fatty acids required by fish to grow, palm oil is also affordable and available at most time. However, Bell *et al* (2002), Ng *et al* (2002) and Ng *et al* (2003) reported that inclusion of palm oil to replace fish oil has demonstrated a similar result to that of other plant oils but only to limited species.

Complete replacement for fish oil by all these oils is not possible due to the lack of essential

fatty acid contents in those plant oils, resulting in the partial replacement. Critical factor which needs to be address in replacing fish oil using vegetable oils is the breaking point of EPA composition without adverse effects on growth and feed utilization efficiency.

Chapter 2

2. General materials and methods

2.1 Experimental fish and location

2.1.1 Fish species

Fish used throughout this research was red sea bream (*Pagrus major*) with different sizes depending on type of experiment. Fish used were1380±40 g (Study I), 3.68±0.09g (Study II), 4.9±0.1g (Study III), 5.2±0.4g (Study IV) and 206.9±3.3g (Study V).

2.1.2 Location of experiments

Feeding experiments were conducted in 100*L* and 200*L* high density polyethelene (HDPE) tanks at Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University. Feed formulation, physico-chemical properties, chemical analysis and sensory analysis were conducted in Laboratory of Aquatic Animal Nutrition, Fisheries Faculty, Kagoshima University.

2.2 Physico-chemical properties analysis

2.2.1 pH measurement

The pH measurements were conducted by using an electronic pH meter probe (IQ150 pH meter, IQ Scientific Instruments, Inc.California). pH was calibrated prior to usage.

2.2.2 K-value and quality deterioration ratio (QDR)

White muscles (3-4 g) were digested with 5 *ml* of 10% trichloroacetic acid (TCA), supernatant was collected, filtered through a filter paper (Advantec no.1, Toyo Roshi Co, Ltd., Tokyo, Japan) and neutralized using 10 M KOH and collected as filtrate 1 (S1). Filtrate 2 (S2) was prepared by mixing 50 *ml* S1 with 50 *ml* alkaline phosphatase (P) and incubated in water

bath (37°C) for 20 min. Prior to injection of samples, the Super Freshness Meter reaction cell was prepared by filling 1 *ml* of air saturated 0.1 M PBS (phosphate buffer saline) into reaction cell and by injecting 10 *ml* S1 (before digestion, total ATP), 10 *ml* E_0 (Xanthine oxidase + Nucleoside phosphorylase) and 20 *ml* S2 (S1+Alkaline phosphatase = dephosphorylated ATP). The k-value was then measured using freshness meter (model KV-202, Central Kagaku Corp., Tokyo, Japan) according to the following formula.

k-value=100*(Inosine + Hypoxanthine)/(ATP+ADP+AMP+IMP+Inosine+Hypoxanthine)

The quality deterioration ratio (QDR) was calculated using the formula QDR=K/H, where K is the k-value of fillet and H is the total storage period after slaughter.

2.2.3 Water-holding capacity (WHC)

Method was adopted from Gomez-Guillen *et al* (2000). Five grams of sample and a filter paper (Advantec no.1, Toyo Roshi Co, Ltd., Tokyo, Japan) were placed into a centrifuge tube (15 *ml*) and centrifuged at 7000 rpm for 10 min (10ºC), and then the filter paper containing aqueous fatty fraction were weighed. Percentage of water loss (WL) from the samples was calculated as follows:

WL (%)=100*(V^2-V^1)/Ws, where V^1 is the weight of filter paper (g), V^2 is the weight of filter paper containing aqueous fatty fraction (g) and Ws is the sample weight (g).

The wet filter paper was then dried to a constant weight at 50ºC in an oven, and percentage of fat loss (FL) was calculated as FL (%)=100*(V^3-V^1)/Ws, where V^1 is the weight of filter paper (g), V^3 is the constant weight of filter paper containing fat (g) and Ws is the sample weight (g).

2.2.4 Rheological (texture profiles) analysis

Texture profiles of the fish fillets were measured on a Rheoner II Creep (model RE-2-33005B, Yamaden. Co, Japan) consisting of four components: a detector (model RE2-33005B-2), measurement meter (model REZ-OP18-01), a cooler (model ETC-3305-1) and a computer auto-analyzer (Model CA-3305) installed with Texture Analysis Program (TAP ver.1.2-a). Samples were obtained from fillet according to Fig. 1.

The temperature of the fresh samples was maintained at 4ºC throughout the analysis and the measurements were set in the central part of the fillet. Analyzed parameters included maximum hardness force and adhesiveness of samples for a period of 48 hrs. Texture profiles were expressed in Fig. 2. Maximum hardness force is expressed as (H) maximum height of force in first plunge (Newton) and adhesiveness is the total area of A_3 (J/m^3).

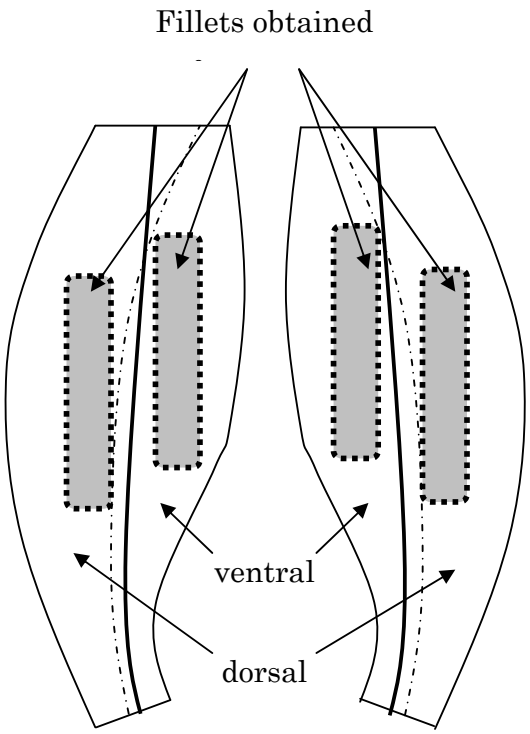


Fig. 1. Schematic drawing of fillets obtained from dorsal and ventral parts of fish

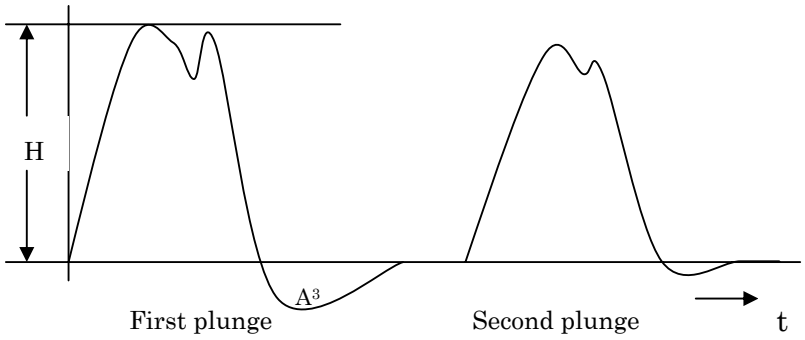


Fig. 2 Calculation of texture profiles according to Texture Analysis Program

2.2.5 Sensory analysis

Sensory analysis was adopted from Amerine *et al* (1965). Fillets from fish were cross-divided into dorso-ventral sections and sliced to obtain uniform sized sashimi of about 30-mm thickness. The slices were hygienically wrapped in aluminum foil and refrigerated at 4ºC for 30 minutes before served to non-trained panelists from the Faculty of Fisheries, Kagoshima University. Sensory evaluation was conducted in a 25ºC air-conditioned room. All panelists were lined 1 m apart and asked to taste and assess odor, texture, freshness and taste of the sashimi slices. The scores grade were 10=excellent, 9=very good, 7=good, 5=medium, 3=poor, 1=very poor and 0=not acceptable.

2.2.6 Fatty acid analysis

Fatty acid composition was analyzed based on the study of Querijero *et al* (1997) with slight modifications. Total lipid (TL) was extracted by homogenizing 0.2 g sample according to Bligh and Dyer (1959). Fatty acid esters were then produced from total lipids aliquots. Samples were then methylated with boron triflouride ($BF_{3)}$ in methanol. Methyl tricosanoate (Nu-Chek

Prep. Inc) was used as internal standard at 1.000 mg/ml hexane. Fatty acid methyl ester was analyzed with a gas chromatograph (Shimadzu GC 17A) with flame ionization detector temperature maintained at 260˚C; carrier gas He at 1 ml/min; column temperature at 200°C; injector temperature at 250°C and helium (He) served as the carrier gas. The samples (1.0 µl) were manually injected into injection port and identified fatty acids were presented as area percentage of total fatty acids.

2.2.7 Thiobarbiturate reactive substances (TBARS)

TBARS was conducted according to a method adapted from Yagi (1987). 0.2g sample, 0.2 ml of 8.1% SDS, 1.5 ml of HCl, 1.5 ml of TBA, 0.05 ml of BHT, and 1 ml of distilled water were added to a test tube. The sample was vortexed and heated in a 90°C water-bath for 60 min. 1 ml of distilled water and 5 ml of n-butanol-pyridine solution was added after sample was cooled for 10 min, mixed thoroughly, and centrifuged at 3,000g for 10 min. The fluorescence reading of upper layer was measured by a fluorometer (532-nm).

2.2.8 Blood chemistry analysis

The micro hematocrit method was adopted from Kawadzu (1981). Glucose, total cholesterol (T-Cho), triglyceride (TG), blood urea nitrogen (BUN) in plasma and albumin, total bilirubin (T-Bil), glutamic oxaloacetic transaminase (GOT), total protein (TSP) in serum were determined by SPOTCHEM EZ SP-4430 system according to Tatsumi *et al* (2000).

2.2.9 Proximate analysis

Homogenized samples were analyzed in duplicates for protein, lipid, ash, and moisture. Protein determination was conducted according to the AOAC method (1990). Lipid content was analyzed according to the method adapted from Bligh and Dyer (1959), while ash content was obtained by combustion in muffle furnace at 550ºC. Moisture, on a 5 g sample of freeze-dried fillet muscle, was determined by oven-drying at 110ºC to constant weight.

2.3 Statistical Analysis

The statistical analysis was performed using an analysis of variance (package super-ANOVA, Abacus19 Concepts, Berkeley, California, USA). Data were expressed as means ±S.E. Homogeneity of variance between treatments (5% level of probability) were analyzed using one-way ANOVA while significant differences of mean values were determined at 5% level of probability using Tukey Test. In Study V, statistical differences in texture between treatments were analyzed using Kruskal-Wallis (SPSS) followed by the paired Mann-Whitney *U* test wherever differences in texture were observed. All statistical analysis were considered significant at ($\alpha = 0.05$).

Chapter 3

Comparison of wild and cultured red sea bream *Pagrus major*

Study I:

Comparative Studies on physico-chemical properties of the muscle between wild and cultured red sea bream (*Pagrus major*) obtained in Kagoshima, Southern Japan

Abstract

This study was aimed to investigate the physico-chemical property differences of muscles in wild and cultured red sea bream obtained from Kagoshima region, southern Kyushu of Japan. Twenty live fish samples comprising 10 wild fish and 10 cultured fish (mean weight 1380±40.0g) were obtained from the Kagoshima Bay fishery and the Azuma Marine Fish hatchery, Kagoshima. All specimens were slaughtered by hypothermia, divided into dorsal and ventral fillets and maintained under 0°C for 48 hours wrapped in Aluminium foil. Monitoring of k-value, pH and muscle rheology was conducted by using the fillets at 6-hour intervals for 48 hrs and sensory analysis were conducted by using samples prepared for Japanese sashimi. In the dorsal muscles, k-values at 24, 30 and 36 hr increased to 5.6, 5.5 and 6.4% respectively in culture fish compared to 3.1, 3.5 and 3.8% respectively in wild fish. The k-value at 48 h was significantly higher ($P<0.05$) in cultured (17.5%) than that in wild (4.5%) ones. Ventral muscles showed slightly higher k-value at 0 hr in both cultured and wild fish, increasing significantly with time. Deterioration rate of sashimi was faster in cultured than wild fish. On the contrary, at 36 hr the pH values were significantly lower in cultured fish (6.0 in dorsal; 6.0 in ventral) compared to wild fish (6.5 in dorsal; 6.9 in ventral). Water losses and fat losses in ventral muscles in both wild and cultured fish were higher compared to those of dorsal muscles. The data of muscle rheology indicated textures of wild fish were harder than those of cultured fish. However, all samples deteriorated at 48 hr. Sensory scores for dorsal freshness, taste, odor and texture were generally similar and product acceptance of sashimi from both wild and cultured fish was not significantly different. Cultured fish showed significantly higher lipid in dorsal and ventral than in wild. Results demonstrated that physico-chemical properties differences between wild and cultured red sea bream were attributed to higher fat accumulation

in cultured fish than in wild fish. Other parameters indicated no big differences between dorsal and ventral muscles of both wild and cultured fish within 48 hr after slaughter. It can be concluded that wild and cultured red sea bream demonstrated similar quality characteristics within 48 hrs after slaughter.

Keywords: Red Sea bream, k-value, rheological analysis, wild and cultured fish

3.1 Introduction

Red sea bream (*Pagrus major*) is one of Japan′s most cultured marine species (Kato *et al.,* 2002) for sashimi. As the natural supply of capture fish continues to decline, aquaculture has often been looked at as the most promising "aqua-based" source for sashimi. Fish quality in terms of freshness determines the quality of the final fish product (Ólafsdóttir *et al.,* 1997). Consequently, the quality of cultured fish in terms of composition (Robb *et al.,* 2002) should be similar or higher compared to fish caught from the wild. The perishable nature of fish and fish products (Ólafsdóttir *et al.,* 2004) therefore calls for clear understanding of the factors, which determine quality characteristics and the deterioration mechanism after harvest. General quality indexes such as the level of freshness, sensory properties (flavor, smell and taste), water-holding capacity and texture determine reasonable evaluations of quality for both wild and cultured based fishes. Various physico-chemical methods were also documented for estimating the level of freshness in fish (Kent *et al.,* 2004).

Saito *et al* (1959) recommended k-value as one of the superior criteria for rapid assessment of fish freshness, and it has been widely used in related areas (Özogul *et al*, 2006). The k-value is a practical biochemical index (Va'zquez-Ortiz *et al.,* 1997) and has been adapted in grading sashimi(Kaminishi *et al.,* 2000; Hamada-Sato *et al.,* 2005), with k-values below 20% regarded as acceptable for human consumption (Ohashi *et al.,* 1991). However, the k-value varies with fish species, slaughter techniques and preservation temperature as well as the duration of storage. Sensory attribute are also used to evaluate acceptability of fish and fish products ensuring quality assurance during processing (Cardello *et al.,* 1982), and fish texture is mainly determined by water holding capacity in fish muscle. Although numerous studies have been conducted to evaluate fatty acid compositions, rigor mortis and freshness of wild and cultured

fish (Morishita *et al.,* 1989; Iwamoto and Yamanaka 1986; Hatae *et al.,* 1989) respectively, very limited updated information exists on the comparative variations in physico-chemical properties of edible muscles from wild and cultured fish fed using dry or extruded diets.

The objective of this study was therefore to compare the physico-chemical variations in edible muscles obtained from wild and cultured red sea bream obtained from Kagoshima area. Specifically, the study focused on k-value, pH, water-holding capacity, texture properties in relation to storage time, proximate composition and sensory evaluation. This evaluation on red sea bream is hoped to give an updated awareness on impacts of aquaculture to Japan's environment and can be used as guidelines in enhancing high quality aquafeeds in future.

3.2 Materials and methods

3.1.1 Experimental fish

A total of 20 fish of mean weight 1380±40 g were used in the current study; 10 wild fish sampled from the Kagoshima Bay during June 2006 and 10 cultured from Azuma Marine Fish Hatchery, Kagoshima Prefecture. The cultured fish, reared in cages, were fed with a commercial (Higashimaru Co., Ltd. Kagoshima, Japan) diet (40-50% crude protein; 11% lipid). The experimental fish were transported alive in High Density Polyethelene (HDPE) tanks with oxygen aeration and held in a flow-through system tank on arrival at Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University. All fish were acclimatized without feeding for 7 days prior to slaughter using hypothermia method, by immersion into slurry-ice cold marine water prepared and maintained at 3 ppt and 0°C according to method adapted from Losada *et al* (2005). The slurry ice mixture was renewed every 30 min to maintain salinity. After acclimatization, the fish were transferred in insulated polystyrene box

with water drainage, weighted, eviscerated and filleted within 6 hr after slaughter. The weights of the sampled fish varied from 1450 g to 1500 g in the wild captured specimens and 1210 g to 1330 g in hatchery cultured fish with a pooled mean of 1380±40.0 g for all the 20 specimens. Filleting was conducted by separating the dorsal and ventral flesh according to Japanese standards to obtain approximately 500 g fillets from each specimen for the analysis in the current study. The fillets were wrapped in aluminum foil and chilled in ice at 0°C during the analysis. Ice changed every 6 hrs.

3.1.2 Physico-chemical properties

3.1.2.1 pH measurement

Monitoring of physico-chemical properties (pH, k-value and muscle rheology) of the dorsal and ventral fillets was conducted at 6 hr-intervals for a period of 48 hr. The pH measurements were conducted by insertion of an electronic pH meter probe (IQ150 pH meter, IQ Scientific Instruments, Inc.California) into both the dorsal and ventral fillets muscles at 6 hr intervals over a 42 hrs period.

3.2.1.2 K-value and quality deterioration ratio (QDR)

White muscles (3-4 g) were digested with 5 *ml* of 10% trichloroacetic acid (TCA), supernatant was collected, filtered through a filter paper (Advantec no.1, Toyo Roshi Co, Ltd., Tokyo, Japan) and neutralized using 10 M KOH and collected as filtrate 1 (S1). Filtrate 2 (S2) was prepared by mixing 50 *ml* S1 with 50ml alkaline phosphatase (P) and incubated in water bath (37°C) for 20 min. Prior to injection of samples, the Super Freshness Meter reaction cell

was prepared by filling 1 *ml* of air saturated 0.1 M PBS (phosphate buffer saline) into reaction cell and by injecting 10 *ml* S1 (before digestion, total ATP), 10 *ml* E_0 (Xanthine oxidase + Nucleoside phosphorylase) and 20 *ml* S2 (S1+Alkaline phosphatase = dephosphorylated ATP). The k-value was then measured using freshness meter (model KV-202, Central Kagaku Corp., Tokyo, Japan) according to the following formula.

k-value=100*(Inosine + Hypoxanthine)/(ATP+ADP+AMP+IMP+Inosine+Hypoxanthine)

The quality deterioration ratio (QDR) was calculated using the formula QDR=K/H, where K is the k-value of fillet and H is the total storage period after slaughter.

3.1.2.3 Water-holding capacity (WHC)

Water-holding capacity of fillet samples was measured according to Gomez-Guillen *et al* (2000). Five grams of sample and a filter paper (Advantec no.1, Toyo Roshi Co, Ltd., Tokyo, Japan) were placed into a centrifuge tube (15 *ml*) and centrifuged at 7000 rpm for 10 min (10ºC), and then the filter paper containing aqueous fatty fraction were weighed. Percentage of water loss (WL) from the samples was calculated as follows:

WL (%)=100*(V^2-V^1)/Ws, where V^1 is the weight of filter paper (g), V^2 is the weight of filter paper containing aqueous fatty fraction (g) and Ws is the sample weight (g).

The wet filter paper was then dried to a constant weight at 50ºC in an oven, and percentage of fat loss (FL) was calculated as FL (%)=100*(V^3-V^1)/Ws, where V^1 is the weight of filter paper (g), V^3 is the constant weight of filter paper containing fat (g) and Ws is the sample weight (g).

3.1.2.4 Rheological (texture profiles) analysis

Texture profiles of the fish fillets were measured on a Rheoner II Creep (model RE-2-33005B, Yamaden.Co, Japan) consisting of four components: a detector (model RE2-33005B-2), measurement meter (model REZ-OP18-01), a cooler (model ETC-3305-1) and a computer auto-analyzer (Model CA-3305) installed with Texture Analysis Program (TAP ver.1.2-a). Samples were obtained from fillet according to Fig. 1. The temperature of the fresh samples was maintained at 4ºC throughout the analysis and the measurements were set in the central part of the fillet. Analyzed parameters included maximum hardness force and adhesiveness of samples for a period of 48 hr. Texture profiles were expressed in Fig. 2. Maximum hardness force is expressed as (H) maximum height of force in first plunge (Newton) and adhesiveness is the total area of A_3 (J/m^3).

3.1.2.5 Sensory Analysis

Sensory analysis was conducted 6 hrs after slaughter. 500 g fillets from both wild and cultured fish were cross-divided into dorso-ventral sections and sliced to obtain uniform sized sashimi of about 30-mm thickness. The slices were hygienically wrapped in aluminum foil and refrigerated at 4ºC for 30 minutes before served to non-trained panelists comprising of 16 students from the Faculty of Fisheries, Kagoshima University. Sensory evaluation was conducted in a 25ºC air-conditioned room. All panelists were lined 1 m apart and asked to taste and assess the sashimi slices according to odor, texture, freshness and taste scores based on method adapted from Amerine *et al* (1965). The scores were then graded as 10 = excellent, 9 = very good, 7 = good, 5 = medium, 3 = poor, 1 = very poor and 0 = not acceptable.

3.1.2.6 Proximate analysis

Homogenized samples of dorsal and ventral muscle as well as the liver were analyzed in duplicates for protein, lipid, ash, and moisture. Protein determination was conducted according to the AOAC method (1990). Lipid content was analyzed according to the method adapted from Bligh and Dyer (1959), while ash content was obtained by combustion in muffle furnace at 550ºC. Moisture, on a 5 g sample of freeze-dried fillet muscle, was determined by oven-drying at 110ºC to constant weight.

3.3 Statistical Analysis

Statistical analysis on the physico-chemical parameters was conducted using One-Way Analysis of Variance (ANOVA) and posthoc tests were done using the Tukey-HSD for differences among treatments. Two-way analysis of variance (ANOVA) was used to analyze proximate composition of dorsal and ventral parts of wild and cultured fish. Data were expressed as means $\pm$ S.E (n= 3x3 individual fish muscles samples). All tests were considered significant at $P<0.05$.

3.4 Results

3.4.1 Physico-chemical properties; pH, k-value, and k-value QDR of fillet samples

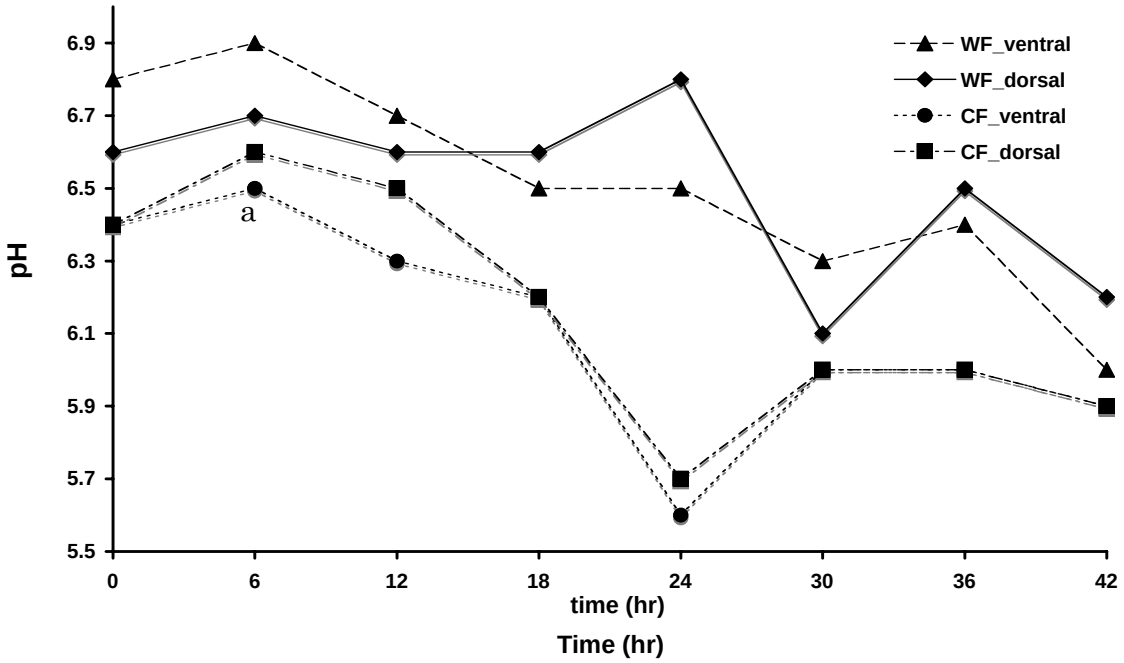


Fig. 3 Changes of pH according to time series for dorsal and ventral muscle fillets of wild and cultured red sea bream

The pH of dorsal and ventral samples decreased with storage time as shown in Fig. 3. In the dorsal samples, there were no significant differences in pH between wild and cultured fish at 0 hr, 6 hr and 12 hr, in which pH ranged from 6.4 to 6.6. The pH decreased gradually until 42 hr for both wild and cultured fish. Results skewed a little at 24 hr and 30 hr in which wild dorsal pH suddenly increased significantly to 6.8 and dropped to 6.1 respectively. Wild dorsal samples indicated significant higher pH value as compared to cultured dorsal ones at 18 hr, 24 hr and 36 hr. In ventral area, pH in wild was also significantly higher than that of cultured samples from 0 hr to 36 hr, in which pH ranged from 5.6 to 6.9. There were no significant differences at 42 hr for both groups.

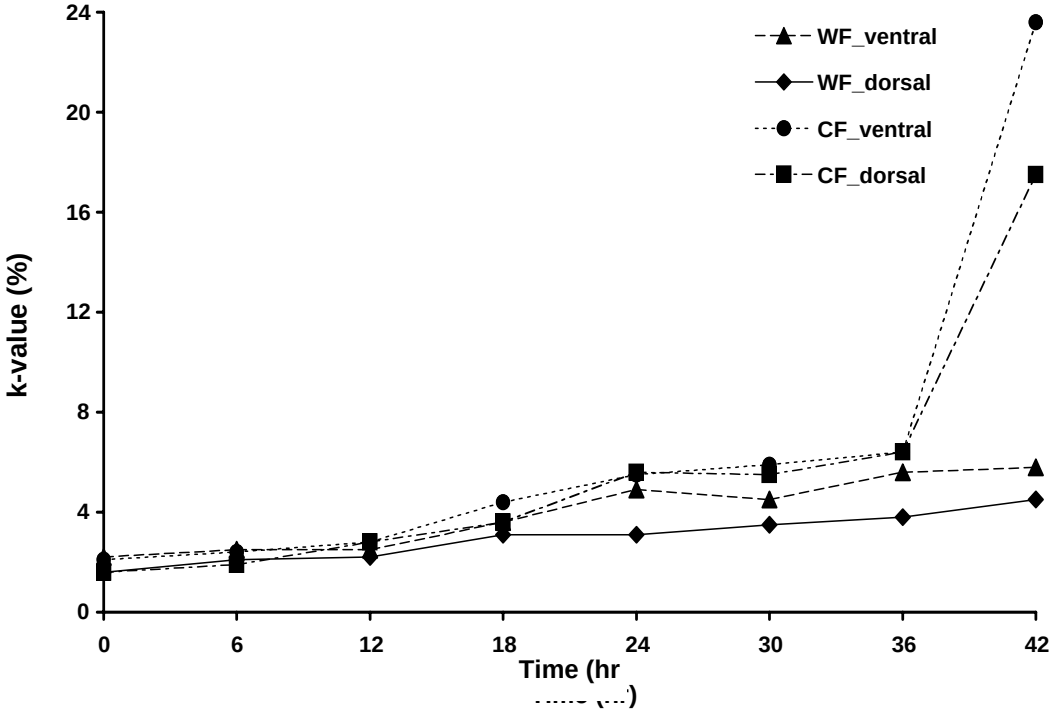


Fig. 4 Changes of k-value according to time series for dorsal and ventral muscle fillets of wild and cultured red sea bream

The k-value in wild and cultured fish samples during cold storage (0ºC) is presented in Fig. 4. In dorsal muscle, the k-value increased from a level of 1.6% to 4.5% in wild fish and 1.6% to 17.5% in cultured fish. The values of dorsal muscles from cultured fish showed significantly higher than those of wild ones after 24 hr. At 42 hr, k-value increased suddenly to 17.5% in cultured muscles. In ventral muscles, k-values gradually increased in both wild and cultured muscles up to 36 hr. However, at 42 hr, a sudden increase of k-value to 23.6% occurred in cultured muscles, which was significantly higher than that of wild ones.

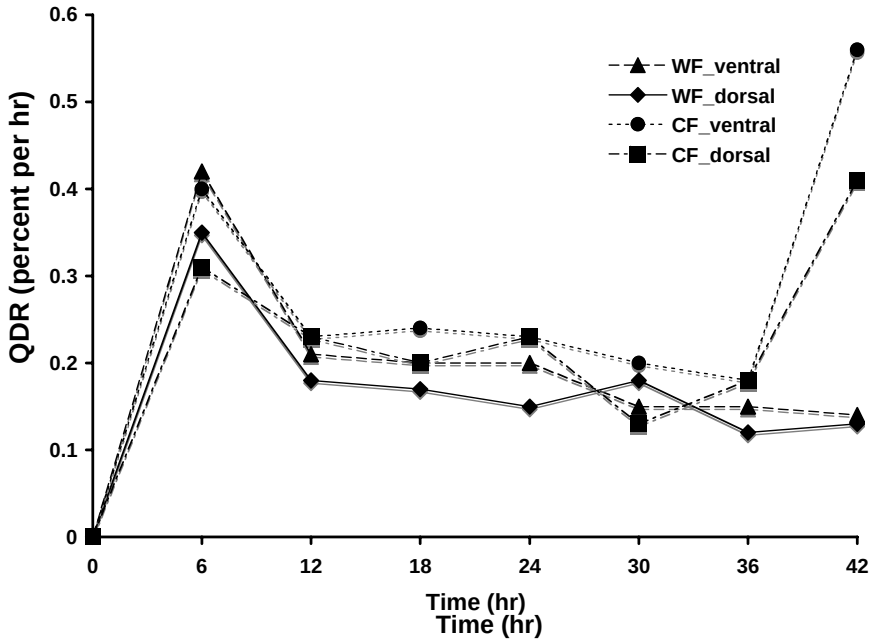
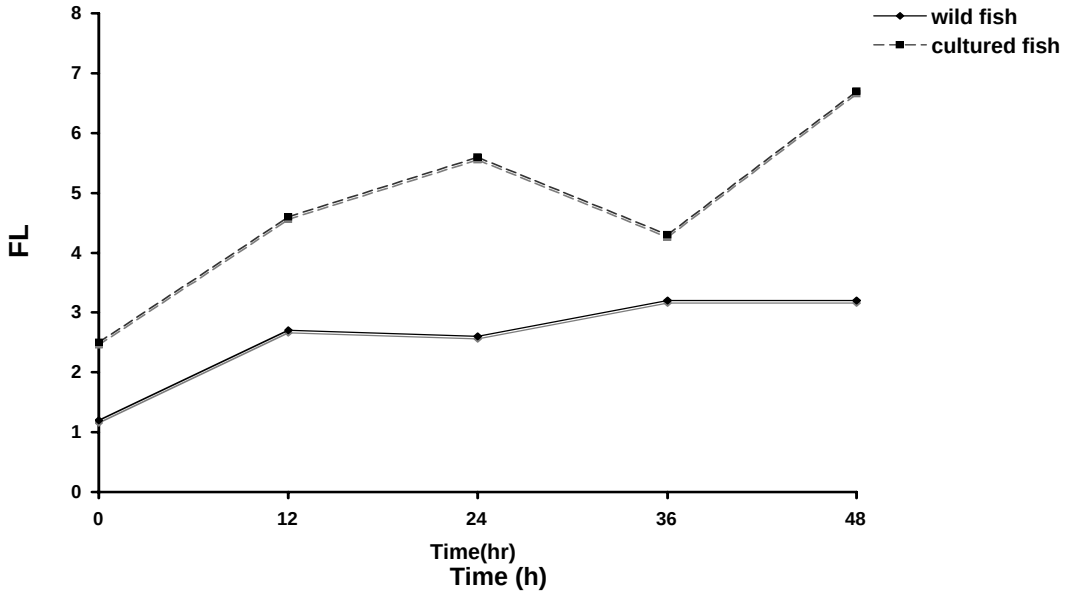


Fig. 5 Changes of quality deterioration ratio according to time series for dorsal and ventral muscle fillets of wild and cultured red sea bream

In terms of QDR (Fig. 5), dorsal and ventral muscles of cultured fish showed higher level of deterioration ratio as compared to wild dorsal and ventral muscles. At 6 hr after slaughter, QDR was high in all samples without significant differences among them. Ratio gradually decreased and remained at constant level up to 36 hr for all treatments. At 42 hr, QDR of cultured fish samples from both dorsal and ventral muscles escalated to 0.41 and 0.56%/h respectively and were significantly higher than those of wild ones.

3.4.2 Water-holding capacity (WHC)

WHC was represented by water loss (WL) and fat loss (FL) in muscle samples, and the changes of dorsal muscles are illustrated in Fig. 6.



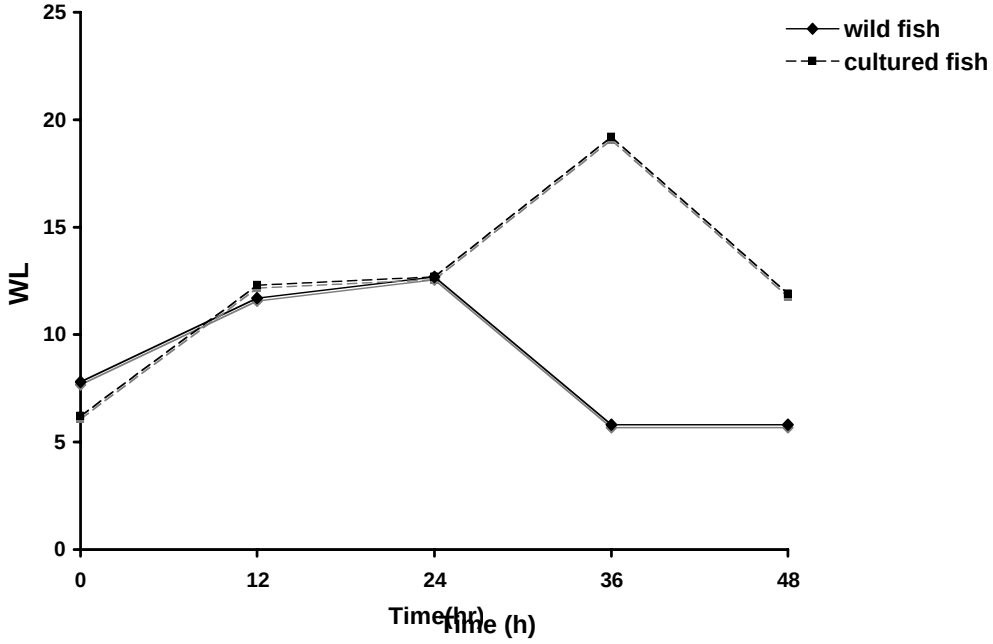


Fig. 6 Changes of water holding capacity according to time series in dorsal muscle fillets of wild and cultured fish

The pattern of WL between wild and cultured dorsal was similar up to 24 hr. However, at 36 hr, WL value of cultured samples increased but that of wild ones dropped to 5.7%. FL values of cultured dorsal were significantly higher than those of wild dorsal up to 36 hr. Comparatively,

WL and FL in ventral fillets of both wild and cultured fish were higher compared to those of dorsal fillets (Figs. 6 and 7). Although no clear pattern in WL was observed between two groups, FL in ventral fillets of wild fish differed significantly from that of cultured one in all periods.

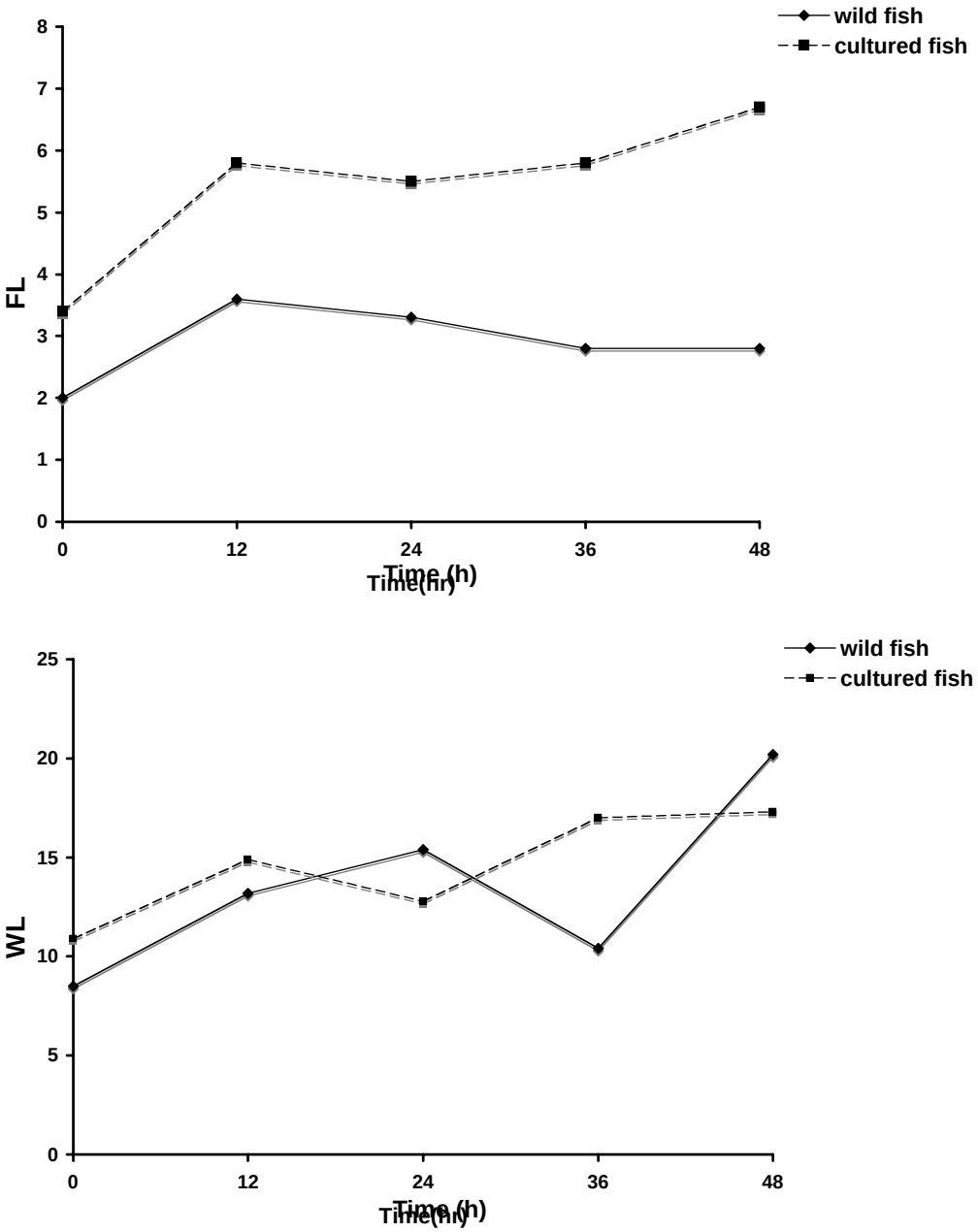


Fig. 7 Changes of water holding capacity according to time series in ventral muscle fillets of wild and cultured fish

3.4.3 Rheological (texture profiles) analysis

Fig. 8 illustrated maximum hardness force in dorsal and ventral muscle fillets of wild and cultured fish. In ventral muscles, the values of wild fish were significantly higher than those of cultured ones from 6 hr to 24 hr, but were similar at 30 hr and 36 hr. Those were leveled off up to 48 hr in cultured samples whereas it dropped zero in wild muscles at 42 hr. In dorsal muscle, the values of wild fish was significantly higher than those of cultured ones at 0, 6, 12, 18, 24, 42 and 48 hr but became similar at 30 and 36 hr.

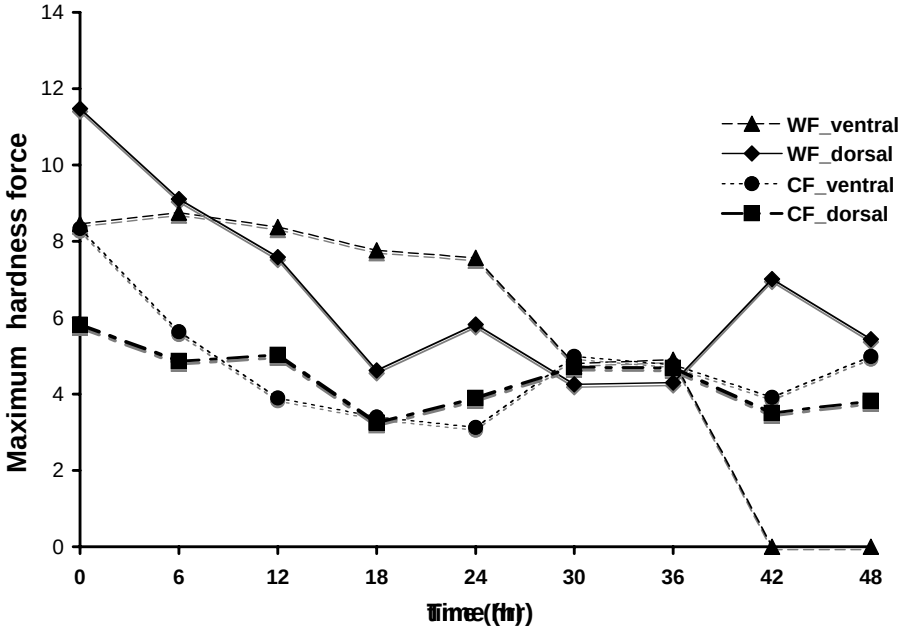


Fig. 8 Maximum hardness force pattern in dorsal and ventral muscle fillets of wild and cultured fish

Dorsal and ventral muscles differed in terms adhesiveness or gumminess of muscles as expressed in Fig.9. Dorsal muscle fillets of both wild and cultured showed more adhesiveness throughout 48 hr with some fluctuations than ventral muscles. Adhesiveness was more pronounced at 0 hr for both samples obtained from dorsal areas. Apparently, the values of

dorsal muscles decreased drastically to less than 5000 J/m^3 after 18 hr. In comparison, ventral areas for both groups were less adhesive and continued at low level until the end of analysis.

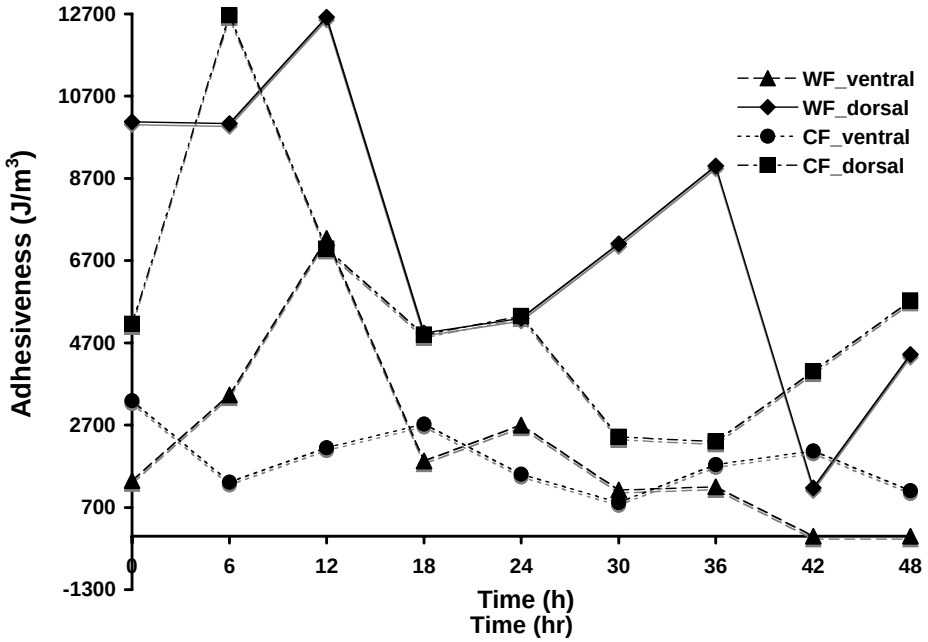


Fig. 9 Adhesiveness according to time series in muscle fillets of wild and cultured fish

3.4.4 Sensory Analysis

The sensory scores from wild and cultured red sea bream are demonstrated in Fig. 10. In dorsal and ventral muscles, freshness, taste, odor and texture scores were good in average without significant differences ($P>0.05$) among all treatments. Results indicated quality of both specimens was well accepted by panelists.

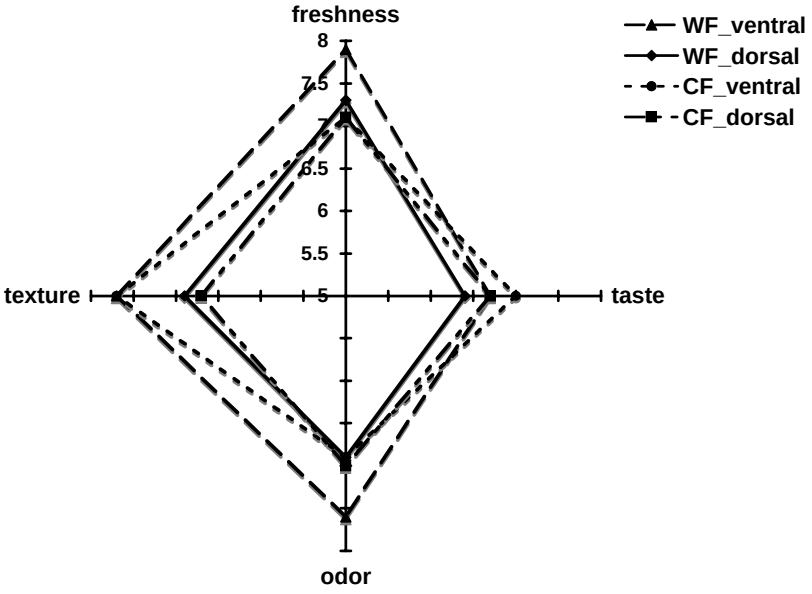


Fig. 10 Sensory analysis of wild and cultured red sea bream sashimi

3.4.5 Proximate analysis

Proximate composition of wild and cultured fish is presented in Table 1. There were no significant differences ($P>0.05$) in crude protein and ash in both dorsal and ventral muscles between wild and cultured fish. Moisture had inverse relationship with lipid content in which higher lipid content decreased moisture level in all fish samples. Results indicated that cultured fish had significantly higher amounts of lipid in both dorsal and ventral areas as compared to wild fish. Lipids contents in dorsal muscles were relatively lower than those in ventral ones in both wild and cultured fish.

Table 1. Proximate composition and major fatty acids contents of wild and cultured fish muscles

Proximate composition[1]	Dorsal muscle		Ventral muscle	
(%)	Wild	Cultured	Wild	Cultured
Crude lipid[2]	2.8±0.2[a]	9.3±0.3[c]	4.9±0.5[b]	14.2±0.0[d]
Crude protein[2])	20.9±0.2	21.7±0.3	22.2±0.3	21.2±0.4
Ash[2]	1.4±0.0[a]	1.3±0.0[a]	1.5±0.0[b]	1.4±0.0[a]
Moisture	74.9±0.1[d]	67.6±0.1[b]	72.3±0.1[c]	63.9±0.1[a]
Major fatty acids (ng/mg dry sample)				
C18:2n-6	768.0±133.0[a]	6728.0±356.00[b]	1033.5±74.5[a]	8043.5±57.5[c]
C18:3n-3	183.5±21.5[a]	1311.5±46.5[b]	277.0±13.0[a]	1427.0±251.0[b]
C20:5n-3	2456.5±0.5[a]	6405.0±323.0[b]	3410.5±15.5[a]	8949.0±125.0[c]
C22:6n-3	5788.5±776.5[a]	14641.0±306.0[b]	6280.5±258.5[a]	16430.0±1126.0[b]
Total PUFA	10359.0±776.0[a]	33418.0±297.0[b]	12698.5±179.5[a]	40479.0±746.0[c]

[1] Values are means ± SE (n=3X3 individual fish muscle samples), and those with the same letters show no significant differences (P>0.05).

[2] dry weight basis

3.5 Discussion

Existence of lipid in fish tissues influences other factors like shelf-life and sensorial preferences. In terms of shelf-life, changes in k-value, pH and water binding capacity by storage time give high impact to fish quality. The k-value relates to fish freshness and it emphasizes on degradation of energy metabolites from ATP to hypoxanthine and inosine in fish products and normally demonstrates a positive relationship between degradation of fish quality and storage time. The present study demonstrated that cultured fish accumulates more lipids in its muscles especially in ventral area. Gradient degradation of freshness in fish is often associated with lipid content in fish. The present study indicated that critical time for deterioration begins at 6 hr after slaughter for both wild and cultured fish, and then became stable from 12 to 36 hr. However, deterioration ratio doubled in dorsal and tripled in ventral at 42 hr. Concurrently, k-value had similar trends for all respective samples. The k-value of cultured ventral muscles at this points exceeded acceptable k-value for "sashimi". Cultured ventral and dorsal muscles showed higher elevation of k-values while wild ventral and dorsal ones demonstrated lower and slower elevation rate against time. Cultured samples also indicated the k-value elevation of more than 300% in both dorsal and ventral muscles at 42 hr. The value of cultured ventral muscles at these points exceeded acceptable k-value for sashimi. These elevations were perhaps due to oxidation of unsaturated fats (Brody, 1999) particularly unsaturated "double bonds" sourced from aquafeeds as observed by Raatikainen *et al* (2005). (Bremner, 2002) suggested that lipoxygenase (LOX) is involved in enzymatic lipid oxidation on C20 fatty acid oxidation that initiates rancidity in raw fish. Therefore, cultured fish commonly known with lipid deposition in adipose tissues (Suzuki *et al.,* 1986; Richards *et al.,* 2002) turns rancid faster than wild fish. Degradation of fish freshness then occurs due to

synergism impact between rancidity and autolysis of ATP (Erikson *et al.,* 1997) which resulted undesirable k-value as reported by Aubourg *et al* (2007). In contrary, wild fish deteriorate at slower pace. From freshness point of view, sashimi from ventral part of cultured fish taken from 42 hr is unsuitable for consumption because of exceeded standard k-value (20%).

pH drop in cultured fish especially in ventral muscle may be due to high production of lactic acid through "ultimate" activities like struggling and force-swimming to escape from being captured as observed by Olsson *et al* (2003a) on halibut. In comparison, wild fish that were exposed to external pressures throughout their life had probably "rested" enough during the acclimatization period. Degradation in fish freshness is often related to low pH which prevents synthesis of ATP (Robb, 2002), resulting in locked rigor mortis, and without ATP, process of rigor and post-rigor becomes more rapid and reduces quality in fish. In relation to this study, deterioration of muscle tissues in cultured fish appeared to be more pronounced as compared to wild fish.

The present study showed that wild fish has better WHC as compared to cultured fish, in which wild fish demonstrated significant lower water loss 36 hr after slaughter while cultured fish illustrated continuously constant high water loss value. Similar observation was also reported by Olsson *et al* (2003b) in Atlantic halibut. In general, water loss and pH have an inverse relationship (Olsson *et al*., 2003b) in which, higher pH in wild fish usually contributes to lower water loss. On the other hand, low water content in cultured fish and low postmortem pH (Lavéty *et al.,* 1988) contribute gaping in Atlantic cod (Espe *et al.,* 2004) and rainbow trout (Mørkøre *et al.,* 2002). Low pH is usually associated with degradation of myofibrillar properties in fish muscle. Myofibrils usually occupy a substantial volume of the muscle (Goodband, 2002) and control the water holding capacity of the whole muscle as described by

Offer and Trinick (1983). In this present study, pH began to degrade after 36 hr (dorsal area) and 30 hr (ventral area) respectively. The pH values were relatively low at this point of time and may influence the increased water loss in cultured fish. This observation corresponds to Olsson *et al* (2003b) that liquid loss (LL) increased with decreasing pH lower than 6.3, whereas at higher pH values, LL was independent on pH.

Fat loss differed significantly in both wild and cultured fish immediately after time of slaughter to 36 hr. Melting properties of triglyceride relate to its fatty acid components, in which high proportion of fatty acids with relatively short chain and low melting point melt easily at lower temperature. This corresponds to the study of Rørå *et al* (2003) that smoked fillet lost relatively higher quantities of lipid at 22ºC. In this study, fats in both wild and cultured fish may consist of low melting fatty acids that promote fat loss at 50ºC. High deposition of lipids (Table 1) in adipose tissues may explain the high amount of fat loss especially in cultured fish.

The present results revealed that wild fish has tougher muscle structure compared to its cultured counterpart even after slaughter (0 hr). On the other hand, dorsal areas of both wild and cultured fish also required a higher degree of force to puncture muscles. Changes of muscle texture differ between dorsal and ventral areas due to degree of lipid deposition in the adipose tissues. Ventral area especially in cultured fish has a higher tendency to retain lipid than dorsal area. Therefore, higher lipid deposition along the connective tissue in this area resulted structural weakening of the muscle, leading to a softer meat texture as observed by Prasad Thakur *et al* (2003) on yellowtail. The swimming behavior also exhibits frequent muscle movement especially at dorsal area but less in ventral area and this may promote deposition of lipid in the belly. Therefore, formation of lipid in the adipose area-surrounding belly of ventral

muscle promotes more rapid lipid hydrolysis and fish deterioration.

Sensory analysis showed that panelists were unable to differentiate between sashimi obtained from dorsal and ventral parts of both wild and cultured fish. High scores of freshness, taste, odor and texture of the sashimi indicated well acceptance by panelists. Our results differed from the study of Grigorakis *et al* (2003), in which wild fish was described as 'more juicy' as compared to cultured counterpart. Grigorakis *et al* (2003) had associated juicy with loosely bound water located in fish flesh and sample preparation by steam-cooking samples for sensory test method may have induced water loss in cultured fish samples. In comparison, our study (Fig. 6 and Fig. 7) demonstrated no significant difference of water loss between cultured and wild fish samples within 24 hrs after slaughter and since water content is still intact in both cultured and wild fish sample, our sensorial taste panelists find no differences in texture and taste in neither cultured nor wild fish. Firmness defines good muscle texture in fish, confirming to Dunajski (1979) and Johnston *et al* (2000), in which firmness in fish texture is influenced by high fiber density. However, texture becomes tenderer with storage time (Skjervold *et al.,* 2001) due to reduced content and strength of the connective tissue (Sato *et al.,* 1986). At this point of time, sashimi quality may deteriorate and unsuitable for consumption.

Comparative studies between wild and cultured fishes conducted on seabass (Alasalvar *et al.,* 2002; Orban *et al.,* 2002), gilthead sea bream (Grigorakis *et al.,* 2002; Grigorakis *et al.,* 2003), salmon (Sylvia *et al.,* 1995; Farmer *et al.,* 2000; Einen and Thomassen, 1998) and Atlantic halibut (Olsson *et al.,* 2003a) demonstrated that there were significant differences between wild and cultured fish in terms of several attributes like lipid content and texture as were reported in this study. Comparatively, cultured fish showed whiter and softer muscles in both dorsal and ventral areas than wild fish, which agreed to the reports by Grigorakis *et al*

(2003) and Jobling *et al* (2002). Softer muscles are often associated with accumulation of fats in adipose tissues. Cultured fish accumulates fats easily in its fish flesh especially in ventral muscles (Suzuki *et al.,* 1986) due to consumption of diets with high total lipid and limited mobility in restricted environment. In comparison, constant mobility (Jutila *et al.,* 2002), prey hunting (Reid *et al.,* 1993) and migration by wild fish develop compositional and textural changes which produce leaner, less-fatty and firmer fish. During force-swimming, wild fish consume energy at dorso-posterior area rather than antero-posterior area resulting greater lipid deposition in ventral muscles as observed by Toussaint *et al* (2005).

It can be concluded that sashimi of both wild and cultured red sea bream is best consumed at approximately 6 hrs after slaughter due to low k-value, high pH, high WHC and good texture chilled under low designated temperature 0 to 4°C. Wild and cultured red sea bream also demonstrated similar quality characteristics within 48 hrs after slaughter and these features can be further enhanced through improvement of diet formulation.

Chapter 4

Effect of vegetable oil replacement on red sea bream *Pagrus major*

Study II:

Influences of palm oil blended with fish oil on growth performances and lipid profiles of red sea bream *Pagrus major*

Study III:

Influences of soya oil blended with fish oil on growth performances and lipid profiles of red sea bream *Pagrus major*

Study II:

Influences of palm oil blended with fish oil on growth performances and lipid profiles of red sea bream *Pagrus major*

Abstract

This study was aimed to investigate the effects of replacing fish oil with palm oil (P) blended with fish oil (F) as the lipid source on growth performance and lipid profiles of red sea bream. A 50-day feeding trial for juveniles (initial body weight 3.68±0.09g) was conducted in triplicates using 4 isocaloric diets containing different ratio of fish oil and palm oil such as {10:0 (10F0P), 8:2 (8F2P), 6:4 (6F4P) and 4:6 (4F6P). Growth performance in terms of specific growth rate (SGR), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and hepatosomatic index (HSI) were taken. Protein, moisture, ash and lipid contents in both whole body and liver, fatty acid compositions in dorsal and ventral muscles, and liver were also compared among treatments. There were no significant differences in SGR of fish fed with 10F0P and 8F2P but the SGR in both treatments were significantly higher than that of 4F6P.

Diet 6F4P and 4F6P groups demonstrated significant lower FI as compared to diet 10F0P and 8F2P groups. Whole body lipid deposition in fish fed diet 4F6P was significantly lower than those of other dietary groups. On the other hand, the dorsal lipid contents gradually decreased with increased palm oil level but there was no significant differencein ventral muscle. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents in both dorsal and ventral muscles gradually declined with increased dietary palm oils. A similar gradient degradation in n-3/n-6 ratio was also demonstrated in both dorsal and ventral muscles although no signs of EFA deficiency occurred throughout the experimental period. In conclusion, diets containing not exceeding 40% palm oil may be a suitable ratio in incorporating palm oil as a fish oil replacement.

Keywords: *Pagrus major*, palm oil, fatty acid composition, growth performances

4.1 Introduction

High dependency on marine fish oil as a major lipid source in aquafeeds has raised major concerns of sustainability for aquaculture in the future due to limited supply of fish oil and expanding global nature of aquaculture products. Therefore, many efforts have been made to find alternative oils in replacing fish oil. Several ongoing research projects that focus on the possibility of using various vegetable oils indicated encouraging results. Soybean oil, rapeseed oil, linseed oil and sunflower oil were reported to give comparative efficiency in growth performances and feed utilization to those fed with fish oil for several species like Atlantic salmon (Tortensen *et a.l*, 2000; Jordal *et al.*, 2007; Ng *et al.*, 2004), gilthead sea bream (Izquierdo *et al.,* 2005), European seabass (Mourente and Bell 2006) and red sea bream (Glencross *et al.,* 2003). However, complete replacement for fish oil is not possible due to the lack of essential fatty acid contents in those plant oils.

Potentials of palm oil in efforts to partially replace marine fish oil have been accepted by researchers in recent years (Ng, 2006). Besides being rich in linoleic acids, and a component of essential fatty acids required by fish to grow, palm oil is also affordable and available. Based on the information available in a few species, inclusion levels of palm oil to replace fish oil are similar to those of other plant oils (Bell *et al.,* 2002; Ng *et al.,* 2000; Ng *et al.,* 2003). However, researches on influences of palm oil inclusion in aquafeeds for Japanese cultured species are not yet available.

Thus, the objective of this study was to examine the influence of partial replacement of fish oil by palm oil on the growth performance of red sea bream, *Pagrus major*, which is one of the most important marine cultured species in Japan (Kato *et al.,* 2002).

4.2 Materials and methods

4.2.1 Experimental fish and feeding protocol

Red sea bream juveniles were obtained from a commercial hatchery (Matsumoto Suisan Ltd., Miyazaki Prefecture) and transported alive to Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University. Fishes were placed in the indoor stock tanks with filtered and aerated sea water, and acclimatized in the tanks a week prior to a feeding trial. During the acclimatization, fishes were fed with commercial pellets (Higashimaru Foods Ltd., Kagoshima, Japan).

Each of fifteen juveniles with mean weight of 3.68±0.09 g was distributed in 12 units of 100-L flow-through tanks. Rearing seawater parameters such as dissolve oxygen, salinity and ammonia were monitored daily. Four experimental diets with different ratio of fish oil : palm oil (Table 2) were fed manually to fish in triplicates twice a day (0800 and 1600hr) until apparent satiation. Test diets contained different ratios of fish oil and palm oil such as 10:0 (10F0P), 8:2 (8F2P), 6:4 (6F4P) and 4:6 (4F6P).Tanks were cleaned and uneaten diets were collected to determine feed intake daily basis.

Test diets were isocaloric, isonitrogenic and isolipidic, and contained 52 % protein, 10% lipid and 14% ash, respectively. Fish were exposed to a photoperiod condition of 12 h light/12 h dark, and water flow was maintained at 2.5 L/min and temperature was at 28.7 ± 1.5°C (mean ± SD) during the rearing period. Fish were weighted in bulk for each tank in every 10 days.

Table 2. Basal ratio of experimental diets containing different levels of palm oil

Ingredients (g/kg DM)	Diets			
	10F0P	8F2P	6F4P	4F6P
Brown fish meal[1]	670.0	670.0	670.0	670.0
Activated gluten	80.0	80.0	80.0	80.0
α-Starch	40.0	40.0	40.0	40.0
Dextrin	40.0	40.0	40.0	40.0
Fish oil	100.0	80.0	60.0	40.0
Palm oil	0.0	20.0	40.0	60.0
Mineral mix[2]	30.0	30.0	30.0	30.0
Vitamin mix[3]	26.5	26.5	26.5	26.5
Stay-C[4]	3.5	3.5	3.5	3.5
α-Cellulose	10.0	10.0	10.0	10.0
Total	1000	1000	1000	1000
Analytical contents (dry matter basis)				
Crude protein (%)	51.0	50.7	52.4	53.8
Total lipid (%)	9.4	10.0	10.2	9.5
Ash (%)	14.3	14.4	14.3	14.7
Gross energy (kcal/g diet)	6.3	6.3	6.3	6.3

[1] Defatted brown fish meal

[2] Mineral mix (g/kg): NaCl, 0.183; $MgSO_4 \cdot 7H_20$, 0.685; $NaH_2PO_4 \cdot 2H_2O$, 0.436; KH_2PO_4, 1.199; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 0.679; Fe Citrate, 0.148; Ca Lactate, 1.635; $AlCl_3 \cdot 6H_2O$, 0.00009, $ZnSO_4 \cdot 7H_2O$, 0.017;$CuCl_2$, 0.00005; $MnSO_4 \cdot 4H_2O$, 0.004; KCl, 0.008; $CoCl_2$, 0.005

[3] Vitamin mix (g/kg): p-aminobenzoic acid, 1.45; biotin, 0.02; myo-inositol, 14.5; nicotinic acid, 2.9; folic acid, 0.05; choline chloride, 29.65

[4] Stay-C: L-Ascorbyl-2-monophosphate-Na/Ca (DSM Nutrition Japan K. K.)

At the end, fish were killed using hypothermia method by immersion into a slurry of ice cold sea water prepared and maintained at 3 ppt and 0°C according to Losada *et al* (2005), and individual weight of fish was taken. Liver samples were removed, pooled and stored at -80°C until analysis. Filleting was conducted by separating the dorsal and ventral flesh according to

Japanese filleting method. Fillets from each treatment were pooled and maintained in ice before freeze-dried for further analysis. Three fish were also randomly sampled from each respective tank and used for carcass proximate analysis.

4.2.2 Proximate analysis

Homogenized samples of whole body and liver were analyzed in duplicates for the analysis of protein, lipid, ash and moisture. Protein was determined by using the Kjeldahl method (AOAC 1990), lipid by Bligh and Dyer (1959) and ash was analyzed by combustion in muffle furnace at 550°C. Moisture was determined on approximately 5 g of minced samples by oven-drying at 110°C to constant weight according to AOAC (1990).

4.2.3 Fatty acid analysis

Fatty acid composition was analyzed based on the study of Querijero *et al* (1997) with slight modifications. Total lipid (TL) was extracted by homogenizing 0.2 g sample according to Bligh and Dyer (1959). Fatty acid esters were then produced from total lipids aliquots. Samples were then methylated with boron triflouride ($BF_{3)}$ in methanol. Methyl tricosanoate (Nu-Chek Prep. Inc) was used as internal standard at 1.000 mg/ml hexane. Fatty acid methyl ester was analyzed with a gas chromatograph (Shimadzu GC 17A) with flame ionization detector temperature maintained at 260˚C; carrier gas He at 1 ml/min; column temperature at 200°C; injector temperature at 250°C and helium (He) served as the carrier gas. The samples (1.0 µl) were manually injected into injection port and identified fatty acids were presented as area percentage of total fatty acids.

4.3 Statistical analysis

The statistical analysis was performed using an analysis of variance (package super-ANOVA, Abacus19 Concepts, Berkeley, California, USA). Data were expressed as means ± S.E. Homogeneity of variance between treatments (5% level of probability) were analyzed using one-way ANOVA while significant differences of mean values were determined at 5% level of probability using Tukey Test.

4.4 Results

4.4.1 Growth performances

Final weight, specific growth rate (SGR) and body weight gain (BWG) in 4F6P were significantly lower than those of 10F0P, 8F2P and 6F4P (Table 3). Although feed conversion ratios (FCR) of all treatments were similar, fish fed with higher level of fish oil (10F0P and 8F2P) indicated significantly higher ($P < 0.05$) feed intake (FI) than other treatments throughout a 50-day experimental period. Results also showed that hepatosomatic indexes (HSI) were similar ($P > 0.05$) among treatments. Survival rate (SR) in all treatments demonstrated at least 96% without any significant differences ($P > 0.05$) among treatments.

Table 3. Growth performances of red sea bream *Pagrus major* fed with different diets

Growth parameters[1]	Diets			
	10F0P	8F2P	6F4P	4F6P
Initial weight (g/fish)	3.8±0.0	3.7±0.0	3.6±0.0	3.7±0.1
Final weight (g/fish)	44.8±0.0^{d}	42.9±0.2^{c}	39.5±0.3^{b}	31.7±0.2^{a}
SGR[2]	5.0±0.0^{b}	4.9± 0.0^{b}	4.8±0.0^{b}	4.3±0.1^{a}
FCR[3]	1.2±0.1	1.2±0.1	1.1±0.1	1.3±0.2
BWG[4]	1091.2±10.1^{c}	1072.2±1.9^{c}	998.7±11.6^{bc}	764.0±36.0^{a}
HSI [5]	1.2±0.0	1.3±0.0	1.3±0.0	1.4±0.2
FI(g/fish/50days)[6]	49.9±1.9^{c}	46.4±0.1^{bc}	35.2±0.1^{a}	34.2±2.5^{a}
SR[7]	100.0±0.0	100.0±0.0	96.7±0.0	100.0±0.0

[1]Values are expressed as mean ± SE (n=3). Data with same alphabets are not significantly different (P>0.05).

[2] SGR, specific growth rate= 100 x (ln final weight- ln initial weight)/(duration)

[3] FCR, feed conversion ratio = dry feed intake(kg)/weight gain(kg)

[4] BWG, body weight gain (%)= 100 x (final weight - initial weight) / (initial weight)

[5] HSI, Hepatosomatic index = 100 × (liver weight/body weight)

[6] FI, Feed intake (g/fish/50days) = (total feed intake (g) / number of fishes) in 50 days feeding period

[7] SR, survival rate (%) = 100 x (initial fish number-dead fish number) / (initial fish number)

4.4.2 Proximate analysis

Whole body data (Table 4) demonstrated no significant differences ($P > 0.05$) among 10F0P, 8F2P and 6F4P diets in lipid contents but lower level was shown in 4F6P. In dorsal muscle, lipid deposition was differed significantly ($P < 0.05$) among treatments with inverse relation to palm oil content. In contrast, ventral muscle demonstrated higher lipid deposition than dorsal muscle without any significant differences among treatments ($P > 0.05$).

Table 4. Lipid, protein, ash, and moisture contents of whole body, muscles and liver in red sea bream *Pagrus major* fed with different diets

Parameters[1]	10F0P	8F2P	6F4P	4F6P
Lipid (%)[2]				
Whole body	9.1±0.3^{b}	10.0±0.1^{b}	10.2±0.1^{b}	7.6±0.3^{a}
Dorsal muscle	14.0±0.0^{d}	12.9±0.12^{c}	11.8±0.1^{b}	10.6±0.1^{a}
Ventral muscle	23.2±0.1	24.3±0.2	24.7±0.3	24.7±0.6
Liver	12.8±0.5	12.7±0.5	11.9±0.3	12.9±0.8
Protein (% whole body)[3]	14.7±0.3^{a}	16.0±0.4^{b}	15.8±0.0^{b}	15.2±0.4^{ab}
Ash (% whole body)[3]	4.1±0.0^{a}	4.6±0.0^{abc}	5.1±0.1^{c}	4.2±0.1^{ab}
Moisture (% whole body)	72.3±0.5^{b}	69.8±0.7^{ab}	68.6±0.1^{a}	72.2±0.8^{b}

[1] Values are expressed as mean ± SE (n=3). Data with same alphabets are not significantly different (P>0.05).

[2] Dry weight basis

[3]Wet weight basis

Lipid contents of liver in all treatments were higher than those of whole body with no significant differences (P > 0.05) among treatments. Ash contents in treatment 6F4P were significantly higher (P < 0.05) than those of 10F0P and 4F6P and moisture contents in 6F4P were significantly lower (P<0.05) than those of 10F0P and 4F6P. On the other hand, protein contents in treatment 8F2P and 6F4Pwere significantly higher than that in 10F0P.

4.4.3 Fatty acid composition

Dietary profiles of fatty acid composition (Table 5) showed dominance of saturated and total poly-unsaturated fatty acids (PUFAs), ranging from 27.3 to 32% and 31.6 to 38.4%, respectively. In contrast, higher inclusion of palm oil increased concentration of total n-6 (particularly C18:2n-6).

Saturates, total PUFAs and monoenes were more prominent in dorsal muscle (Table 6) and ventral muscle (Table 7), and liver (Table 8). With dietary increased palm oil, saturates

and monoenes levels elevated in ventral muscles and liver while gradual decline of total n-3 fatty acids was shown in dorsal and ventral muscles. On the other hand, total n-6 concentrations were not affected by dietary treatment, except in liver.

Table 5. Fatty acid composition (% of total fatty acid) in experimental diets

Types of fatty acid[1]	Diets			
	10F0P	8F2P	6F4P	4F6P
14:0	5.2	4.4	3.1	1.9
16:0	17.0	21.9	24.5	25.9
18:0	5.1	4.2	4.4	3.5
∑Saturated	27.3	30.5	32.0	31.3
16:1n-9	6.7	5.8	4.3	2.3
18:1n-5	0.1	0.2	0.2	0.0
18:1n-9	8.7	8.9	11.1	12.7
20:1n-9	0.3	0.2	0.4	0.6
22:1n-7	0.1	0.2	0.1	0.2
22:1n-9	0.8	0.5	0.7	0.5
22:1n-11	1.1	0.9	0.1	0.5
∑Monoenes	17.8	16.7	16.9	16.8
18:2n-6	6.2	6.7	7.0	8.0
20:4n-6	0.7	0.9	1.0	0.7
22:4n-6	4.0	5.8	5.3	6.6
∑n-6fatty acids	10.9	13.4	13.3	15.3
18:3n-3	0.1	0.1	0.0	0.7
18:4n-3	0.2	0.2	0.8	0.6
20:4n-3	0.8	0.4	0.3	0.3
20:5n-3	9.5	3.8	3.3	2.1
22:5n-3	2.0	1.3	1.2	1.0
22:6n-3	14.9	13.2	12.7	12.0
∑n-3 fatty acids	27.5	19.0	18.3	16.7
∑PUFA[2]	38.4	32.4	31.6	32.0
∑n-3HUFA[3]	27.2	18.7	17.5	15.4
∑n-3/n-6 ratio[4]	2.5	1.4	1.4	1.1
∑EPA+DHA[5]	24.4	17.0	16.0	14.1

[1] Values are expressed as means ±S.E (n=3). Same superscripts are not significant different ($P > 0.05$).

[2] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[3] Total n-3HUFA is expressed as sum of n-3 fatty acids in carbons more than 20

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of Eicosapentaenoic Acid (C20:5n-3) and Docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 6. Fatty acid composition (% of total fatty acid) in dorsal muscle of red sea bream *Pagrus major* fed with different diets

Types of fatty acid[1]	Dorsal			
	10F0P	8F2P	6F4P	4F6P
14:0	3.5±0.1[d]	3.0±0.1[c]	2.2±0.1[b]	1.8±0.0[a]
16:0	22.3±10.5	23.0±0.3	25.2±0.2	25.9±1.7
18:0	7.8±0.1[a]	8.5±0.0[b]	8.4±0.2[ab]	4.2±0.3[ab]
∑ Saturated	33.6±0.6	34.5±0.2	35.8±0.4	31.9±1.4
16:1n-9	6.4±0.1[d]	5.7±0.0[c]	4.7±0.2[b]	3.6±0.0[a]
18:1n-9	19.2±0.2[a]	22.9±0.1[b]	25.2±0.0[c]	27.6±0.5[d]
20:1n-9	1.9±0.4[b]	1.5±0.0[ab]	1.3±0.1[ab]	0.8±0.0[a]
22:1n-7	0.2±0.0[b]	0.2±0.0[b]	0.2±0.0[ab]	0.1±0.0[a]
22:1n-9	0.5±0.0[d]	0.5±0.1[c]	0.3±0.0[b]	0.2±0.0[a]
∑ Monoenes	28.2±0.4[a]	30.8±0.1[b]	31.7±0.3[b]	32.3±0.6[b]
18:2n-6	3.1±0.1[a]	3.3±0.2[a]	5.2±0.2[b]	6.4±0.1[c]
18:3n-6	0.3±0.0[b]	0.2±0.0[a]	0.2±0.0[a]	nd[2]
20:4n-6	1.5±0.2[b]	1.3±0.0[b]	1.2±0.2[b]	0.3±0.0[a]
22:3n-6	0.2±0.0[b]	0.2±0.0[b]	0.1±0.0[a]	nd
22:4n-6	6.4±0.4[b]	6.7±0.1[b]	5.8±0.2[ab]	4.5±0.4[a]
22:5n-6	0.2±0.0[b]	0.2±0.0[b]	0.1±0.0[a]	nd
∑n-6fatty acids	11.9±0.5	12.7±0.3	12.6±0.2	11.7±0.5
18:3n-3	nd	0.3±0.0[b]	0.3±0.0[ab]	0.2±0.0[a]
18:4n-3	0.4±0.0[d]	0.3±0.0[c]	0.3±0.0[b]	0.2±0.0[a]
20:4n-3	0.5±0.0[c]	0.4±0.0[b]	0.3±0.0[a]	0.2±0.0[a]
20:5n-3	6.6±0.1[b]	5.7±0.4[ab]	4.7±0.7[ab]	4.1±0.2[a]
22:5n-3	2.7±0.2[b]	2.4±0.1[ab]	2.0±0.3[ab]	1.7±0.0[a]
22:6n-3	12.1±0.1[b]	11.0±0.1[ab]	9.8±0.9[ab]	8.3±0.4[a]
∑n-3 fatty acids	22.6±0.1	20.0±0.5	17.0±2.9	14.8±0.6
∑ PUFA[3]	34.6±0.4	32.7±0.3	29.6±3.1	26.4±1.6
∑n-3/n-6 ratio[4]	1.9±0.1	1.6±0.1	1.4±0.3	1.3±0.1
∑ EPA+DHA[5]	18.8±0.2	16.7±0.3	14.3±2.8	12.4±0.6

[1] Values are expressed as means ± S.E (n=3). Same superscripts are not significant different (P > 0.05).

[2] nd = Not detected

[3] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of eicosapentaenoic Acid (C20:5n-3) and docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 7. Fatty acid composition (% of total fatty acid) in ventral muscle of red sea bream *Pagrus major* fed with different diets

Types of fatty acid[1]	Ventral			
	10F0P	8F2P	6F4P	4F6P
14:0	3.2±0.0	2.8±0.1	2.6±0.3	2.6±0.1
16:0	21.8±0.2^{a}	23.9±0.2^{a}	27.5±10.8^{b}	27.6±0.5^{b}
18:0	7.4±0.2	8.6±0.1	8.6.±0.2	7.6±0.5
∑saturated	32.3±0.4^{a}	35.2±0.2^{ab}	38.2±1.3^{b}	37.8±0.9^{b}
16:1n-9	6.1±0.1^{c}	5.3±0.2^{bc}	4.9±0.2^{ab}	4.2±0.3^{a}
18:1n-9	16.8±0.4^{a}	23.4±0.3^{b}	28.2±0.2^{c}	30.7±0.5^{d}
20:1n-9	1.7±0.1^{c}	1.9±0.1^{c}	1.2±0.0^{b}	0.7±0.1^{a}
22:1n-9	0.5±0.0^{d}	0.3±0.0^{c}	0.3±0.0^{b}	0.2±0.0^{a}
∑monoenes	25.0±0.4^{a}	30.9±0.2^{b}	34.5±0.2^{c}	35.7±0.4^{c}
18:2n-6	2.9±0.0^{a}	3.9±0.2^{ab}	4.3±0.1^{b}	7.3±0.4^{c}
20:4n-6	1.5±0.0^{b}	1.3±0.0^{ab}	0.7±0.1^{a}	0.7±0.2^{a}
22:4n-6	6.7±0.3^{b}	5.4±0.1^{a}	6.3±0.1^{ab}	5.3±0.3^{a}
∑n-6	11.1±0.3^{a}	10.6±0.2^{a}	11.6±0.1^{ab}	13.5±0.4^{b}
18:3n-3	nd[2]	0.3±0.0	0.2±0.0	0.2±0.0
18:4n-3	0.5±0.0^{c}	0.3±0.0^{b}	0.3±0.0^{b}	0.2±0.0^{a}
20:4n-3	0.7±0.0	0.4±0.0^{c}	0.3±0.6	0.2±0.0^{a}
20:5n-3	6.3±0.3^{c}	5.2±0.1^{b}	4.8±0.2^{ab}	4.1±0.1^{a}
22:5n-3	3.6±0.1^{b}	2.0±0.4^{a}	1.54±0.0^{a}	1.5±0.3^{a}
22:6n-3	11.8±0.2^{c}	10.1±0.2^{b}	10.3±0.3^{b}	8.7±0.3^{a}
∑n-3	22.8±0.2^{c}	18.2±0.4^{b}	17.3±0.1^{b}	14.8±0.6^{a}
∑PUFA[3]	33.8±0.1^{b}	28.8±0.3^{a}	28.9±0.3^{a}	28.3±0.1^{a}
∑n-3/n-6 ratio[4]	2.1±0.1^{c}	1.8±0.1^{bc}	1.5±0.0^{b}	1.1±0.1^{a}
∑EPA+DHA[5]	18.0±0.1^{c}	15.3±0.0^{b}	15.2±0.1^{b}	12.8±0.4^{a}

[1] Values are expressed as means ±S.E (n=3). Same superscripts are not significant different (P > 0.05).

[2] nd = Not detected

[3] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of eicosapentaenoic acid (C20:5n-3) and docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 8. Fatty acid composition (% of total fatty acid) in liver of red sea bream *Pagrus major* fed with different diets

Types of fatty acid [1]	liver			
	10F0P	8F2P	6F4P	4F6P
14:0	2.8±0.2^{b}	2.0±0.2^{ab}	1.1±0.4^{ab}	1.3±0.1^{a}
16:0	20.5±0.8^{a}	23.5±0.7^{ab}	23.6±0.2^{ab}	25.0±0.42^{b}
18:0	9.1±0.2	9.5±0.2	9.1±0.1	10.0±0.2
∑Saturated	32.4±0.7^{a}	34.9±0.6^{ab}	34.8±0.4^{ab}	36.3±0.2^{b}
16:1n-9	6.4±0.3^{b}	6.4±0.3^{b}	6.2±0.0^{b}	4.9±0.1^{a}
18:1n-5	13.8±0.7	16.2±0.7	14.4±0.4	16.2±0.1
18:1n-9	9.8±0.0^{a}	12.6±0.1^{b}	15.1±0.3^{c}	17.3±0.2^{d}
20:1n-9	2.2±0.1^{b}	1.8±0.1^{ab}	1.9±0.1^{ab}	1.5±0.1^{a}
22:1n-7	0.2±0.0^{b}	0.2±0.0^{ab}	0.1±0.0^{a}	0.1±0.0^{a}
22:1n-9	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0
24:1	0.2±0.0	0.2±0.0	0.2±0.0	nd[2]
∑Monoenes	32.7±0.9^{a}	37.5±0.5^{b}	37.9±0.5^{b}	39.9±0.2^{b}
18:2n-6	2.3±0.2^{a}	2.2±0.2^{a}	3.4±0.3^{ab}	3.8±0.3^{b}
18:3n-6	0.4±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:4n-6	0.7±0.2^{b}	0.7±0.1^{ab}	0.6±0.1^{ab}	0.2±0.1^{a}
22:3n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
22:4n-6	16.6±0.6^{b}	10.6±1.0^{a}	9.4±0.6^{a}	9.0±0.4^{a}
22:5n-6	0.3±0.1^{b}	0.1±0.1^{a}	0.1±0.0^{a}	0.1±0.0^{a}
∑n-6	20.3±0.7^{b}	13.5±1.6^{a}	13.7±0.3^{a}	12.6±0.9^{a}
18:3n-3	0.3±0.1	0.2±0.1	0.3±0.0	0.2±0.1
18:4n-3	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.0
20:4n-3	0.4±0.0^{b}	0.4±0.1^{b}	0.4±0.1^{b}	0.2±0.1^{a}
20:5n-3	1.8±0.1^{a}	1.7±0.2^{a}	2.0±0.2^{ab}	2.5±0.1^{a}
22:5n-3	1.3±0.3	1.6±0.1	1.7±0.0	1.6±0.1
22:6n-3	9.0±0.4^{a}	9.1±0.3^{ab}	10.6±0.5^{ab}	11.2±0.4^{b}
∑ n-3	12.6±0.2^{a}	13.0±0.3^{a}	15.0±0.4^{b}	15.7±0.4^{b}
∑PUFA[3]	33.0±1.0	25.2±3.0	28.6±0.7	28.3±1.3
∑n-3/n-6 ratio[4]	0.6±0.0	1.2±0.3	1.1±0.0	1.3±0.1
∑EPA+DHA[5]	10.7±0.5^{a}	10.8±0.1^{a}	12.5±0.4^{ab}	13.7±0.3^{b}

[1] Values are expressed as means ±S.E (n=3). Same superscripts are not significant different (P > 0.05).

[2] nd = Not detected

[3] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of eicosapentaenoic Acid (C20:5n-3) and docosahexanoic acid (C22:6n-3) as essential fatty acids

The incremental palm oil in diets intensified palmitic (C16:0) and linoleic acid (C18:2n-6) concentrations in fish samples. Oleic acid accounted in dorsal and ventral muscles, and liver gradually increased with higher inclusion of palm oil while inverse

relationship was demonstrated with monoenes compared to that in diets. Proportions of total n-3 decreased significantly between 10F0P and 4F6P in dorsal and ventral muscles but relatively inverse in liver. Similar pattern was observed in ratio of n-3 to n-6 in same samples. Declining of n-3 in both dorsal and ventral muscles was due to the decline of C20:5n-3 and C22:6n-3 concentrations respectively.

4.5 Discussion

Results from this current experiment corroborate influences of dietary palm oil on fish growth performance. It is likely that growth performances of red sea bream juveniles impeded gradually with incremental dietary palm oil although there are no significant differences among 10F0P, 8F2P and 6F4P. The declination in growth could be caused by decline of docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) from diets. The inclusion of palm oil at 60% reduced EPA by 7.4% and DHA by 2.9% compared to their concentrations in 10F0P, which significantly reduced SGR and BWG. However, replacement of fish oil by palm oil up to 60% did not alter yellow tail (*Seriola quinqueradiata*) growth (Watanabe, 2002) and 50% for Atlantic salmon (*Salmo salar*) as reported by Bell *et al* (2002). Previous studies using up to 15% palm oil blended with other vegetable oils may also replace 60% fish oil with no marked effect on growth performance in European seabass (Richard *et al.*, 2006). Other vegetable oils too like soybean oil, linseed oil and rapeseed oil may substitute up to 60% of fish oil (Izquierdo *et al.,* 2005) for gilthead sea bream and linseed oil, rapeseed oil and olive oil (Mourente *et al.,* 2005) for European seabass. As marine fish cannot synthesize n-3 HUFA in vivo, the good growth in fish is highly contributed from DHA and EPA available in diets. 4F6P diet consisted of only 12.0%

DHA and 2.1% EPA, which resulted in lowest SGR and BWG. In addition, DHA concentrations in dorsal and ventral muscles, and liver also illustrated similar patterns to dietary DHA concentrations with slightly lower values in fish samples compared to diet. Thus, reduction of marine fish oil inclusion does markedly contribute to declining DHA and EPA in both diet and muscle. Nevertheless, fish oil replacement with palm oil is possible as long as the portion of fish oil included could meet the essential fatty acid required by fish although the determination of optimal supplement level for palm oils in the diet would be depended upon some factors such as species, age, size and so on.

Higher feed intake (FI) demonstrated in dietary 10F0P and 8F2P during the trial suggests higher lipid deposition in whole body especially at dorsal area. Lipid content was high in treatment 10F0P, 8F2P and 6F4P with no significant differences among treatments although FI in 6F4P was significantly lower. Deposition of lipid also doubled in ventral muscle compared to those in dorsal one and this is regarded as common in cultured fish (Suzuki *et al.,* 1986) due to anteroposterior swimming mechanism (Toussaint *et al.,* 2005). Gradual declining of FI specifically in 6F4P and 4F6P indicate reduction of dietary acceptance with at least 40% palm oil, which may be due to the palatability preferences by fish. However, no tendency of liver inflammation in fish fed with incremental dietary palm oil as shown in HSI in all treatments. Instead, FCR and SR notably indicated that fish regardless of treatments grew economically and were in considerably healthy condition.

Replacing fish oil by feeding palm oil to fish reduced EPA and DHA contents in dorsal and ventral muscles and this conforms to Ghioni *et al* (1999) study on turbot, Mourente and Bell (2006) on European seabass, Bell *et al* (2002) on Atlantic salmon and Ng *et al* (2003) on African catfish. Gradient reduction of fatty acid compositions particularly DHA and EPA

in both diet and muscles suggest inherent complexity due to interactions between various fatty acids and catabolism mechanism (Robin and Skalli, 2006), and Stubhaug *et al* (2005) also observed similar complexity in Atlantic salmon fed with rapeseed oil. Excessive palmitic (C16:0) and linoleic (C18:2n-6) acids due to incremental palm oil may have interrupted the β-oxidation metabolism which caused inherent complexity. In addition, red sea bream may also have lower ability to synthesize these fatty acids *de novo* from palm oil based diet. Izquierdo *et al* (2005) has reported similar observation on gilthead sea bream and Regost *et al* (2003) on turbot regarding inability of these fish in synthesizing C18:2n-6 into C20:4n-6.

This study also showed inverse relationship between inclusion level of palm oil and body EPA and DHA. Those concentrations decreased in both dorsal and ventral muscles in fish fed with higher inclusion of palm oil. Concurrently, similar gradient degradation in n-3/n-6 ratio was also demonstrated in both dorsal and ventral muscles, and this observation relatively conforms to reports by Torstensen *et al* (2004) on salmon. Reduction of EPA and increment of n-6 in fish muscle when fed with palm oil may leverage complication in fish health. However, such adverse effects were not detected in all treatments. Although the highest inclusion of palm oil resulted in lowest SGR and BWG, symptoms of EFA deficiency indicated in Huang *et al* (2008) report on red sea bream were not detected in fish throughout the experimental period. According to Takeuchi *et al* (1991), juvenile red sea bream requires EFA comprising 1% EPA and 0.5% DHA. All test diets used in this study met the 0.5% DHA requirement but indicated lower EPA. This conforms to Huang *et al* (2007) observation that DHA showed higher efficacy than EPA by using dietary canola oil on red sea bream. In addition to that, n-3 HUFA in the test diets of this study ranging from

1.54 to 2.72% is still within the requirement of most marine fish species (0.5 to 1.9%) as reported by Xue *et al* (2006). Therefore, encouraging growth performance shown in dietary 8F2P and 6F4P may have been sufficiently sourced from n-3HUFA within limited rations in test diets.

Increase of ash content in fish with incremental dietary palm oil except in treatment 6F4P (40% palm oil inclusion) in this study illustrated similar ash content pattern up to 50% palm oil inclusion in European seabass as reported by Bell *et al* (2002). Although this presumably reflects improvement of mineral utilization in fish with inclusion of palm oil, the underlying mechanism determining interaction between palm oil and mineral utilization in fish is still unclear and requires further studies in future.

It can be concluded that diets containing not more than 40% palm oil blended with 60% fish oil may be a suitable ratio for red sea bream. Growth performances, EFA deposition in both dorsal and ventral muscles and absence of detrimental EFA deficiencies indicated the possibility of incorporating palm oil in red sea bream juvenile diets as partial replacement for fish oil.

Study III:

Influences of soya oil blended with fish oil on growth performances and lipid profiles of red sea bream *Pagrus major*

Abstract

Continuous increase in fish feed prices due to deterioration of fish oil source and quality catalyses more research efforts to study soya oil as alternatives for fish oil. Reputed as rich in linolenic acid (C18:2n-6) gives soya oil a competitive edge as a Arachidonic acid (ARA) precursor. Therefore, a 50-day feeding trial was conducted to evaluate effects of dietary soya oil inclusion on growth performances and lipid composition of juvenile red sea bream. Four types of iso-nitrogenous experimental diets formulated in this feeding trial consisted of {10:0 (10F0S), 8:2 (8F2S), 6:4 (6F4S) and 4:6 (4F6S)}. All diets were fed to triplicate groups of 15 tails red sea bream *Pagrus major* with an initial average weight of 4.9±0.1g twice daily to apparent satiation. Our results demonstrated that inclusion of soya oil as a lipid source to partially replace fish oil in red sea bream indicated highest BWG in 10F (837.2±2.2%), followed by 8F2S (832.3±3.5%), 6F4S (807.1±8.1%) and 4F6S (682.0±17.2%) respectively without any significant differences among 10F, 8F2S and 6F4S. Soya oil inclusion too gradually decreased BWG and SGR; both BWG and SGR in 4F6S were significantly lower than other treatments. Feed conversion ratio (FCR) and feed intake (FI) illustrated no significant differences among treatments. Survival rate of all treatments exceeded 90% although hepatosomatic index (HSI) in 4F6S was significantly higher than other treatment and serum GOT increased gradually with higher inclusion of soya oil. Lipid deposition in whole body was highest in fish fed with dietary 10F and decreased to elevated concentration of soya oil in diets. Ventral muscles had doubled amount of lipid deposition as compared to dorsal muscles. Dominance of saturates among total fatty acid composition particularly C16:0 was similarly observed in dorsal, ventral and lipid. Saturates, monoenes, n-3 and ratio of n-3/n-6 observed similar gradient degradation in dorsal, ventral and liver. Inverse relationship of

inclusion level of soya oil on eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) in both dorsal and ventral including liver was also observed. In conclusion, diets containing not exceeding 40% soya oil may be a suitable ratio in incorporating soya oil as fish oil replacement.

4.1 Introduction

Red sea bream, *Pagrus major* is a very important species for aquaculture ventures in East Asia including China, Japan, and Korea, due to its high market value and desirable taste (Ren *et al.,* 2007). Regarded as an alternative protein source besides meat, this species is cultivated to meet the ever increasing demand by consumers on fresh marine fish due to the diminishing landings of red sea bream from capture fisheries. However, aquaculture expansion including this species is hampered by continuos increase in fish feed prices due to deterioration of fish oil source (Barlow, 2000) and quality. Thus, the scenario catalyses more research efforts to study vegetable oils as alternatives for fish oil (Sargent *et al.,* 2002; Torstensen *et al.,* 2000; Rodriguez *et al.,* 2002; Almaida-Pagán *et al.,* 2007). Fish oil, the main source of essential fatty acids (EFA) is rich with omega-3 fatty acid especially DHA (C22:6n-3) and EPA (C20:5n-3). In addition, ARA (C20:4n-6) sourced from omega-6 fatty acids is also important to meet the growth requirement of fish. As production pathways of these EFA are still unknown yet; all EFA must be provided through proper diet. Therefore, omega-3 can be sourced directly from fish oil while ARA is sourced from vegetable oil. To date, some documented results demonstrated possibility of using various vegetable oils to substitute fish oil like palm oil, rapeseed oil, linseed oil and sunflower oil. Results showed the efficacies of these oils in growth performances and feed utilization in Atlantic salmon (Bell *et al.,* 2002), gilthead sea bream (Izquierdo *et al.,* 2005), European seabass (Mourente and Bell 2006) and red sea bream (Glencross *et al.,* 2003) respectively.

Soya oil is reputed as a potential substitute for fish oil whereby it constitutes about half of worldwide edible vegetable oil. Its richness in linolenic acid (C18:2n-6) gives soya oil a

competitive edge as an arachidonic acid (ARA) precursor. Little information is available on suitability of using soya oil as a substitute in feeds used for red sea bream. Therefore, this study was conducted to examine efficacy of partial substitution of fish oil by using soya oil on red sea bream's growth performance, body proximate composition, muscle and liver fatty acid composition profiles. In addition, health status was also examined to determine whether consumption of dietary incremental soya oil could affect red sea bream's health condition.

4.2 Materials and methods

4.2.1 Experimental fishes and feeding protocols

The dietary trial was carried out at Marine Finfish Hatchery, Kagoshima University. Red sea bream juveniles with mean weight of 4.9±0.1g were obtained from a commercial hatchery, Matsumoto Suisan, Miyazaki Prefecture, transported alive in HDPE tank aerated with oxygen and acclimatized in indoor rearing tanks a week prior to experiment. Fishes were fed with commercial pellet (Higashimaru Foods, Kagoshima, Japan). Fifteen juveniles were distributed equally in 12units of 100-L flow-thru tanks in triplicates. Four experimental diets (Table 9) with different ratio of fish oil: soya oil was fed manually at feeding ration (0800 and 1600hr) to fish until near satiation. Tanks were cleaned and uneaten diets were collected to determine feed intake.

Table 9. Basal ratio of experimental diets containing different levels of soya oil

Ingredients (g/kg DM)	Diets			
	10F0S	8F2S	6F4S	4F6S
Brown fish meal[1]	670.0	670.0	670.0	670.0
Activated gluten	80.0	80.0	80.0	80.0
α -Starch	40.0	40.0	40.0	40.0
Dextrin	40.0	40.0	40.0	40.0
Fish oil	100.0	80.0	60.0	40.0
Soya oil	0.0	20.0	40.0	60.0
Mineral mix[2]	30.0	30.0	30.0	30.0
Vitamin mix[3]	26.5	26.5	26.5	26.5
Stay-C[4]	3.5	3.5	3.5	3.5
α-Cellulose	10.0	10.0	10.0	10.0
Total	1000	1000	1000	1000
Analytical contents (dry matter basis)				
Crude protein (%)	55.8	55.7	55.4	55.7
Total lipid (%)	12.3	11.6	11.6	11.4
Ash (%)	13.9	14.5	13.9	11.1
Gross energy (kcal/g diet)	6.4	6.4	6.4	6.3

[1] Defatted brown fish meal

[2] Mineral mix (g/kg): NaCl, 0.183; $MgSO_4 \cdot 7H_20$, 0.685; $NaH_2PO_4 \cdot 2H_2O$, 0.436; KH_2PO_4, 1.199; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 0.679;Fe Citrate, 0.148; Ca Lactate, 1.635; $AlCl_3 \cdot 6H_2O$, 0.00009, $ZnSO_4 \cdot 7H_2O$, 0.017;$CuCl_2$, 0.00005; $MnSO_4 \cdot 4H_2O$, 0.004;KCl, 0.008; $CoCl_2$, 0.005

[3] Vitamin mix (g/kg): p-aminobenzoic acid, 1.45; biotin, 0.02; myo-inositol, 14.5; nicotinic acid, 2.9; folic acid, 0.05; choline chloride, 29.65

[4] Stay-C: L-Ascorbyl-2-monophosphate-Na/Ca (DSM Nutrition Japan K. K.)

The diets were formulated to contain 55.4-55.8g kg^{-1} protein, 11.4-12.3g kg^{-1} lipid and 11.1-14.5g kg^{-1} ash. Fishes were also exposed to a photoperiod condition of 12 h light/12 h dark, water flow (filtered seawater) maintained at 2.5 *L*/min in which water temperature was 28.7 ± 1.5°C (mean ± SD). Fish sampling was done in every 10 days interval where weight was expressed as bulk weight (g) of all fishes in each respective tank. At end of

experiment, fishes were killed using hypothermia method, by immersion into slurry-ice cold marine water prepared and maintained at 3 ppt and 0°C according to method adapted from Losada *et al* (2005) and individual weight of fish was taken. Livers were removed, pooled and stored at -80°C until further analysis was performed. Filleting was conducted by separating the dorsal and ventral flesh according to Japanese standards. Fillets from each treatment were pooled and maintained in ice before freeze-dried and used in further analysis. Three fishes were also randomly sampled from each respective tank and used for proximate analysis.

4.2.2 Proximate composition analysis

Homogenized samples of whole body and liver were analyzed in duplicates were analyzed for protein, lipid, ash, moisture and dry matter. Protein was determined by using the Kjeldahl method (AOAC 1990); lipid by Bligh and Dyer (1959); ash was analyzed by combustion in muffle furnace at 600°C. Moisture was determined on approximately 5 g of minced muscle, by oven-drying at 110°C to constant weight, according to AOAC (1990) while results of dry matter were expressed as 100 minus moisture (in g of water/100 g of muscle).

4.2.3 Fatty acid composition analysis

Fatty acid composition analysis was performed on homogenized samples of dorsal fillet, ventral fillet and liver. Total lipid (TL) was extracted by homogenizing 2 g sample according to Bligh and Dyer (1959). Fatty acid esters (FAME) were then produced from total lipids aliquots. Fatty acids were methylated with BF_3 in methanol. Methyl tricosanoate

(Nu-Chek Prep. Inc) was used as internal standard at 1.000mg/ml Hexane. Fatty acid methyl esters (FAME) was analyzed with a gas chromatograph (Shimadzu GC 17A) with flame ionization detector temperature maintained at 260˚C; carrier N_2 gas at 1ml/min; column temperature at 200°C; injector temperature at 250°C and Helium (He) served as the carrier gas. 0.1μl sample including 0.1µl air was manually injected into injection port and identified fatty acids were presented as area percentage of total fatty acids.

4.2.4 Blood chemistry analysis

The micro hematocrit method by Kawadzu (1981) was used for the determination of hematocrit level. Glucose, total cholesterol (T-Cho), triglyceride (TG), blood urea nitrogen (BUN) in plasma and albumin, total bilirubin (T-Bil), glutamic oxaloacetic transaminase (GOT), total protein (TSP) in serum were determined by SPOTCHEM EZ SP-4430 system according to Tatsumi *et al* (2000).

4.3 Statistic analysis

The statistical analysis was performed using ANOVA. Data was expressed as means $\pm$ S.E. Homogeneity of variance between treatments ($P<0.05$) were analysed using one-way Analysis of Variance (ANOVA) while significance of differences were determined using Tukey-Test. Effects of dietary treatments were considered significant at $P<0.05$.

4.4 Results

4.4.1 Growth performances

Specific growth rate (SGR) and body weight gain (BWG) in 10F, 8F2S and 6F4S noted insignificant differences among treatments except in 4F6S (Table 10). However, inclusion of soya oil gradually decreased BWG and in 4F6S, both BWG and SGR were significantly lower than other treatments. Feed conversion ratio (FCR) and feed intake (FI) for all treatments were similar ranging from 1.1 to 1.2 and 45.9 to 54.6 respectively throughout the 50-days experimental period. Survival rate was relatively high in all treatments ranging from 90 to 96.5% without any significant differences among treatments. Hepatosomatic index (HSI) in 4F6S was significantly higher than other treatments.

4.4.2 Proximate composition

Inclusion of soya oil as fish oil replacement in diets indicated significant differences on growth performance and of red sea bream. Although diets formulated similarly contained proximate composition (55.4-55.8 g kg^{-1} protein, 11.4-12.3g kg^{-1} lipid and 11.1-14.5g kg^{-1} ash) as shown in Table 9, significant differences in proximate composition were illustrated in Table 11.

Table 10. Growth performances of red sea bream *Pagrus major* fed with different diets

Growth parameters[1]	Diets			
	10F0S	8F2S	6F4S	4F6S
Initial weight (g/fish)	4.9±0.0	5.0±0.1	5.0±0.0	4.9±0.0
Final weight (g/fish)	49.3±0.6^{b}	43.3±1.2^{a}	42.3±0.6^{a}	40.5±1.7^{a}
SGR[2]	4.5±0.0^{b}	4.5± 0.0^{b}	4.4±0.0^{b}	4.3±0.1^{a}
FCR[3]	11.1±0.1	1.1±0.0	1.1±0.0	1.2±0.0
BWG[4]	837.2±2.2^{b}	832.3±3.5^{b}	807.1±8.1^{b}	682.0±1.2^{a}
HSI [5]	1.28±0.1^{a}	1.7±0.0^{a}	1.8±0.0^{a}	2.2±0.0^{b}
FI(g/fish/50days)[6]	54.6±5.5	49.0±0.1	45.9±0.9	49.2±3.1
SR[7]	96.5±3.5	96.5±3.5	96.5±3.5	90.0±3.1

[1]Values are expressed as mean ± SE (n=3). Data with same alphabets are not significantly different (P>0.05).

[2] SGR, specific growth rate= 100 x (ln final weight- ln initial weight)/(duration)

[3] FCR, feed conversion ratio = dry feed intake(kg)/weight gain(kg)

[4] BWG, body weight gain (%)= 100 x (final weight - initial weight) / (initial weight)

[5] HSI, Hepatosomatic index = 100 × (liver weight/body weight)

[6] FI, Feed intake (g/fish/50days) = (total feed intake (g) / number of fishes) in 50 days feeding period

[7] SR, survival rate (%) = 100 x (initial fish number-dead fish number) / (initial fish number)

Whole body demonstrated a gradual decline without any significant differences in 10F, 8F2S and 6F4S with lipid retention (31.0%, 30.6% and 28.1% respectively); however a significant lower lipid content was shown in 4F6S at 26.6%. Table 11 also illustrated plateau deposition patterns of lipid in both dorsal and ventral muscles including liver. Lipid deposition was lower in dorsal as compared to ventral and liver. Dorsal muscles contained lipid ranging from 8.0% to 10.2% while concentration doubled from 20.4% to 23.1% in ventral and 21.8% to 27.9% in liver. Current result also indicated that lipid elevated with soya oil increment. Ash, protein and moisture contents demonstrated inconsistent pattern among treatments; ash contents at 14.1-15.3%WM, moisture at 67.8-69.2% and protein at 48.2-53.08%WM respectively.

Table 11. Lipid, protein, ash, and moisture contents of whole body, muscles and liver in red sea bream *Pagrus major* fed with different diets

Parameters[1]	10F0S	8F2S	6F4S	4F6S
Lipid (%)[2]				
Whole body	31.0±0.1^{b}	30.6±0.2^{b}	28.1±0.5^{a}	26.6±0.2^{a}
Dorsal muscle	10.2±0.1	10.0±0.3	8.0±0.9	10.0±0.1
Ventral muscle	20.4±0.4	22.7±0.7	22.6±0.6	23.1±0.1
Liver	21.8±0.9	23.9±1.9	23.9±1.1	27.9±0.9
Protein (% whole body)[3]	48.2±0.1^{a}	48.9±0.0^{b}	53.0±0.0^{d}	51.8±0.1^{c}
Ash (% whole body)[3]	14.4±0.0^{ab}	14.1±0.2^{a}	15.3±0.1^{c}	14.6±0.1^{b}
Moisture (% whole body)	67.8±0.1^{a}	68.7±0.0^{b}	69.2±0.2^{b}	69.0±0.2^{b}

[1] Values are expressed as mean ± SE (n=3). Data with same alphabets are not significantly different (P>0.05).

[2] Dry weight basis

[3]Wet weight basis

4.4.3 Fatty acid composition

Profiles of fatty acid composition in dietary (Table 12) reflected expected results of determined concentration of soya oil inclusion. Saturates and monoenes dominated total fatty acid composition ranging from 22-24.2% and 29.2-32.0% respectively. Concentration increment of C16:0 increased from 16.2% to 19.3%, C18:1n-7 from 9.0% to 17.2% and C18:2n-6 from 4.2% to 5.0% affirmed positive prototype with higher inclusion of soya oil.

Saturates dominated total fatty acid compositions in dorsal (Table 13), ventral (Table 14) and liver (Table 15) of fish although higher inclusion of soya oil significantly decreased saturates concentration. Similar pattern was also observed in monoenes, n-6 and n-3. Concentration of all fatty acids except C18:2n-6 decreased gradually Therefore, it can be notably observed that determined soya oil intensified linoleic acid concentrations (C18:2n-6) in all samples. Significant declining of total PUFAs and ratio n-3:n-6 accounted

in both dorsal and ventral muscle was also observed. Concentrations of essential fatty acids (FA) like ARA, EPA and DHA too declined to incremental soya oil which resulted inverse amount of all respective EFAs as mentioned earlier.

4.4.4 Blood chemistry

Current result as illustrated in Table 16 also demonstrated degradation of hemoglobin (Ht) count in blood serum of fishes fed with incremental soya oil. Other serum biochemical properties like Hb, Glu, T-Cho, Bun, T-bil and GPT found no significant differences among treatments. However, GOT elevated significantly from 2% inclusion of soya oil in dietary at 77.3 IU/l, 79.0 IU/l and 83.8IU/l respectively without any significant differences among treatments. These three treatments were significantly higher than GOT in dietary 10F (54.0 IU/l).

Table 12. Fatty acid composition (% of total fatty acid) in experimental diets

Types of fatty acid[1]	Diets			
	10F0S	8F2S	6F4S	4F6S
14:0	3.2	3.3	2.1	2.4
16:0	16.3	17.6	18.2	19.3
18:0	2.5	2.8	2.7	2.5
∑ Saturated	22.0	23.7	23.0	24.2
16:1n-9	5.0	4.1	3.3	3.5
18:1n-5	0.4	0.4	0.3	0.2
18:1n-9	9.0	14.4	15.9	17.2
20:1n-9	4.0	3.9	2.9	2.0
22:1n-7	nd	nd	nd	nd
22:1n-9	0.2	0.2	0.2	0.5
20:1n-11	0.5	0.4	0.5	0.8
∑ Monoenes	19.1	23.4	23.1	24.2
18:2n-6	4.2	4.7	5.3	5.0
20:4n-6	0.9	0.9	0.9	0.8
22:4n-6	8.3	6.9	5.8	4.4
∑ n-6fatty acids	13.4	12.5	12.0	10.2
18:3n-3	nd	0.9	1.3	1.8
18:4n-3	1.2	0.9	0.7	0.5
20:4n-3	0.4	0.3	0.3	0.3
20:5n-3	9.4	3.8	2.9	1.8
22:5n-3	1.0	0.9	0.9	1.0
22:6n-3	12.4	10.7	10.5	11.0
∑ n-3 fatty acids	24.4	17.5	16.6	16.4
∑PUFA[3]	37.8	30.3	29.0	26.8
∑n-3HUFA[3]	23.2	15.7	14.6	14.1
∑n-3/n-6 ratio[4]	1.8	1.4	1.4	1.5
∑EPA+DHA[5]	21.8	14.5	13.4	12.8

[1] Values are expressed as means ±S.E (n=3). Same superscripts are not significant different (P > 0.05).

[2] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[3] Total n-3HUFA is expressed as sum of n-3 fatty acids in carbons more than 20

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of Eicosapentaenoic Acid (C20:5n-3) and Docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 13. Fatty acid composition (% of total fatty acid) in dorsal muscle of red sea bream *Pagrus major* fed with different diets

Types of fatty acid[1]	Dorsal			
	10F0S	8F2S	6F4S	4F6S
14:0	2.9±0.2^{b}	2.6±0.2^{b}	1.7±0.1^{a}	2.7±0.0^{a}
16:0	25.0±0.2^{c}	23.2±0.3^{b}	18.6±0.2^{a}	17.6±0.3^{a}
18:0	6.4±0.3	7.0±0.1	6.6±0.1	6.4±0.2
∑ Saturated	34.2±0.1^{c}	32.7±0.2^{c}	26.8±0.6^{b}	25.3±0.2^{a}
16:1n-9	5.5±0.2^{c}	5.0±0.2^{bc}	3.5±0.4^{ab}	3.2±0.2^{a}
18:1n-9	26.1±3.0	25.5±0.1	25.4±0.7	25.9±0.6
20:1n-9	6.6±0.5^{c}	5.0±0.9^{bc}	3.6±0.2^{ab}	2.0±0.5^{a}
22:1n-7	nd	nd	nd	nd
22:1n-9	0.5±0.1	0.5±0.0	0.4±0.0	0.2±0.1
∑ Monoenes	38.6±3.3	35.9±0.5	33.0±0.8	30.7±0.1
18:2n-6	3.1 ± 0.1^{a}	3.3 ± 0.2^{a}	5.2 ± 0.2^{b}	6.4± 0.1^{c}
18:3n-6	0.3 ± 0.0^{c}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	nd
20:4n-6	1.5 ± 0.1	1.3 ± 0.0	1.2 ± 0.3	1.3± 0.0
22:3n-6	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	nd
22:4n-6	6.4 ± 0.4	6.7 ± 0.1	5.8± 0.1	5.5 ± 0.4
22:5n-6	0.5 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	nd
∑n-6fatty acids	12.0 ± 0.5	11.8 ± 0.7	12.8 ± 0.1	13.2± 0.9
18:3n-3	0.9±0.0^{a}	1.0±0.1^{a}	1.2±0.3^{a}	2.2±0.3^{b}
18:4n-3	0.7±0.0^{c}	0.6 ± 0.4	0.4±0.1^{b}	0.3±0.0^{a}
20:4n-3	nd	nd	nd	nd
20:5n-3	7.3±0.2^{d}	6.0±0.1^{c}	5.2±0.1^{b}	3.8±0.1^{a}
22:5n-3	1.8±0.0	1.8±0.1	1.5±0.2	1.4±0.0
22:6n-3	7.9±0.1	7.9±0.2	8.1±0.4	7.5±0.0
∑n-3 fatty acids	18.6±0.3^{c}	17.3±0.1^{b}	16.4±0.2^{ab}	15.2+0.2^{a}
∑ PUFA[3]	30.6±0.8	29.1±0.3	29.2±0.1	28.4±0.6
∑n-3/n-6 ratio[4]	1.6±0.0^{c}	1.5±0.1^{bc}	1.3±0.1^{ab}	1.2±0.1^{a}
∑ EPA+DHA[5]	15.2±0.2	13.9±0.3	13.3±2.8	11.3±0.6

[1] Values are expressed as means ± S.E (n=3). Same superscripts are not significant different (P > 0.05).

[2] nd = Not detected

[3] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of eicosapentaenoic Acid (C20:5n-3) and docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 14. Fatty acid composition (% of total fatty acid) in ventral muscle of red sea bream *Pagrus major* fed with different diets

Types of fatty acid[1]	Ventral			
	10F0S	8F2S	6F4S	4F6S
14:0	2.6±0.3^{c}	2.5±0.1^{bc}	1.8±0.1^{ab}	1.3±0.0^{a}
16:0	23.8±0.3^{b}	19.7±0.4^{a}	19.1±0.2^{a}	18.6±0.4^{a}
18:0	6.5±0.2	6.5±0.1	6.7±0.1	7.0±0.1
∑ Saturated	32.9±0.2^{b}	28.7±0.3^{a}	27.6±0.4^{a}	26.8±0.5^{a}
16:1n-9	5.5±0.2^{b}	4.9±0.3^{b}	3.3±0.1^{a}	2.7±0.0^{a}
18:1n-9	30.7±4.8	24.0±0.8	26.1±-.6	21.0±3.9
20:1n-9	4.4±0.4^{bc}	4.8±0.4^{c}	3.0±0.2^{b}	0.8±0.4^{a}
22:1n-7	nd	nd	nd	nd
22:1n-9	0.5±0.1^{b}	0.4±0.1^{ab}	0.3±0.0^{a}	0.3±0.0^{a}
∑ Monoenes	40.1±3.3	34.1±0.0	32.6±0.5	28.7±0.0
18:2n-6	3.0± 0.0^{a}	4.4 ± 0.5^{a}	4.0 ± 0.3^{a}	7.3 ± 0.4^{b}
18:3n-6	0.3 ± 0.0^{b}	0.2 ± 0.0^{a}	nd	nd
20:4n-6	1.5 ± 0.1^{c}	1.3 ± 0.1^{bc}	0.7 + 0.1^{a}	0.5 + 0.3^{ab}
22:3n-6	nd	nd	nd	nd
22:4n-6	6.6 ± 0.3^{c}	5.4 ± 0.2^{b}	6.3± 0.1^{c}	2.2 ± 0.1^{a}
22:5n-6	1.0 ± 0.0^{b}	0.7 ± 0.0^{a}	nd	nd
∑n-6fatty acids	12.4 ± 0.3^{b}	12.0 ± 0.5^{ab}	11.0 + 0.3^{ab}	9.9 + 0.5^{a}
18:3n-3	0.6±0.1^{a}	1.2±0.1^{b}	1.6±0.1^{c}	2.0±0.1^{d}
18:4n-3	0.7±0.1^{b}	0.6±0.1^{b}	0.4±0.0^{a}	0.3±0.0^{a}
20:4n-3	0.7±0.1^{c}	0.7±0.0^{c}	0.9±0.0^{b}	0.5±0.0^{a}
20:5n-3	8.6±0.2^{d}	7.3±0.4^{c}	5.1±0.1^{b}	3.8±0.0^{a}
22:5n-3	1.9±0.1^{b}	2.0±0.2^{b}	1.6±0.1^{ab}	1.4±0.0^{a}
22:6n-3	11.6±0.2^{b}	11.0±1.0^{b}	8.7±0.1^{ab}	7.3±0.3^{a}
∑n-3 fatty acids	24.0±0.5^{c}	22.7±1.7^{bc}	18.1±0.4^{ab}	15.0±0.3^{a}
∑ PUFA[3]	36.4±0.3^{c}	34.6±2.2^{bc}	28.8±0.1^{ab}	25.2±0.3^{a}
∑n-3/n-6 ratio[4]	2.0±0.1^{b}	1.9±0.1^{b}	1.7±0.1^{ab}	1.5±0.1^{a}
∑ EPA+DHA[5]	20.2±0.2^{c}	18.3±0.3^{b}	13.8±2.8^{a}	11.1±0.6^{a}

[1] Values are expressed as means ± S.E (n=3). Same superscripts are not significant different (P > 0.05).

[2] nd = Not detected

[3] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of eicosapentaenoic Acid (C20:5n-3) and docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 15. Fatty acid composition (% of total fatty acid) in liver of red sea bream *Pagrus major* fed with different diets

Types of fatty acid[1]	Liver			
	10F0S	8F2S	6F4S	4F6S
14:0	1.5±0.1^{b}	1.5±0.0^{b}	1.4±0.0^{b}	0.9±0.0^{a}
16:0	23.9±0.2^{d}	22.5±0.2^{c}	21.0±0.1^{b}	19.3±0.1^{a}
18:0	14.5±0.9	15.8±0.2	14.4±0.2	14.7±0.4
∑ Saturated	39.8±1.2^{b}	39.9±0.1^{b}	36.8±0.4^{ab}	34.9±0.6^{a}
16:1n-9	4.3±0.1^{c}	3.8±0.2^{bc}	3.3±0.3^{ab}	2.4±0.1^{a}
18:1n-9	23.5±0.3	25.5±1.0	25.4±1.1	23.0±0.2
20:1n-9	3.3±0.2^{b}	3.0±0.1^{ab}	2.4±0.2^{ab}	2.2±0.1^{a}
22:1n-7	nd	nd	nd	nd
22:1n-9	2.2±0.1^{d}	1.7±0.0^{c}	1.4±0.1^{b}	0.9±0.0^{a}
∑ Monoenes	33.1±0.6^{b}	33.9±0.8^{b}	30.5±1.1^{ab}	28.7±0.3^{a}
18:2n-6	1.2±0.0^{a}	4.3±0.1^{b}	7.8±0.1^{c}	12.6±0.0^{d}
18:3n-6	nd	nd	nd	nd
20:4n-6	nd	nd	nd	nd
22:3n-6	nd	nd	nd	nd
22:4n-6	1.1±0.2	1.0±0.0	0.9±0.1	1.1±0.0
22-6:5n	nd	nd	nd	nd
∑n-6fatty acids	2.8±0.1^{a}	5.8±0.2^{b}	9.4±0.3^{c}	14.4±0.0^{d}
18:3n-3	0.1±0.0^{a}	0.3±0.3^{ab}	0.4±0.1^{ab}	0.6±0.1^{b}
18:4n-3	nd	nd	nd	nd
20:4n-3	0.6±0.1	0.5±0.1	0.4±0.0	0.4±0.1
20:5n-3	4.7±0.2^{b}	4.5±0.1^{b}	3.8±0.3^{ab}	3.5±0.1^{a}
22:5n-3	1.1±0.1	1.2±0.1	1.3±0.1	1.1±0.1
22:6n-3	12.5±0.2^{b}	11.2±0.4^{ab}	9.6±0.4^{a}	10.0+0.2^{a}
∑n-3 fatty acids	18.9±0.6^{b}	17.6±0.3^{ab}	15.4±0.6^{a}	15.5±0.1^{a}
∑ PUFA[3]	22.3±0.7^{a}	23.7±0.9^{a}	25.1±0.9^{a}	30.4±0.1^{b}
∑n-3/n-6 ratio[4]	6.8±0.1^{c}	3.0±0.1^{b}	1.7±0.0^{a}	1.1±0.0^{a}
∑ EPA+DHA[5]	17.2±0.2^{c}	15.7±0.3^{b}	13.4±2.8^{a}	13.5±0.6^{a}

[1] Values are expressed as means ± S.E (n=3). Same superscripts are not significant different (P > 0.05).

[2] nd = Not detected

[3] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of eicosapentaenoic Acid (C20:5n-3) and docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 16. Blood serum biochemical analysis of red sea bream *Pagrus major* fed with four different levels of soya oil inclusion in diets

Parameters[1,2]	10F0S	8F2S	6F4S	4F6S
Ht (%)	43.9±0.0[b]	37.6±0.6[a]	39.1±0.9[a]	38.7±0.2[a]
Hb (g/dl)	4.9±0.1	4.7±0.0	4.9±0.1	4.7±0.0
GLU (IU/l)	51.0 ±0 .0	53.5±2.5	48.5±3.5	48.5±0.5
T-CHO (IU/l)	262.5±44.5	289.5±21.5	289.5±17.5	329.5±2.5
BUN(mg/dl)	<5	<5	<5	<5
T-Bil (IU/l)	0.4±0.1	0.3±0.0	0.40±0.1	0.4±0.0
GOT (IU/l)	54.0±1.0[a]	77.3±0.3[b]	79.0±2.0[b]	83.8±1.8[b]
GPT (IU/l)[3]	<10	<10	<10	<10

[1]Values are expressed as means ± S.E (n=3). Same superscripts are not significant different (P > 0.05).

[2]Ht = hematocrit (%);Hb = hemoglobin (g/dl); GLU = glucose (IU/l); T-CHO = total cholesterol (IU/l); BUN = blood urea nitrogen (mg/dl); T-Bil = total bilirubin (IU/l); GOT = glutamic oxaloacetic transaminase(IU/l); GPT =glutamate pyruvate transaminase(IU/l)

[3]IU/l= international unit liter^{-1}

4.5 Discussion

Results from this study affirm potential ability of red sea bream in acquiring soya oil at limited amount for optimal growth and health maintenance. Economically accepted, FCR and FI showed no significant differences among treatments. Nevertheless, 10F, 8F2S and 6F4S showed acceptable growth rate but at degrading gradient whereas 4F6S demonstrated slowest SGR (4.2±0.1) and lowest BWG (682.0±17.2%). Degradations may be originated from (i) presence of abnormal fatty acids; (ii) selectivity mechanisms of available fatty acids; (iii) accumulation of lipid droplets and (iv) immunomodulators response.

Incremental soya oil in diets amends presence of fatty acids, reduces EFAs and subsequently degrades growth performances including health status in fish. Results demonstrated that saturates dominated the total fatty acid composition of dorsal, ventral and liver (Table 5 to Table 7). However, concentration level declined at gradual gradient in all samples. Others like monoenes, n-6 except C18:2n-6 and n-3 too demonstrated similar

declining pattern as saturates. Similar observations using soyabean oil dietary were reported by Xue *et al.,* (2006) on Japanese seabass and Izquierdo *et al.,* (2005) on gilthead sea bream. Declining pattern indicated above implies fish difficulties in metabolizing concentrated soya oil-based diets. EFAs mainly consist of n-3 (C18:3n-3, C20:5n-3 and C22:6n-3) and n-6 (C18:2n-6 and C20:4n-6). The current study confirms expected degradation of n-3 EFAs with higher inclusion of soya oil in diets although linolenic acid (C18:3n-3) elevated in dorsal, ventral and also liver. C18:3n-3 importance as a source for EPA (C20:5n-3) and further elongation transforms it to DHA (C22:6n-3). Nevertheless, results showed that elevated C18:3n-3 in diets did not contribute much to elevation of EPA and DHA; instead EPA and DHA declined further. In addition to that, decline of essential fatty acids concentration in dorsal and ventral may be inhibited by receptors responsible in producing enzymes. This state could probably prohibit further elongation of C18:3n-3 to C20:5n-3; then further elongation to C22:6n-3. Therefore, low amount of C22:6n-3 in dorsal, ventral and liver with higher inclusion of soya oil was accounted and therefore suggest inability of the liver to function under such abnormality in lipid esterification but instead, liver has to reposition its function to β-oxidation metabolism. In relation to this inhibitory pattern, significant decline of BWG and SGR in 4F6S compared to other treatments occurred. This confirmed poor growth rate in fish due to reduced amount of EFA like C22:6n-3 required by fish. Nevertheless, 60% inclusion of soya oil suggests acceptability in terms of growth performance for fish and this conforms to Montero *et al.,* (2003) study on gilthead sea bream using 60% soybean oil replacing fish oil. In other words, fatty acid composition in fish muscles and also liver depend on each respective fish metabolism pathway in diluting incorporated dietary fatty acids as pointed out by Robin *et al* (2003).

Elevated C18:2n-6 from dietary also suggests reposition of liver's function to β-oxidation metabolism instead of elongating C18:3n-3. Liver which functions as a regulator in

fat metabolism obviously showed that liver has to function at a more vigorous manner to either desaturate or elongate fatty acids especially at higher inclusion of soya oil (Table 5 to Table 7). This mechanism derived here conforms to Robin and Skalli (2006) on relative incorporation theory that explains influences of catabolism mechanism by diet characteristics and possible interactions between various fatty acids. Liver's function may protract during the metabolism including catabolism. Excessive C18:2n-6 from determined inclusion of soya oil will then further depress desaturation and elongation of fatty acid; and this was observed in decline of desaturates fatty acids like C20:4n-6 in all samples. This observation obviously reflects the inability of fish to desaturate and elongate selected fatty acids within feeding period of 50days. Due to selectivity metabolic rate, these excessive soya oil based fatty acids are synthesized into β-oxidation instead of triglicerides (TAG). Observation by Stubhaug *et al (*2005) too showed increased β-oxidation in Atlantic salmon with incremental rapeseed oil although this species has high resistance to β-oxidation (Bell *et al.,* 2001; Bell *et al.,* 2002).

Therefore, inherent complexities occurred in liver as a result of excessive concentration of C18:2n-6 for β-oxidation metabolism pathways and low C_{18} resulted deficiencies in HUFA like EPA, DHA and ARA which is similar to Ghioni *et al* (1999) observation on turbot. Lost in ability to convert C18:2n-6 as expressed by red sea bream in this study confirms other observations by Mourente and Tocher (1994), Sargent *et al.,* (2002) and Mourente *et al.,* (2005) on marine species.

The plateau pattern of lipid deposition in dorsal, ventral and also liver (Table 3) did not indicate possibilities of fat accumulation with higher inclusion of soya oil. Although inclusion of soya oil at 60% may still be acceptable in terms of fat accumulation, HSI in fish fed with dietary 60% soya oil elevated significantly from other treatments. However, elevation of HSI does not necessary indicates correlation of inflammatory response to degree of fat accumulation (Stubhaug *et al.,* 2005) but instead due to implication of desaturation and elongation

mechanism within liver itself. Apparently, rigorous β-oxidation metabolism due to excessive C18:2n-6 in liver has exhausted and eventually inflamed this organ.

Blood chemical properties are important indicators to fish health status. Degradation of hemoglobin (Ht) count in blood plasma obviously demonstrates impact of DHA decline in fish and its importance in maintenance of red blood cell membrane properties as confirmed by Waagboo *et al* (1995). Higher inclusion of soya oil also seemed to inhibit RBC in generating new cells most probably due to higher β-oxidation metabolism. Elevated GOT according to higher inclusion of soya oil indicates symptom of liver congestion. GOT and GPT have been used in fish for the detection of liver damage in flounder (Jung *et al.,* 2003; Hernandez Hernandez *et al* (2007) but little information has been reported on effects of determined vegetable oil dietary on red sea bream. Substitution of soya oil as lipid source may also have created a temporary shocking condition for fish and therefore causes impaired enzyme to meet liver's aggressive efforts in β-oxidation metabolic. Therefore, prolonged high intake of soya oil may induce these functional organs to deteriorate and subsequently confers poor health to fish.

Substituting fish oil with soya oil at limited ratio as lipid source demonstrated acceptable growth performances and optimum health status. However, prolonged high intake of soya oil may contribute some negative influences to fish hepatic metabolism due to increased β-oxidation metabolism. This study concluded that dietary 6F4S may contribute to optimal fish growth and acceptable health status in red sea bream.

Chapter 5

Effect of vegetable oil on oxidation stability in red sea bream (*Pagrus major*)

Study IV

Palm oil efficacy in oxidation stability with oxidized fish oil on growth performances and fatty acid composition in red sea bream (*Pagrus major*) juveniles

Abstract

This study was aimed to investigate the effects of replacing fish oil (F) with palm oil (P) blended with oxidized fish oil as the lipid source on growth performance and lipid profiles of red sea bream. A 50-day feeding trial on red sea bream (initial body weight 5.2±0.2g) was designed and conducted in triplicates using 8 isocaloric diets containing two degrees of oxidized fish oil (peroxide values of 2.2 and 86.8 $meqkg^{-1}$) blended with palm oil at different levels. Formulated experimental diets used consist of 100%fish oil 2.2$meqkg^{-1}$ (10F0P), 60%fish oil 2.2meqkg-1 + 40%palm oil (6F4P), 50%fish oil 2.2$meqkg^{-1}$ + 50%palm oil (5F5P), 40%fish oil 2.2meq/kg + 60% palm oil (4F6P), 100%fish oil 86.8 $meqkg^{-1}$ (Ox10F0P), 60%fish oil 86.8 $meqkg^{-1}$ + 40%palm oil (Ox6F4P), 50%fish oil 86.8 $meqkg^{-1}$ + 50%palm oil (Ox5F5P) and 40%fish oil 86.8 $meqkg^{-1}$ + 60% palm oil (Ox4F6P). Growth performance indexes like body weight gain (BWG), feed conversion ratio (FCR) and hepatosomatic index (HSI) were taken at end of trial. Results indicated that specific growth rate (SGR) in all treatments was not significantly different ($P>0.05$) except in 10F0P, Ox5F5P and Ox4F6P respectively, Ox5F5P and Ox4F6P were significantly lower ($P<0.05$) than 10F0P. Feed conversion ratio (FCR) increased with higher inclusion of palm oil in both non oxidized and oxidized fish oil. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents in both dorsal and ventral muscles gradually declined with increased dietary palm oils. Total PUFA increased Thiobarbiturate reactive substances (TBARS). Incremental palm oil also did not affect hemoglobin (Hb), glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) but increased blood urea nitrogen (BUN) significantly particularly in Ox4F6P. In conclusion, the replacement of oxidized fish oil using palm oil resulted in an acceptable growth performance at limited level. Incorporation of palm oil not more than 40% is suggested as replacement for both non oxidized and oxidized fish oils.

Keywords: *Pagrus major*, fish oil oxidation, palm oil, oxidative stress

5.1 Introduction

Lipid like fish oil in aquafeeds has high tendency to oxidize and it leads to development of undesirable influence on fish metabolism in several ways. Peroxidation of fish oil resulted detrimental conditions to fish. It complicates several pathological conditions in fish (Tocher *et al.,* 2003) which leads to a growing interest to manipulate fatty acid compositions of oxidized fish oil by substituting with other vegetable source like palm oil. Potential of palm oil in efforts to partially replace marine fish oil have been accepted by researchers in recent years (Ng, 2006). Besides being rich in linoleic acids, and a component of essential fatty acids required by fish to grow, palm oil is also affordable and available. Although the information is available in few species, inclusion of palm oil to replace fish oil has demonstrated a similar result to that of other plant oils (Bell *et al.,* 2002; Ng *et al.,* 2000; Ng *et al.,* 2003). However, research on influences of palm oil inclusion with oxidized fish oil in aquafeeds for Japanese cultured species is not yet available.

The present study was therefore conducted to investigate whether palm oil can influences fatty acid compositions stability in diets, fish muscle and liver. The effects of dietary oxidized fish oil with incremental palm oil on growth performance, proximate compositions, fatty acid compositions and blood chemistry were determined. Finally, the levels of muscles and liver peroxidation were also measured using thiobarbituric acid reactive substances (TBARS), reactive oxygen metabolites (ROM) and biological antioxidant potential (BAP).

5.2 Materials and methods

5.2.1 Experimental fishes and feeding protocols

The dietary trial was carried out at Marine Finfish Hatchery, Kagoshima University. Red sea bream juveniles with mean weight of 5.2$\pm$0.4 g were obtained from a commercial hatchery,

Matsumoto Suisan, Miyazaki Prefecture, transported alive in HDPE tank aerated with oxygen and acclimatized in indoor rearing tanks a week prior to experiment. Fishes were fed with commercial pellet (Higashimaru Foods, Kagoshima, Japan). Twelve juveniles were distributed equally in 24 units of 100-L flow-thru tanks in triplicates. Eight experimental diets (Table 17) with different ratio of fish oil, fish oil:palm oil, oxidized fish oil, oxidized fish oil: palm oil were formulated consisting of 100%fish oil 2.2meq/kg (10F), 60%fish oil 2.2meq/kg + 40%palm oil (6F4P), 50%fish oil 2.2meq/kg + 50%palm oil (5F5P), 40%fish oil 2.2meq/kg + 60%palm oil (4F6P), 100%fish oil 51.4meq/kg (Ox10F), 60%fish oil 52.3meq/kg + 40%palm oil (Ox6F4P), 50%fish oil 86.8meq/kg + 50%palm oil (Ox5F5P), 40%fish oil 86.8meq/kg + 60%palm oil (Ox4F6P). Fresh marine fish oil without any antioxidant stabilization was obtained from Nihon Suisan Kabushiki Ltd (Japan) and oxidized by heating oil at 70°C with vigorous aeration for 24 h, with the degree of oxidation monitored by determination of the peroxide value at 8 h intervals until a peroxide value of 86.8 meq/kg was reached. These feeds were fed manually at feeding ration (08:00 and 16:00) to fish until near satiation. Tanks were cleaned and uneaten diets were collected to determine feed intake. The diets were formulated to contain 43.2-46.8 g kg^{-1} protein, 18.1-19.3g kg^{-1} lipid and 14.6-15.6g kg^{-1} ash. Fishes were also exposed to a photoperiod condition of 12 h light/12 h dark, water flow (filtered seawater) maintained at 2.5 *l*/min in which water temperature was 28.7 ± 1.5°C (mean ± SD). Fish sampling was done in every 10 days interval where weight was expressed as bulk weight (g) of all fishes in each respective tank. At end of experiment, fishes were killed using hypothermia method, by immersion into slurry-ice cold marine water prepared and maintained at 3 ppt and 0°C according to method adapted from Losada *et al* (2005) and individual weight of fish was taken. Livers were removed, pooled and stored at −80°C until further analysis was performed. Filleting was conducted by separating the dorsal and ventral flesh according to Japanese standards.

Table 17. Basal ratio of test diets containing different levels of non oxidized and oxidized palm oil inclusion for red sea bream *Pagrus major*

Ingredients(g/kg diet)	Non oxidized groups				Oxidized groups			
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P
Brown fish meal	670	670	670	670	670	670	670	670
Activated gluten	80	80	80	80	80	80	80	80
Starch	40	40	40	40	40	40	40	40
Dextrin	40	40	40	40	40	40	40	40
Fish oil[*1]	100	60	50	40	100	60	50	40
Palm oil	0	40	50	60	0	40	50	60
Mineral Mix[*2]	30	30	30	30	30	30	30	30
Vitamin min[*3]	26.5	26.5	26.5	26.5	26.5	26.5	26.5	26.5
AMP-Na/Ca	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Alpha-cellulose	10	10	10	10	10	10	10	10
Proximate composition								
Moisture	10.8	14.9	13.6	11.2	13.3	13.5	12.2	12.6
Crude protein (% in D.M.)	45.7	44.3	45.5	46.8	45.6	43.2	45.9	44.3
Crude fat (% in D.M.)	18.2	18.1	18.4	19.3	18.7	18.8	18.5	18.4
Ash (% in D.M.)	15.3	14.6	14.9	15.6	15.4	15.1	15.6	15.3

*1; marine fish oil (with no antioxidant stabilization)

*2: Vitamin mixture(g/kg): β-carotene 32.09; Vitamin D_3 3.23; $NaHSO_3.3H_2O(K_3)$ 15.28;

DL-α-Tochopherol acetate 128.32;

Thiamine-nitrate 19.25; Riboflavin 64.13;Pyridoxine-HCl 15.28; Cyanocobalamine 0.03; d-Biotin 1.92; Inositol 1283.04; Niacine (Nicotic acid) 256.58; Ca Panthothenate 89.83; Folic acid 4.81; Choline chloride 2623.10; p-Aminobenzoic acid 127.75.

*3: Mineral mixture(g/kg): NaCl 359.30; $MgSO_4$. $7H_2O$ 1266.74; $NaHPO_4.2H_2O$ 806.35; KH_2PO_4 2217.32; $CA(H_2PO_4)_2.2H_2O$ 1255.66; Fe Citrate 274.60; Ca Lactate 3023.67; $Al(OH)_3$ 1.73; $ZnSO_4.7H_2O$ 33.01; $CuSO_4$ 0.92; $MnSO_4.5H_2O$ 7.40; $Ca(lO_3)_2$ 1.39; $CoSO_4.7H_2O$

Fillets from each treatment were pooled and maintained in ice before freeze-dried and used in further analysis. Three fishes were also randomly sampled from each respective tank and used for proximate analysis.

5.2.2 Proximate composition analysis

Homogenised samples of whole body and liver were analyzed in duplicates were analysed for protein, lipid, ash, moisture and dry matter. Protein was determined by using the Kjeldahl method (AOAC, 1990); lipid by Bligh and Dyer (1959); ash was analysed by combustion in muffle furnace at 600°C. Moisture was determined on approximately 5 g of minced muscle, by oven-drying at 110°C to constant weight, according to (AOAC, 1990) while results of dry matter were expressed as 100 minus moisture (in g of water/100 g of muscle).

5.2.3 Fatty acid composition analysis

Fatty acid composition analysis was performed based on the study of Querijero *et al* (1997) with slight modifications on homogenized samples of dorsal fillet, ventral fillet and liver. Total lipid (TL) was extracted by homogenizing 2 g sample according to Bligh and Dyer (1959). Fatty acid esters (FAME) were then produced from total lipids aliquots. Fatty acids were methylated with BF_3 in methanol. Methyl tricosanoate (Nu-Chek Prep. Inc) was used as internal standard at 1.000 mg/ml Hexane. Fatty acid methyl esters (FAME) was analyzed with a gas chromatograph (Shimadzu GC 17A) with flame ionization detector temperature maintained at 260˚C; carrier N^2 gas at 1ml/min; column temperature at 200°C; injector temperature at 250˚C and Helium (He) served as the carrier gas. 0.1µl sample including 0.1µl air was manually injected into injection port and identified fatty acids were presented as area percentage of total fatty acids.

5.2.4 TBARS analysis

Analysis of Thiobarbiturate Reactive Substances (TBARS) was conducted according to a method adapted from Yagi (1987). 0.2g sample, 0.2 ml of 8.1% SDS, 1.5 ml of HCl, 1.5 ml of TBA, 0.05 ml of BHT, and 1 ml of distilled water were added to a test tube. The sample was vortexed and heated in a 90°C water-bath for 60 min. 1 ml of distilled water and 5 ml of n-butanol-pyridine solution was added after sample was cooled for 10 min, mixed thoroughly, and centrifuged at 3,000g for 10min. The fluorescence reading of upper layer was measured by a fluorometer with 532-nm excitation.

5.2.5 Blood chemistry analysis

The micro hematocrit method by Kawadzu (1981) was used for the determination of hematocrit level. Glucose, total cholesterol (T-Cho), triglyceride (TG), blood urea nitrogen (BUN) in plasma and albumin, total bilirubin (T-Bil), glutamic oxaloacetic transaminase (GOT), total protein (TSP) in serum were determined by SPOTCHEM EZ SP-4430 system according to Tatsumi *et al* (2000).

5.2.6 BAP and ROM analysis

Biological antioxidant potential (BAP) and reactive oxygen metabolites (ROM) were determined using free radical analytical system (model FRAS4, H & D Ltd, Serial no: 330892, Wismerll, Italy). Plasma was centrifuged using high speed refrigerated micro centrifuge (TOMY, MX-160, serial 32039219) at 4°C, 3000 rpm for 10 mins. Reagents thiocyanate derivative (R1) and $FeCl_3$ (R2) were used as reagents in detecting potentials and reactive metabolites levels in plasma.

5.3 Statistic analysis

The statistical analysis was performed using ANOVA. Data was expressed as means $\pm$ S.E. Homogeneity of variance between treatments ($P < 0.05$) were analysed using one-way Analysis of Variance (ANOVA) while significance of differences were determined using Tukey-Test.

Effects of dietary treatments were considered significant at P <0 .05.

5.4 Results

5.4.1 Growth performances

Body weight gain (BWG) in 10F0P, 6F4P, Ox10F0P and Ox6F4P showed no significant difference (P>0.05) among treatments although 10F0P demonstrated highest BWG and decreased with incremental palm oil blended with fresh fish oil and oxidized fish oil (Table 18) 5F5P, 4F6P, Ox5F5P and Ox4F6P showed no significant difference (P>0.05) among treatments but significantly different (P<0.05)from 10F0P. Specific growth rate (SGR) in all treatments was not significantly different (P>0.05) except in 10F0P, Ox5F5P and Ox4F6P respectively, Ox5F5P and Ox4F6P were significantly lower (P<0.05) than 10F0P. Feed conversion ratio (FCR) increased with higher inclusion of palm oil in both non oxidized and oxidized fish oil. In non oxidized fish oil, 4F6P showed significantly higher FCR than lower inclusion of palm oil. There were no significant differences among treatments in oxidized fish oil. Survival rate (SR) was similar without any significant differences (P>0.05) among all treatments ranging from 80.5% to 94.4%. However, SR in fish fed with oxidized fish oil showed lower value as compared to SR in fish fed with non oxidized fish oil. Feed intake (FI) in 10F0P, 6F4P and 5F5P were significantly higher (P<0.05) than in other treatments. Hepatosomatic index (HSI) in 10F0P was significantly (P<0.05) lower than Ox5F65 and Ox4F6P.

5.4.2 Proximate analysis

Table 17 illustrated proximate composition of eight experimental diets in this trial. Crude protein amounted between 43.2 to 46.8% (dry weight); crude lipid between 18.1 to 19.3% (dry weight) and ash ranged from 14.6% to 15.6% (dry weight). Moisture fluctuated among treatments ranging from 10.8% to 14.9%. Gross energy in diets illustrated similar energy level

ranging from 6.5-6.6 kcal/g among treatments.

Although diets were similarly formulated on proximate composition, significant differences in proximate composition of whole body and muscle (Table 19). Whole body data demonstrated no significant difference ($P > 0.05$) among all treatments except in 10F0P (36.8%) and Ox4F6P (32.7%). No significant difference was demonstrated among treatments in dorsal muscle. In contrast, ventral muscle demonstrated more than twice-fold lipid deposition than dorsal muscle with significant higher contents in 4F6P and Ox4F6P. Liver also showed similar trend as in ventral muscles. Ash contents in treatment 10F were significantly higher ($P < 0.05$) than those in Ox5F5P and Ox4F6P. On the other hand, protein content increased gradually with incremental palm oil particularly in oxidized fish oil groups. Protein content was significantly highest ($P < 0.05$) in Ox5F5P and Ox4F6P.

5.4.3 Fatty acid composition in diet

Inclusion of palm oil in non oxidized and oxidized fish oil in diets was strongly reflected in fatty acid composition in diets (Table 20). Saturates, mononenes and n-3 fatty acids dominates total composition in which higher inclusion of palm oil gradually increased saturates but showed a tendency of inverse gradient with n-3 series. n-6 series demonstrated a gradual increase which was dominated with incremental linoleic acid (C18:2n-6). In n-3, EPA and DHA showed a gradient decrease in composition with incremental palm oil blended with either non oxidized or oxidized fish oil.

Table 18. Growth performances of red sea bream *Pagrus major* fed with different diets

	Non oxidized group				Oxidized group				Significance[6]		
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P	Ox	Po	Interaction
B.W.G.[*1,4] (%)	756.9±34.9^{d}	735.4±20.6^{cd}	654.9±28.6^{abc}	628.9±10.9^{ab}	699.0±40.4^{bcd}	687.3±18.4^{bcd}	630.1±68.6^{ab}	550.2±29.8^{a}	*[6]	*	NS
SGR[*2](%)	4.3±0.1^{c}	4.2±0.1^{bc}	4.0±0.1^{abc}	4.0±0.0^{abc}	4.2±0.1^{bc}	4.1±0.0^{bc}	4.0±0.2^{ab}	3.7±0.1^{a}	*	*	NS
FCR[*3]	1.4±0.1^{a}	1.5±0.1^{ab}	1.5±0.1^{ab}	1.6±0.1^{ab}	1.5±0.1^{ab}	1.5±0.1^{ab}	1.6±0.1^{ab}	1.6±0.1^{b}	NS	*	NS
SR[*4](%)	91.7±8.3	88.9±9.6	94.4±4.8	91.7±8.4	86.1±4.8	88.9±4.8	80.5±4.8	83.3±8.4	*	NS	NS
FI[*5](g/fish)	55.4±3.0^{c}	52.0±1.7^{bc}	53.3±1.7^{c}	42.7±3.7^{a}	43.3±2.7^{a}	45.3±0.9^{ab}	41.8±3.5^{a}	43.2±3.7^{a}	*	*	*

[1]Values are expressed as mean ± SE (n=3). Data with same alphabets are not significantly different (P>0.05).

[2] SGR, specific growth rate= 100 x (ln final weight- ln initial weight)/(duration)

[3] FCR, feed conversion ratio = dry feed intake(kg)/weight gain(kg)

[4] BWG, body weight gain (%)= 100 x (final weight - initial weight) / (initial weight)

SR, survival rate (%) = 100 x (initial fish number-dead fish number) / (initial fish number)

[5] FI, Feed intake (g/fish/50days) = (total feed intake (g) / number of fishes) in 50 days feeding period

[6] Values are results of two way ANOVA analysis. Data with * are significantly different (P<0.05) and NS is expressed as not significantly different (P>0.05)

5.4.4 Fatty acid composition in dorsal

Fatty acid composition in Table 21 showed that saturates, mononenes and n-3 dominated total fatty composition in dorsal muscle. Incremental palm oil has increased C16:0, C18:1n-9 and C18:2n-6 at gradient level in non-oxidized and oxidized fish oil blends. In contrary, total n-6 increased significantly while total n-3 decreased significantly with higher inclusion of palm with decreasing EPA and DHA trend in all treatments. 10F0P, 6F0P and Ox10F0P demonstrated highest EPA while Ox5F0P and Ox4F6P showed significantly lowest amount. On the other hand, DHA in 10F0P, 6F4P, 5F5P and Ox10F0P showed significantly higher while 4F6P, Ox6F4P, Ox5F5P and Ox4F6 demonstrated lower DHA. In comparison, total content (∑) of polyunsaturated fatty acids (PUFA) differed from total % of fatty acid composition. Results showed that total content in 10F0P was significantly highest than other treatments. Other treatments had no significant differences among treatments although there is a declining trend with higher inclusion of palm oil.

5.4.5 Fatty acid composition in ventral

Saturates, mononenes and n-3 dominated the total fatty composition in ventral muscle (Table 22). Incremental palm oil blended with either non oxidized or oxidized fish oil had significantly increased C18:1n-9 and C18:2n-6. In contrary, total n-6 increased significantly while total n-3 decreased significantly with higher inclusion of palm with decreasing EPA and DHA trend in all treatments. DHA showed gradient decrease without significant differences among treatments although EPA amounts significantly decreased with higher inclusion of palm oil particularly in 5F5P, 4F6P, Ox6F4P, Ox5F5P and Ox4F6P. Results showed that total content in all non oxidized fish oil blended with incremental palm oil was not significantly different among treatments. OX10F0P, Ox6F4P, Ox5F5P and Ox4F6P were significantly lower than

10F0P.

5.4.6 Fatty acid composition in liver

Saturates and monoenes dominated the total fatty composition (Table 23). In oxidized fish oil treatments, incremental palm oil increased saturates than in non oxidized fish oil treatments. Similar trend was shown in n-6 with incremental C18:2n-6 with higher inclusion of palm oil. Results showed no significant differences of total n-3 among treatments. However, EPA and DHA decreased gradually with higher inclusion of palm oil in both non oxidized and oxidized fish oils. EPA in Ox6F4P, Ox5F5P and Ox4F6P decreased significantly compared to other treatments. DHA showed gradient decrease without significant differences among treatments. PUFA content in 10F0P, 6F4P, 5F0P, 4F6P, Ox10F0P and Ox6F4P were significantly higher than Ox5F5P and Ox4F6P.

5. 4.7 TBARS assay

In Table 24, diets containing non oxidized fish oils blended with palm oil showed gradient decrease in TBARS while TBARS in diets using oxidized fish oils showed inverse results. Similar pattern was also shown in liver. In comparison, TBARS was significantly highest in treatment 10F0P in dorsal and ventral muscles. Levels decreased gradually with incremental palm oil in both non oxidized and oxidized treatments. Treatments 6F4P, 5F5P, 4F6P, Ox10F0P, Ox6F4P and Ox5F5P in dorsal and ventral muscles showed no significant differences among treatments although there is a descending trend with higher inclusion of palm oil.

5.4.8 Blood chemistry

Results in Table 25 showed effect of blending palm oil with non oxidized and oxidized fish oil. Treatments 10F0P and Ox10F0P showed highest hemoglobin (Hb) level although there

were no significant differences ($P > 0.05$) among treatments. Hb gradually decreased with inclusion of palm oil as partial replacement for fish oil in both non oxidized and oxidized fish oil. Blood urea nitrogen (BUN) also gradually increased with incremental palm oil with a significantly ($P < 0.05$) highest amount in Ox4F6P. Glutamic oxaloacetic transaminase (GOT) and Glutamate pyruvate transaminase (GPT) also demonstrated similar incremental pattern but with no significant differences ($P > 0.05$) among treatments in both non oxidized and oxidized fish oil.

5.4.9 BAP and ROM analysis

Results (Fig. 11) demonstrated that 6F4P and 5F5P showed highest antioxidant potential with lowest oxidative stress in blood. In comparison, 10F (control) showed lower BAP with lower oxidative stress while Ox5F5P and 4F6P demonstrated higher level of BAP and d-ROMs. However, both components in this matrix marked acceptable levels of both antioxidant and oxidative stress in blood. On the other hand, Ox10F, Ox6F4P and Ox4F6P illustrated lowest potential to antioxidant with highest concentration of d-ROMs.

Table 19. Proximate analysis of whole body in red sea bream *Pagrus major* fed with different diets

	Non oxidized group				Oxidized group				Significance[2]		
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P	Ox	Po	Interaction
Crude Fat [*1](%)	36.8±1.8[b]	35.2±0.5[ab]	34.6±1.3[ab]	34.6±1.2[ab]	36.8±1.3[b]	37.1±1.3[b]	33.9±0.6[ab]	32.7±0.4[a]	NS[1]	*	NS
Moisture (%)	66.4±1.1	67.7±0.7	66.9±0.9	66.2±0.9	66.2±0.6	66.8±0.2	67.4±0.7	69.8±0.8	*	*	*
Ash (% in D.M.)	11.9±0.8[a]	12.6±0.9[ab]	14.0±0.4[b]	12.8±0.9[ab]	13.0±0.5[ab]	13.3±0.4[ab]	14.1±0.3[b]	14.2±0.4[b]	*	*	NS
Protein (% in D.M.)	45.8±1.9[a]	49.3±0.4[bc]	48.8±0.8[b]	47.9±1.0[ab]	47.5±0.6[ab]	48.9±0.3[b]	52.0±1.8[cd]	52.9±0.1[d]	*	*	*

Values are expressed as mean ± SE(n=3). Data with same alphabets are not significantly different (P>0.05).
[2]Dry weight basis
[3] Values are results of two way ANOVA analysis. Data with * are significantly different (P<0.05) and NS is expressed as not significantly different (P>0.05

Table 20: Fatty acid composition (% of total fatty acid) in experimental diets

	Non oxidized group				Oxidized group			
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P
14:0	4.1	3.6	3.3	3.2	4.9	4.1	3.8	3.6
16:0	22.0	27.4	27.5	29.1	20.6	25.9	26.3	29.5
18:0	5.0	5.7	5.7	5.9	4.8	5.2	5.1	5.4
∑Saturated	31.1	36.7	36.5	38.2	30.2	35.2	35.3	38.5
16:1n-7	5.4	4.5	4.2	4.1	6.2	4.9	4.6	4.5
18:1n-9	11.9	17.2	17.6	19.0	14.6	17.9	18.2	19.3
18:1n-7	3.3	1.7	1.5	1.3	2.2	2.0	2.1	2.0
20:1n-9	3.8	1.8	0.9	0.6	4.3	1.9	1.2	1.1
22:1n-11	3.0	1.7	1.5	1.2	4.7	2.8	2.7	2.2
22:1n-9	0.4	0.3	0.2	0.2	0.4	0.3	0.2	0.2
∑Monoenes	27.8	27.2	25.9	26.4	32.4	29.7	29.0	29.3
18:2n-6	2.8	4.1	4.4	4.5	3.6	4.8	5.2	5.6
20:2n-6	nd	nd	nd	nd	nd	nd	nd	nd
20:4n-6	1.6	1.3	1.3	1.0	1.4	1.3	1.3	0.3
∑n-6fatty acids	4.4	5.4	5.7	5.5	5.1	6.1	6.5	5.9
18:3n-3	1.1	0.7	0.6	0.5	0.9	0.5	0.5	0.4
20:4n-3	0.3	0.2	nd	nd	0.2	0.1	0.1	nd
20:5n-3	9.2	7.5	7.2	5.9	8.2	7.3	6.7	6.3
22:5n-3	1.4	1.4	1.4	1.2	1.6	1.5	1.6	1.5
22:6n-3	13.3	11.7	10.7	8.6	12.7	12.6	12.3	11.5
∑n-3 fatty acids	25.2	21.5	20.9	16.2	23.7	22.0	21.2	19.6
∑n-3:PUFA	0.5	0.4	0.4	0.3	0.4	0.4	0.4	0.3
∑n-3:n-6 Fatty acids	5.9	3.9	3.6	3.0	5.0	3.5	3.1	3.3
∑EPA:DHA	0.8	0.8	0.7	0.8	0.8	0.8	0.7	0.7

[1] Values are results of two way ANOVA analysis. Data with * are significantly different ($P<0.05$) and NS is expressed as not significantly different ($P>0.05$)

Table 21. Fatty acid composition (% of total fatty acid) in dorsal of red sea bream *Pagrus major* fed with different diets

	Non oxidized group				Oxidized group				Significance[1]		
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P	Ox	Po	Interaction
14:0	2.8±0.0^{d}	2.2±0.0^{bc}	2.0±0.1^{abc}	1.9±0.0^{a}	3.0±0.0^{d}	2.2±0.1^{c}	2.1±0.0^{abc}	1.9±0.0^{ab}	*	NS	NS
16:0	21.6±0.9^{a}	24.4±0.3^{ab}	25.2±0.9^{b}	25.3±1.8^{b}	21.7±1.2^{a}	24.7±0.6^{b}	25.2±1.9^{b}	26.2±1.3^{b}	*	NS	NS
18:0	6.1±0.1^{a}	6.2±0.2^{a}	6.4±0.0^{ab}	6.7±0.1^{ab}	6.4±0.0^{ab}	6.9±0.3^{ab}	6.8±0.1^{ab}	7.1±0.3^{b}	*	*	NS
∑Saturated	30.5±1.0	32.7±0.5	33.6±0.7	33.9±1.7	31.1±1.1	33.8±0.4	34.1±1.8	35.3±1.6	NS	*	NS
16:1n-7	4.9±0.1^{d}	4.0±0.2^{bc}	3.6±0.0^{a}	3.6±0.0^{a}	5.3±0.1^{e}	4.1±0.0^{c}	3.8±0.0^{ab}	3.8±0.0^{ab}	*	*	NS
18:1n-9	18.9±0.5^{a}	22.2±0.3^{ab}	22.9±1.0^{ab}	24.2±0.8^{bc}	19.3±1.7^{a}	24.6±0.7^{bc}	24.3±0.2^{bc}	27.3±1.7^{c}	*	*	NS
18:1n-7	0.2±0.0	0.2±0.1	0.8±0.9	0.7±0.9	1.1±1.2	0.1±0.0	0.1±0.0	0.1±0.0	NS	NS	NS
20:1n-9	4.7±0.0^{d}	3.0±0.3^{c}	1.4±0.4^{a}	1.2±0.3^{a}	4.5±0.5^{d}	2.6±0.1^{bc}	1.5±0.0^{ab}	1.2±0.2^{a}	*	NS	NS
22:1n-11	2.6±0.1^{e}	1.6±0.2^{d}	1.3±0.2^{c}	0.9±0.0^{ab}	2.4±0.0^{e}	1.3±0.0^{bc}	1.0±0.0^{abc}	0.8±0.0^{a}	*	*	NS
22:1n-9	0.4±0.0^{c}	0.3±0.0^{b}	0.2±0.0^{ab}	0.2±0.0^{a}	0.4±0.0^{c}	0.2±0.0^{ab}	0.2±0.0^{ab}	0.2±0.0^{a}	*	NS	NS
∑Monoenes	31.6±0.5	31.3±0.8	30.1±0.4	30.8±0.2	33.0±1.2	33.0±0.6	31.0±0.1	33.4±1.9	*	NS	NS
18:2n-6	2.1±0.1^{a}	4.2±0.1^{b}	4.6±0.0^{cd}	5.1±0.0^{e}	2.3±0.1^{a}	4.5±0.1^{bc}	5.0±0.1^{de}	5.6±0.1^{f}	*	*	NS
20:2n-6	0.2±0.0	0.2±0.1	0.1±0.0	0.1±0.1	0.1±0.1	0.1±0.0	0.0±0.1	0.1±0.0	NS	*	NS
20:4n-6	1.5±0.2	1.7±0.1	1.6±0.0	1.6±0.0	1.8±0.0	1.6±0.1	1.8±0.0	1.4±0.3	NS	NS	NS
∑n-6fatty acids	3.8±0.3^{a}	6.1±0.3^{b}	6.3±0.0^{bc}	6.9±0.2^{bc}	4.2±0.2^{a}	6.3±0.0^{bc}	6.8±0.2^{bc}	7.1±0.3^{c}	NS	*	NS
18:3n-3	0.7±0.0^{c}	0.5±0.1^{cd}	0.4±0.0^{bcd}	0.4±0.0^{abc}	0.6±0.1^{de}	0.3±0.0^{ab}	0.3±0.0^{ab}	0.2±0.0^{a}	*	*	NS
18:4n-3	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	nd	nd	NS	*	NS
20:4n-3	0.6±0.0	0.5±0.0	0.3±0.1	0.3±0.1	0.4±0.2	0.4±0.0	0.3±0.1	0.2±0.1	*	NS	NS
20:5n-3	8.8±0.2^{d}	7.7±0.2^{bcd}	7.1±0.2^{bc}	6.9±0.2^{bc}	8.3±0.0^{cd}	6.7±0.1^{bc}	6.5±0.2^{ab}	4.9±1.1^{a}	*	*	NS
22:5n-3	2.4±0.1^{bcd}	2.3±0.0^{bd}	2.3±0.1^{b}	2.1±0.0^{b}	2.4±0.1^{bcd}	2.1±0.1^{ab}	2.1±0.0^{b}	1.6±0.2^{a}	*	*	*
22:6n-3	14.4±0.4^{d}	13.9±0.3^{cd}	13.8±0.3^{cd}	12.5±0.5^{abc}	14.5±0.5^{d}	13.2±0.5^{ab}	13.1±0.3^{bcd}	11.6±0.1^{a}	*	*	*
∑ n-3 fatty acids	27.1±0.7^{e}	25.1±0.0^{cde}	24.1±0.0^{bcd}	22.3±0.9^{bc}	26.2±0.4^{de}	21.7±0.7^{b}	22.2±0.7^{bc}	18.6±1.4^{a}	*	*	NS
∑n-3:PUFA	0.4±0.0^{e}	0.4±0.0^{cde}	0.4±0.0^{bcde}	0.4±0.0^{bcd}	0.4±0.0^{de}	0.3±0.0^{ab}	0.4±0.0^{bc}	0.3±0.0^{a}	*	*	NS
∑n-3:n-6 Fatty acids	7.3±1.0^{b}	4.0±0.2^{a}	3.7±0.0^{a}	3.1±0.0^{a}	6.1±0.3^{b}	3.3±0.1^{a}	3.1±0.0^{a}	2.5±0.0^{a}	*	*	NS
∑EPA:DHA	0.7±0.0^{b}	0.6±0.0^{ab}	0.6±0.1^{ab}	0.6±0.0^{a}	0.7±0.0^{b}	0.6±0.0^{ab}	0.6±0.0^{ab}	0.5±0.1^{a}	*	NS	NS

[1] Values are results of two way ANOVA analysis. Data with * are significantly different (P<0.05) and NS is expressed as not significantly different (P>0.05)

Table 22. Fatty acid composition (% of total fatty acid) in ventral of red sea bream *Pagrus major* fed with different diets

	Non oxidized group				Oxidized group				Significance[1]		
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P	Ox	Po	Interaction
14:0	3.0±0.1^{bc}	2.6±0.3^{abc}	2.3±0.2^{abc}	2.2±0.2^{ab}	3.1±0.1^{c}	2.7±0.4^{abc}	2.3±0.0^{abc}	2.2±0.1^{a}	*	NS	NS
16:0	22.3±0.1	23.5±2.4	24.4±1.5	24.8±1.4	21.0±1.7	22.4±0.8	24.4±0.1	25.5±0.1	*	NS	NS
18:0	6.0±0.1	6.5±0.7	6.5±0.1	6.7±0.4	6.5±0.1	6.5±0.4	6.7±0.1	7.0±0.2	NS	NS	NS
∑Saturated	31.3±0.0^{ab}	32.6±3.4^{ab}	33.3±1.1^{ab}	33.7±1.1^{ab}	30.6±1.6^{a}	31.5±0.7^{ab}	33.4±0.2^{ab}	34.7±0.4^{b}	NS	NS	NS
16:1n-7	5.1±0.1^{b}	4.4±0.5^{ab}	4.0±0.0^{a}	3.8±0.0^{a}	5.4±0.1^{b}	4.7±0.6^{ab}	3.9±0.1^{a}	3.8±0.1^{a}	*	NS	NS
18:1n-9	17.9±0.5^{a}	22.0±2.1^{c}	23.7±1.1^{abc}	25.1±0.6^{bc}	19.2±1.3^{ab}	22.8±3.0^{abc}	24.7±0.6^{bc}	26.6±0.8^{c}	*	NS	NS
18:1n-7	2.0±0.1	1.2±0.1	0.8±1.0	0.1±0.1	1.1±1.1	0.1±0.1	0.2±0.1	0.1±0.1	*	NS	NS
20:1n-9	4.3±0.0^{b}	2.3±0.4^{ab}	2.0±0.6^{ab}	1.7±0.2^{ab}	3.9±0.9^{ab}	2.9±1.5^{ab}	1.8±0.4^{ab}	1.2±0.0^{a}	*	NS	NS
22:1n-11	3.1±0.5^{c}	1.7±0.1^{ab}	1.4±0.1^{ab}	1.2±0.2^{ab}	2.4±0.1^{bc}	1.9±0.7^{abc}	1.2±0.1^{ab}	0.8±0.0^{a}	*	NS	NS
22:1n-9	0.3±0.0^{bc}	0.2±0.0^{abc}	0.2±0.0^{abc}	0.2±0.0^{ab}	0.3±0.0^{c}	0.3±0.1^{c}	0.2±0.0^{a}	0.2±0.0^{a}	*	NS	NS
∑Monoenes	32.7±1.1	31.9±3.2	32.1±0.6	32.1±0.6	32.4±1.1	32.7±0.1	31.9±1.0	32.7±1.0	NS	NS	NS
18:2n-6	2.2±0.3^{a}	4.2±0.1^{b}	4.9±0.1^{bc}	5.1±0.2^{bc}	2.2±0.1^{a}	3.9±0.9^{b}	5.2±0.2^{bc}	5.7±0.1^{c}	*	NS	NS
20:2n-6	0.1±0.0	0.1±0.1	0.0±0.1	0.1±0.1	0.1±0.1	0.1±0.0	0.2±0.1	0.1±0.0	NS	NS	NS
20:4n-6	1.5±0.2	1.1±0.5	1.6±0.0	1.0±0.8	1.8±0.0	1.7±0.1	1.6±0.1	1.7±0.0	NS	*	NS
∑n-6fatty acids	3.8±0.2^{a}	5.5±0.4^{bc}	6.5±0.1^{cd}	6.3±0.6^{cd}	4.0±0.1^{ab}	5.8±0.8^{c}	6.9±0.2^{cd}	7.4±0.1^{d}	*	*	NS
18:3n-3	0.7±0.0^{c}	0.5±0.0^{abc}	0.4±0.0^{abc}	0.4±0.0^{abc}	0.6±0.0^{bc}	0.4±0.2^{abc}	0.3±0.1^{ab}	0.3±0.0^{a}	*	*	NS
18:4n-3	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.0±0.1	0.1±0.0	NS	NS	NS
20:4n-3	0.6±0.0^{b}	0.4±0.1^{ab}	0.3±0.1^{ab}	0.4±0.0^{ab}	0.5±0.0^{ab}	0.4±0.1^{ab}	0.3±0.0^{ab}	0.3±0.0^{a}	*	NS	NS
20:5n-3	9.2±0.0^{c}	8.3±1.8^{bc}	7.2±0.2^{ab}	6.9±0.4^{ab}	8.4±0.3^{bc}	7.2±0.9^{ab}	6.1±0.2^{a}	5.7±0.0^{a}	*	*	NS
22:5n-3	2.4±0.0	2.8±0.5	2.3±0.1	2.0±0.0	2.3±0.0	2.1±0.3	2.0±0.2	1.8±0.1	NS	NS	NS
22:6n-3	14.4±0.1	13.4±2.3	12.9±0.2	11.9±0.4	13.5±0.2	12.5±1.1	11.7±0.7	11.3±0.3	NS	NS	NS
∑n-3 fatty acids	27.5±0.2^{c}	25.5±3.5^{bc}	23.2±0.4^{abc}	21.8±0.8^{ab}	25.4±0.5^{bc}	22.6±2.6^{ab}	20.6±1.2^{a}	19.4±0.4^{a}	*	*	NS
∑n-3:PUFA	0.4±0.0	0.4±0.1	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.3±0.0	0.3±0.0	*	NS	NS
∑n-3:n-6 Fatty acids	7.1±0.2^{c}	4.3±1.0^{a}	3.4±0.0^{a}	3.5±0.6^{a}	6.2±0.1^{bc}	4.0±1.3^{ab}	2.8±0.3^{a}	2.5±0.1^{a}	*	NS	NS
∑EPA:DHA	0.7±0.0	0.7±0.0	0.6±0.1	0.7±0.0	0.7±0.0	0.7±0.0	0.6±0.0	0.6±0.0	*	NS	NS

[1] Values are results of two way ANOVA analysis. Data with * are significantly different (P<0.05) and NS is expressed as not significantly different (P>0.05)

Table 23. Fatty acid composition (% of total fatty acid) in liver of red sea bream *Pagrus major* fed with different diets

	Non oxidized group				Oxidized group				Significance[1]		
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P	Ox	Po	Interaction
14:0	1.8±0.1	1.6±0.2	1.4±0.4	1.2±0.2	2.0±0.3	1.7±0.3	1.7±0.2	1.4±0.1	*	NS	NS
16:0	25.4±0.9	23.6±3.5	23.1±1.1	24.8±1.3	24.7±2.9	27.0±2.3	28.8±1.8	28.0±0.7	NS	*	NS
18:0	17.1±0.1^{a}	10.6±0.8^{ab}	11.9±1.5^{bc}	12.5±4.2^{bc}	12.3±2.5^{bc}	14.3±0.9^{bc}	13.7±0.3^{bc}	16.3±0.0^{c}	*	*	NS
∑Saturated	34.4±1.0^{a}	33.9±4.5^{a}	36.4±2.2^{ab}	37.4±5.4^{ab}	39.0±5.1^{ab}	42.7±3.5^{ab}	44.2±2.3^{ab}	45.7±0.8^{b}	*	NS	NS
16:1n-7	4.9±0.6	3.6±0.3	3.4±0.3	3.1±0.0	7.8±4.6	3.7±0.4	3.4±0.2	3.5±0.3	NS	NS	NS
18:1n-9	20.7±0.5^{c}	21.8±2.1^{a}	22.9±0.2	25.2±2.1^{abc}	21.2±0.6^{a}	24.9±1.4^{abc}	22.4±0.5^{ab}	26.8±0.4^{bc}	*	NS	*
18:1n-7	0.8±0.6	0.1±0.1	0.2±0.0	0.1±0.1	0.2±0.1	0.2±0.2	0.1±0.0	0.2±0.2	NS	NS	NS
20:1n-9	3.9±0.1^{bc}	3.3±0.0^{abc}	2.4±0.8^{a}	2.4±0.4^{a}	4.1±0.1^{c}	3.1±0.3^{abc}	3.4±0.2^{abc}	2.8±0.1^{ab}	*	NS	NS
22:1n-11	1.9±0.1^{c}	1.2±0.1^{abc}	0.9±0.5^{ab}	0.7±0.2^{b}	1.7±0.1^{bc}	1.0±0.3^{abc}	0.9±0.1^{ab}	0.7±0.0^{a}	*	NS	NS
22:1n-9	0.3±0.1	0.4±0.2	0.2±0.1	0.3±0.0	0.6±0.4	0.3±0.0	0.3±0.0	0.3±0.0	NS	NS	NS
∑Monoenes	32.6±0.7^{b}	30.5±2.3^{ab}	30.0±1.8^{a}	31.8±2.5^{b}	35.7±4.5^{ab}	33.3±2.7^{ab}	30.6±1.0^{ab}	34.3±0.8^{ab}	NS	*	NS
18:2n-6	1.4±0.1^{a}	3.1±0.3^{b}	3.0±0.0^{b}	3.2±0.2^{b}	1.5±0.3^{a}	2.5±0.0^{b}	3.8±0.2^{c}	3.2±0.1^{bc}	*	NS	*
20:2n-6	0.1±0.1^{a}	0.3±0.0bc	0.2±0.0b	0.3±0.0^{b}	0.2±0.0^{a}	0.2±0.0^{b}	0.4±0.0^{c}	0.3±0.0^{b}	*	*	*
20:4n-6	1.6±0.1	1.9±0.4	2.2±0.3	2.4±0.2	1.9±0.2	1.7±0.5	1.7±0.1	1.4±0.3	NS	*	NS
∑n-6fatty acids	3.2±0.1^{a}	5.3±0.7^{bc}	5.5±0.3^{c}	5.8±0.4^{c}	3.6±0.4^{ab}	4.5±0.6^{abc}	5.9±0.3^{c}	4.9±0.3^{bc}	NS	*	NS
18:3n-3	0.2±0.0	0.3±0.1	0.4±0.3	0.1±0.0	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.1	NS	NS	NS
18:4n-3	0.1±0.0^{ab}	0.1±0.0^{b}	0.1±0.0^{ab}	0.1±0.0^{ab}	0.1±0.0^{ab}	0.1±0.0^{ab}	0.1±0.0^{ab}	0.0±0.0^{a}	*	*	NS
20:4n-3	0.4±0.2	0.5±0.1	0.4±0.0	0.4±0.0	0.5±0.1	0.3±0.1	0.3±0.0	0.2±0.0	NS	*	NS
20:5n-3	4.9±0.1^{b}	4.7±1.1^{ab}	4.6±0.1^{ab}	4.5±0.3^{b}	4.1±0.8^{ab}	3.0±0.9^{ab}	2.6±0.5^{ab}	2.2±0.0^{b}	NS	*	NS
22:5n-3	2.1±0.2^{ab}	2.5±0.5^{b}	2.3±0.3^{ab}	2.5±0.4^{b}	2.1±0.3^{ab}	1.7±0.5^{ab}	2.1±0.3^{ab}	1.1±0.2^{a}	NS	*	NS
22:6n-3	11.7±0.3	10.7±1.2	10.6±0.7	9.6±1.4	10.7±3.2	8.5±4.8	8.0±1.8	5.2±1.6	NS	*	NS
∑n-3 fatty acids	19.4±0.6	18.8±6.0	18.4±0.7	16.8±2.2	17.6±4.4	13.5±6.2	13.2±2.6	8.8±1.8	*	NS	NS
∑n-3:PUFA	0.4±0.0	0.4±0.1	0.4±0.0	0.4±0.0	0.3±0.0	0.3±0.1	0.3±0.0	0.2±0.0	NS	*	NS
∑n-3:n-6 Fatty acids	5.8±0.1^{e}	3.8±0.6^{bcd}	3.5±0.2^{bcd}	3.4±0.2^{cd}	4.8±0.5^{de}	2.8±0.7^{abc}	2.1±0.3^{ab}	1.7±0.2^{a}	*	*	NS
EPA:DHA	0.5±0.0	0.5±0.0	0.4±0.1	0.4±0.0	0.5±0.0	0.5±0.0	0.4±0.0	0.5±0.1	NS	NS	NS

Table 24. TBARS in red sea bream *Pagrus major* fed with different diets

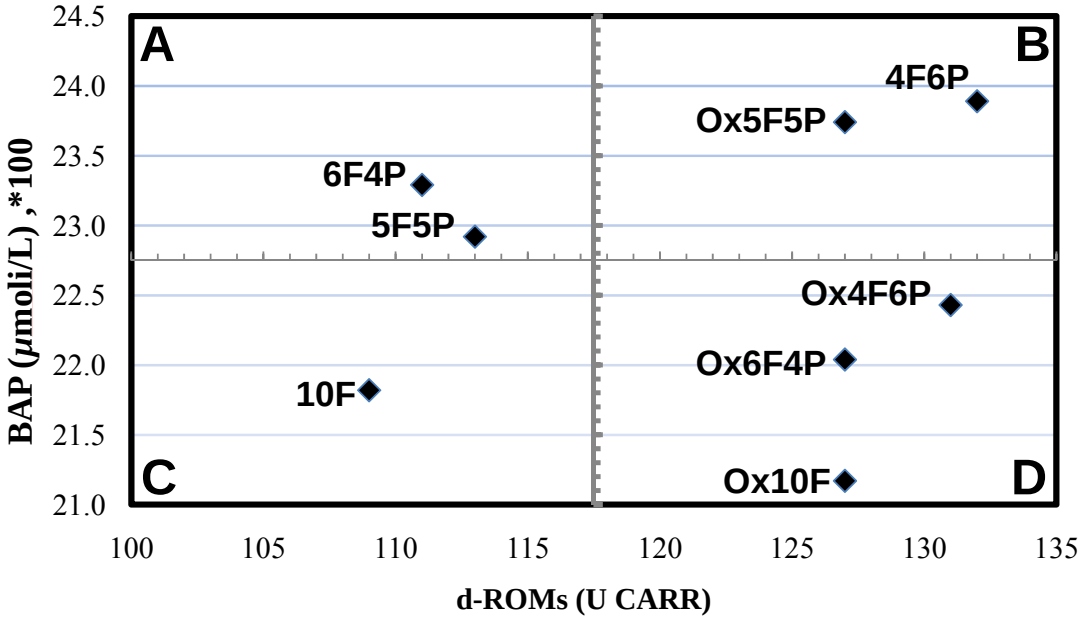
TBARS	Non oxidized group				Oxidized group				Significance[1]		
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P	Ox	Po	Interaction
Diet (nmol/g)	37.87±3.69^{c}	33.69±4.76^{bc}	27.78±4.05^{ab}	22.03±1.80^{a}	21.69±0.34^{a}	22.48±1.92^{a}	29.23±3.87^{ab}	37.37±3.07^{c}	*	NS	NS
Liver (nmol/g)	22.10±2.58^{bc}	17.00±0.34^{ab}	15.62±1.06^{a}	14.90±2.40^{a}	15.92±4.01^{ab}	14.48±2.34^{a}	16.55±1.10^{ab}	23.62±1.67^{c}	*	NS	NS
Muscle (nmol/g)	16.72±3.37^{b}	12.72±1.00^{ab}	8.16±2.80^{a}	11.46±3.50^{ab}	9.53±3.20^{ab}	9.69±1.72^{ab}	7.75±1.71^{a}	5.57±1.94^{a}	*	NS	NS

[1] Values are results of two way ANOVA analysis. Data with * are significantly different (P<0.05) and NS is expressed as not significantly different (P>0.05)

Table 25. Blood chemistry in red sea bream *Pagrus major* fed with different diets

	Non oxidized group				Oxidized group				Significance[1]		
	10F	6F4P	5F5P	4F6P	Ox10F	Ox6F4P	Ox5F5P	Ox4F6P	Ox	Po	Interaction
Ht (%)	40.49±4.96	36.57±2.01	38.92±2.61	45.16±4.43	44.16±2.64	46.35±3.18	46.43±4.54	44.78±4.98	*	NS	NS
Hb (g/dl)	5.47±0.83	4.77±0.60	4.40±0.66	4.60±0.17	4.60±0.17	5.00±1.23	4.68±0.46	4.03±0.59	NS	NS	NS
GLU	45.20±1.79	49.40±7.44	45.80±2.49	42.00±2.12	50.25±4.92	44.20±6.69	42.60±4.16	42.20±5.40	*	NS	NS
T-CHO	380.60±34.67	288.20±68.75	280.40±64.28	352.80±38.19	375.00±35.83	346.80±47.11	262.20±60.35	304.60±37.71	*	NS	NS
BUN	5.33±0.58	5.50±0.71	6.50±0.71	6.00±0.00	5.00±0.00	6.40±0.55	6.00±1.41	7.00±0.00	*	NS	NS
T-Bil	0.46±0.19	29.40±24.29	0.63±0.21	0.32±0.08	0.40±0.08	0.46±0.11	0.40±0.10	0.47±0.06	NS	NS	NS
GOT	36.75±20.65	45.75±10.63	52.80±28.31	80.80±16.50	88.00±29.78	92.00±27.40	123.50±27.31	112.75±53.66	*	NS	NS
GPT	18.00±8.49	23.25±3.40	23.00±2.83	24.40±3.97	28.00±1.41	25.33±4.73	30.00±1.41	26.50±0.71	NS	*	*

[1] Values are results of two way ANOVA analysis. Data with * are significantly different ($P<0.05$) and NS is expressed as not significantly different ($P>0.05$)



Matrix A: High antioxidant potential and low reactive oxygen metabolites (highly preferred)
Matrix B: High antioxidant potential and high reactive oxygen metabolites (acceptable)
Matrix C: Low antioxidant potential and low reactive oxygen metabolites (acceptable)
Matrix D: Low antioxidant potential and high reactive oxygen metabolites (not acceptable)

Fig. 11. BAP and d-ROMs in red sea bream *Pagrus major* blood fed with different diets

5.5 Discussion

Knowledge of rearing red sea bream using oxidized oil supplemented with palm oil is still scarce. Generally, feeding the oxidized oil to fish could caused oxidative stress to the liver as indicated by increased concentration of lipid peroxidation (Brandsch *et al.,* 2004). Results demonstrated that feeding fish with oxidized oil with inclusion of maximum 40% palm oil showed excellent growth with specific growth rate (SGR) or more than 4.0%. Tocher *et al* (2003) also reported that SGR varying between 2.9 and 3.7 in sea bream and turbot fed with oxidized oil showed good SGR. Inclusion of palm oil as replacement for either non oxidized or oxidized oils has a slight stimulatory effect on fish growth. Importantly, it is noteworthy to express that inclusion of palm oil at more than 40% reduced EPA and DHA contents in diets which contributed to the poor growth in Ox4F6P. The use of oxidised oil in diet Ox4F6P therefore produced fish with very low content of n-3 PUFA (Estévez *et al.,* 1997). However, the finding that growth performances by fish fed the oxidized oil in Ox4F6P did not differ much from fish fed with oxidized oil is an indicator that fish growth was not seriously subjected to lipid peroxidation.

Effects of good growth performance above by most of the treatments may be due to the equalization of their fatty acid composition. Fatty acids (FA) in diets are generally responsible on the acceptable growth trend as mentioned above. However, replacing fish oil by feeding palm oil blended with oxidized oil to fish reduced EPA and DHA contents in dorsal and ventral muscles and this conforms to Ghioni *et al* (1999) study on turbot, Mourente and Bell (2006) on European seabass, Bell *et al* (2002) on Atlantic salmon and Ng *et al* (2003) on African catfish. Gradient reduction of fatty acid compositions particularly DHA and EPA in both diet and muscles suggest inherent complexity due to interactions between various fatty acids and catabolism mechanism (Robin and Skalli 2006). Stubhaug *et al* (2005) also observed similar complexity in Atlantic salmon fed with rapeseed oil.

In addition to that, FA from these diets is also responsible in rancidity that leads to lipid peroxidation. In this study, total PUFA (% of total fatty acids) in diets gradually decreased in composition with incremental palm oil in both non oxidized or oxidized oils treatments. However, actual amounts of unsaturated fatty acids in diets were observed to decline more in non oxidized treatments but less in oxidized oil blended with palm oil. These differences may be due to the complex mixture of FA from fish oil and palm oil blend used in the diets as suggested by Sargent *et al* (1999).

Effects of dietary palm oil can also be observed in liver. Dietary with 60% palm oil inclusion in non oxidized oil increased HSI. Similar pattern is also observed in fish fed diets using oxidized oil suggesting liver dysfunction. Excessive palmitic (C16:0) and linoleic acid (C18:2n-6) as shown in diets, muscles and liver due to incremental palm oil may have interrupted the β-oxidation metabolism. In addition, red sea bream may also have lower ability to synthesize these fatty acids *de novo* from palm-oil based diet. Izquierdo *et al* (2005) has reported similar observation on gilthead sea bream and Regost *et al* (2003) on turbot regarding inability of these fish in synthesizing C18:2n-6 into C20:4n-6. As this may be species related, more research need to be undertaken to determine relationship of oxidized oil and palm oil blend to β-oxidation metabolism.

Increase in blood urea nitrogen (BUN), glutamic oxaloacetic transaminase (GOT) and Glutamate pyruvate transaminase (GPT)) with incremental palm oil are evidences that inclusion of palm oil may trigger some catabolism mechanism which conforms to Robin and Skalli (2006) study on European sea bass. Fish fed with highest fish oil inclusion also suggest that fish oil is essential in Hb multiplication. Although mechanism of palm oil inclusion on fish health particularly in blood chemistry are not unexplainable, result may suggests that inclusion of palm oil at a certain level may be detrimental to fish health due to Hb degradation.

Results show that TBARS corroborates with actual content of PUFA in diets, dorsal and

ventral muscles and also in liver. TBARS which is associated as a major indicator of oxidative stress declines with decrease in PUFA. This observation suggests that leverage of oxidation is according to amount of PUFA available in diets which agrees to Brody (1999) and Brember (2002) suggestions that rancidity is formed from oxidation particularly in unsaturated "double bonds" sourced from aquafeeds as observed by Raatikainen *et al* (2005). Nevertheless, an increase in TBARS reflects the fact that levels of PUFA seem to be the main factor affecting lipid oxidation in fish. Lipid deposition may also contribute to levels of PUFA. As muscle lipid is susceptible to oxidation (Zhong *et al.,* 2007), higher deposition in ventral muscle compared to dorsal muscles in this study suggests increased level of PUFA. In this study, diets used are considerably rich in fats due to the total lipid amounting about 18% fish oil which conditioned fish to be more susceptible to lipid oxidation (Alvarez *et al.,* 1998). However, further studies are required to investigate the relationship between oxidized oils, PUFA synthesis and deposition in fish.

Besides TBARS, oxidative stress occurrence in fish can be assessed by using BAP and d-ROMS parameters. According to Pasquini *et al* (2008), BAP is a test to evaluate the plasma antioxidant biological potential as the capacity of the plasma sample to reduce ferric ions to ferrous ions. This test determines the ability of fish in hindering reactivity to free radicals and other scavengers. On the other hand, d-ROMs test provides a measure of the whole oxidant capacity of plasma against the N,N-diethylparapehenylendiamine in acidic buffer and determines markers and amplifiers of free radicals induced oxidative damage. Both parameters are required to distinguish the ability of fish "real time" in whether oxidative stress depends on an increased production of free radicals and/or a decreased efficacy to oxidative stress. As indicated in Fig.11, BAP and d-ROMs showed the equilibrium balance of each treatment in respective matrix groups. In this study, results showed that treatments 6F4P and 5F5P demonstrated the highest antioxidant potentials with lowest level of oxidative stress as

compared to control. The equilibrium of BAP and d-ROMs positioned both of two treatments in matrix A which is considered as the best position for high biological anti-oxidant potentials in counteracting to oxidative stress level. The control which consists of 10F was positioned to matrix C while Ox5F5P and 4F6P in matrix B. Both matrix demonstrated reverse levels of concentration of both BAP and d-ROMs from one another; however both matrix showed acceptable level of potentials against oxidative stress although degradation occured in either BAP or d-ROMs respectively. Treatments using oxidized fish oils except Ox5F5P demonstrated degrading conditions (matrix D) with lowest antioxidant potentials with highest exposure to oxidative stress. As this is the first report that measured the range of d-ROMs and BAP on cultured fish fed with non oxidized and oxidized fish oils, it showed evidence that there is a strong difference of antioxidant potentials to oxidative stress in fish. These tests had been successfully validated in humans, pig (Ballerini *et al*., 2003), rabbit (Oriani *et al*., 2001) and dog (Pasquini *et al*., 2008) whereby results showed that both BAP and d-ROMS expressed pattern of antioxidants *in vivo* related to oxidative stress. Apparently, there is no information available on either utilization of oxidized fish oils and inclusion of palm oil as replacement of fish oil in fish. However, results indicated a positive trend that inclusion of palm oil up to 50% maximum may be able to synergize with non oxidized fish oil and developed encouraging antioxidant potentials to oxidative stress except with oxidized fish oil. Positioning of each respective treatment in different matrix may also be attributed from content of PUFA. High content of PUFA particularly unsaturated "double bonds" in control (10F) suggest possibility of a more rapid oxidation rate than others. This may induced the fish in developing a decrease efficacy to antioxidant potentials. In comparison, non oxidized fish oil with palm oil inclusion demonstrated a better equilibrium of fish ability to counteract on oxidative stress.

In conclusion, the replacement of oxidized fish oil using palm oil resulted in an acceptable growth performance at limited level, a decrease in lipid oxidation in both dorsal and ventral

muscles and high survival rate for fish. Therefore, the incorporation of palm oil not more than 40% in both non oxidized and oxidized oils is promising, although more research is needed.

Chapter 6

Effect of vegetable oil on physico-chemical properties in red sea bream *Pagrus major*

Study VI:

Efficacy of using vegetable oils on physico-chemical properties and fatty acid composition in red sea bream *Pagrus major*

Abstract

This study was aimed to investigate the effects of replacing fish oil with palm oil (P) and soya oil (S) blended with fish oil (F) as the lipid source on growth performance and lipid profiles of red sea bream. A120-days feeding trial for red sea bream (initial body weight 200±0.2g) was conducted in triplicates using 5 isocaloric diets containing different ratio of fish oil:palm oil and fish oil:soya oil such as {10:0 (10F0P), 7:3 (7F3P), 6:4 (6F4P), 7:3 (7F3S) and 6:4 (6F4S). Protein, moisture, ash and lipid contents in both whole body, dorsal and muscle including liver were also investigated. All specimens were slaughtered by hypothermia, divided into dorsal and ventral fillets and maintained under 0°C for 72 hours wrapped in aluminium foil. Monitoring of k-value, pH and muscle rheology was conducted by using the fillets at 18-hour intervals for 72 hours and sensory analysis were conducted by using samples prepared for sashimi. Deterioration rate of sashimi was faster in dietary 7F3P. Textures of fish fed with fish oil blended with vegetable oil were tougher. WHC and All samples deteriorated at 48h. K-value changed significantly at 36h onwards while sensory scores on sashimi observed no significant differences of these samples prepared from all treatments. Replacing fish oil by either feeding palm oil or soya oil to fish reduced EPA and DHA and also n-3/n-6 ratio contents in muscles. EPA and DHA were lowest at 40% inclusion of both palm oil and soya oil in muscles. As all test diets used in this study met the 0.5% DHA with lower EPA with n-3 HUFA in the test diets of this study ranging from 1.39 to 1.96%.

It can be concluded that sashimi of fish fed with diets containing not more than 40% palm oil or soya oil blended with 60% fish oil are consumable within 24hrs without deterioration of k-value, high pH and high WHC and contained acceptable beneficial DHA and EPA.

Keywords: Red Sea bream, k-value, rheological analysis, vegetable oil

6.1 Introduction

Red sea bream, *Pagrus major*, one of Japan's most cultured marine species usually served as a sashimi cuisine. Classified as a healthy fish-based cuisine, it is necessary for sashimi dealers to maintain sashimi´s freshness especially those obtained from cultured red sea bream as measured by (Ólafsdóttir *et al.,* 1997). Sashimi produced from cultured red sea bream may perish easily and requires proper handling so that it can meet quality standards acceptable by consumers. Fish quality parameters like freshness, sensory properties (flavor, smell and taste), water-holding capacity and texture are normally used to evaluate acceptability of fish and fish products ensuring quality assurance during processing (Cardello *et al.,* 1982) while fish texture is mainly determined by water holding capacity in fish muscle. Numerous studies have been conducted to evaluate variations in quality attributes between wild and cultured fish and also among cultured fish.

However, no information exists on the variations in physico-chemical properties of sashimi produced from fish fed with various vegetable oil source blended with fish oil. Therefore, the objective of this study was therefore to compare the physico-chemical variations in the sashimi of red sea bream fed with fish oil blended with either palm oil or soya oil at different level. Specifically, the study aimed to investigate quality attributes in sashimi of red sea bream based on k-value, ph, water-holding capacity and texture properties in relation to storage time, proximate composition, fatty acid composition and to relate the physico-chemical variations to autolysis mechanisms slaughter.

6.2 Materials and methods

6.2.1 Experimental fishes and feeding protocols

The dietary trial was carried out at Marine Finfish Hatchery, Kagoshima University. Red sea bream juveniles with mean weight of 206.9±3.3g were obtained from a commercial hatchery, Matsumoto Suisan, Miyazaki Prefecture, transported alive in HDPE tank aerated with oxygen and acclimatized in indoor rearing tanks a week prior to experiment. Fishes were fed with commercial pellet (Higashimaru Foods, Kagoshima, Japan). Five fishes were distributed equally in 10 units of 200-L flow-thru tanks in duplicates. Five experimental diets (Table 26) with different ratio of fish oil: palm oil and fish oil: soya oil were fed manually at feeding ration (08:00 and 16:00) to fish until near satiation. Water in tanks was maintained at 18±1.5 ºC for a total period of 120 days. Fishes were also exposed to a photoperiod condition of 12 L/12 D, water flow (filtered seawater) maintained at 2.0 L/min. Fish sampling was done in every 10 days interval where weight was expressed as bulk weight (g) of all fishes in each respective tank.

At end of experiment, fishes were killed using hypothermia method, by immersion into slurry-ice cold marine water prepared and maintained at 3 ppt and 0°C according to method adapted from Losada *et al* (2005); the slurry ice mixture was renewed every 30 minutes to maintain salinity. Blood was collected from the caudal vein by puncturing caudal peduncle using heparinised syringe and fresh blood stored in ice until further blood chemistry analysis was performed.

Livers were also removed, pooled and stored at -80°C until further analysis was performed. Samples were then transferred in insulated polystyrene box with water drainage to the Laboratory of Aquatic Animal Nutrition, weighted, eviscerated and filleted within 6 hrs after

slaughter. Filleting was conducted on edible muscles according to Japanese standards and specimens were pooled, weighted and maintained in ice. The fillets were wrapped in aluminum foil and chilled in ice at 0°C during the analysis. Ice was changed at every 6 hr until 72 hr. Other fillets were freeze-dried and used for proximate analysis.

Table 26. Basal ratio of experimental diets containing different levels of palm oil and soya oil

Ingredients (g/kg DM)	Diets				
	10F0P0S	7F3P	6F4P	7F3S	6F4S
Brown fish meal[1]	670.0	670.0	670.0	670.0	670.0
Activated gluten	80.0	80.0	80.0	80.0	80.0
α-Starch	40.0	40.0	40.0	40.0	40.0
Dextrin	40.0	40.0	40.0	40.0	40.0
Fish oil	100.0	70.0	60.0	70.0	60.0
Palm oil	0.0	30.0	40.0		
Soya oil				30.0	40.0
Mineral mix[2]	30.0	30.0	30.0	30.0	30.0
Vitamin mix[3]	26.5	26.5	26.5	26.5	26.5
Stay-C[4]	3.5	3.5	3.5	3.5	3.5
α-Cellulose	10.0	10.0	10.0	10.0	10.0
Total	1000	1000	1000	1000	1000
Analytical contents (dry matter basis)					
Crude protein (%)	47.7	49.6	49.6	47.3	48.8
Total lipid (%)	17.5	17.1	10.2	9.4	8.8
Ash (%)	14.3	14.4	16.4	15.4	16.9
Gross energy (kcal/g diet)	6.5	6.5	6.3	6.3	6.3

[1] Defatted brown fish meal
[2] Mineral mix (g/kg): NaCl, 0.183; $MgSO_4 \cdot 7H_20$, 0.685; $NaH_2PO_4 \cdot 2H_2O$, 0.436; KH_2PO_4, 1.199; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 0.679;Fe Citrate, 0.148; Ca Lactate, 1.635; $AlCl_3 \cdot 6H_2O$, 0.00009, $ZnSO_4 \cdot 7H_2O$, 0.017;$CuCl_2$, 0.00005; $MnSO_4 \cdot 4H_2O$, 0.004;KCl, 0.008; $CoCl_2$, 0.005
[3] Vitamin mix (g/kg): p-aminobenzoic acid, 1.45; biotin, 0.02; myo-inositol, 14.5; nicotinic acid, 2.9; folic acid, 0.05; choline chloride, 29.65
[4] Stay-C: L-Ascorbyl-2-monophosphate-Na/Ca (DSM Nutrition Japan K. K.)

6.2.2 Physico-chemical properties

6.2.2.1 pH measurement

Monitoring of physico-chemical properties (pH, k-value and muscle rheology) of the edible

fillets was conducted at 18 hr-intervals for a period of 72 hr. The pH measurements were conducted by insertion of an electronic pH meter probe (IQ150 pH meter) into the muscles at 18 hr intervals over a 72 hr period.

6.2.2.2 k-value

White muscles (3-4 g) were digested with 5ml of 10% trichloroacetic acid (TCA), supernatant was collected, filtered through a filter paper (Advantec no.1, Toyo Roshi Co, Ltd., Tokyo, Japan) and neutralized using 10M KOH and collected as filtrate 1 (S1). Filtrate 2 (S2) was prepared by mixing 50ml S1 with 50ml Alkaline phosphatase (P) and incubated in water bath (37°C) for 20min. Prior to injection of samples, the Super Freshness Meter reaction cell was prepared by filling 1ml of air saturated 0.1M PBS (Phosphate buffer saline) into reaction cell; by injecting 10ml S1 (before digestion, total ATP), 10ml E_0 (Xanthine oxidase + Nucleoside phosphorylase) and 20ml S2 (S1+Alkaline phosphatase = dephosphorylated ATP). The k-value was then measured using freshness meter (model KV-202, Central Kagaku Corp., Tokyo, Japan). Calculation of k-value is as follows:

k-value=100*(Inosine+Hypoxanthine)/(ATP+ADP+AMP+IMP+Inosine+Hypoxanthine)

The quality deterioration ratio (QDR) was calculated using the formula QDR=K/H, where K is the k-value of fillet and H is the total storage period after slaughter.

6.2.2.3 Water-holding capacity (WHC)

Water-holding capacity of fillet samples was measured according to Gomez-Guillen *et al* (2000). Five grams of sample and a filter paper (Advantec no.1, Toyo Roshi Co, Ltd., Tokyo, Japan) were placed into a centrifuge tube (15 ml), and those were centrifuged (7000 rpm, 10ºC, 10 min) and then the filter paper containing aqueous fatty fraction were weighed. Percentage of

water loss (WL) from the samples was calculated as follows:

WL (%)=100*(V_2-V_1)/Ws, where V_1 is the weight of filter paper (g), V_2 is the weight of filter paper containing aqueous fatty fraction (g) and Ws is the sample weight (g).

The wet filter paper was then dried to a constant weight at 50ºC in an oven, and percentage of fat loss (FL) was calculated as FL (%)=100*(V_3-V_1)/Ws, where V_1 is the weight of filter paper (g), V_3 is the constant weight of filter paper (g) and Ws is the sample weight (g)

6.2.3 Rheological (Texture profiles) analysis

Texture profiles of the fish fillets were measured on a Rheoner II Creep (model RE-2-33005B) consisting of four components: a detector (model RE2-33005B-2), measurement meter (model REZ-OP18-01), a cooler (model ETC-3305-1) and a computer auto-analyzer (Model CA-3305) installed with Texture Analysis Program (TAP ver.1.2-a). Samples were obtained from fillet according to Fig. 1. The temperature of the fresh samples was maintained at 4ºC throughout the analysis and the measurements were set in the central part of the fillet. Analyzed parameters included maximum hardness force and adhesiveness of samples for a period of 72hrs. Maximum hardness force is expressed as (H) Maximum height of force in first plunge (Newton) and Adhesiveness is the Total area of A^3 (J/m^3).

6.2.4 Sensory Analysis

In the sensory analysis, the 500 g fillets from all treatments were sliced to obtain uniform sized sashimi of about 30-mm thickness. The slices were hygienically wrapped in aluminum foil and refrigerated at 4ºC for 30 min before served to non-trained panelists comprising of 17

students from the Faculty of Fisheries, Kagoshima University. Sensory evaluation was conducted in a 25ºC air-conditioned room. All panelists were lined 1m apart and asked to taste and assess the sashimi slices according to odor, texture, freshness and taste scores based on method adapted from Amerine *et al* (1965). The scores were then graded as excellent(10), very good(9), good(7), medium(5), poor(3), very poor(1) and not acceptable(0).

6.2.5 Fatty acid composition analysis

Fatty acid composition analysis was performed based on the study of Querijero *et al.,* (1997) with slight modifications on homogenized samples of dorsal fillet, ventral fillet and liver. Total lipid (TL) was extracted by homogenizing 2 g sample according to Bligh and Dyer (1959). Fatty acid esters (FAME) were then produced from total lipids aliquots. Fatty acids were methylated with BF_3 in methanol. Methyl tricosanoate (Nu-Chek Prep. Inc) was used as internal standard at 1.000mg/ml Hexane. Fatty acid methyl esters (FAME) was analyzed with a gas chromatograph (Shimadzu GC 17A) with flame ionization detector temperature maintained at 260˚C; carrier N^2 gas at 1ml/min; column temperature at 200°C; injector temperature at 250°C and Helium (He) served as the carrier gas. 0.1µl sample including 0.1µl air was manually injected into injection port and identified fatty acids were presented as area percentage of total fatty acids.

6.2.6 Proximate analysis

Homogenized samples of muscle as well as the liver were analyzed in duplicates for protein, lipid, ash, and moisture. Protein determination was conducted according to the AOAC method (1990). Lipid content was analyzed according to the method adapted from Bligh & Dyer (1959), while ash content was obtained by combustion in muffle furnace at 550ºC. Moisture, on a 5 g sample of minced fillet muscle, was determined by oven-drying at 110ºC to constant weight.

6.3 Statistic analysis

The statistical analysis was performed using ANOVA. Data was expressed as means $\pm$ S.E. Homogeneity of variance between treatments ($P<0.05$) were analysed using one-way Analysis of Variance (ANOVA) while significance of differences were determined using Tukey-Test. Effects of dietary treatments were considered significant at $P<0.05$. In Study V, texture was analyzed using Kruskal-Wallis (SPSS) to test one-way analysis of variance by ranks. This was followed by Mann-Whitney *U* test to assess distribution of two samples.

6.4 Results

6.4.1 Physico-chemical properties; pH, k-value, and k-value QDR of fillet samples

The pH of fillet samples shown in Fig. 11 demonstrated that there were significant differences of pH among samples except at 18 hr. At 18 hr, results skewed and dropped drastically to less than 5.4. There were significant differences from 36 hr to 72 hr for all groups. pH were significantly higher in 7F3P muscle fillets followed with 10F0P0S compared to other treatments at 36 hr to 54 hr. pH declined significantly for all samples at 72 hr with highest pH in treatment 7F3S and followed with treatments 10F0P0S, 6F4P and 6F4S while 7F3P indicated significantly lowest pH among treatments.

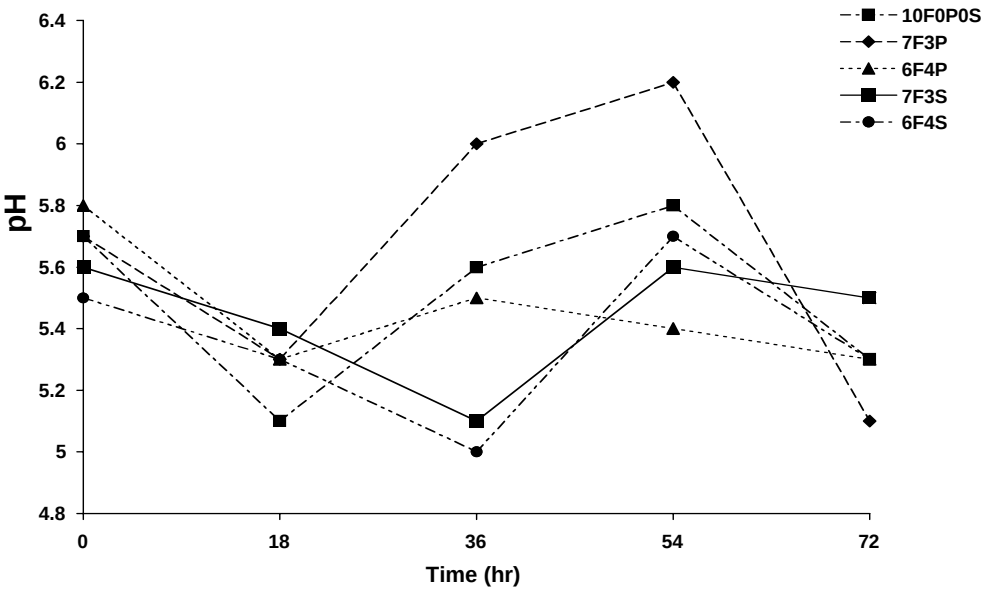


Fig. 12. Changes of pH according to time series for muscle fillets of cultured red sea bream

The k-value in fish samples during cold storage (0ºC) for a total period time of 72 hr are presented in Fig. 12. There were no significant differences of k-value among treatments at 0 hr and 18 hr which ranged from 3.4 to 3.9%. k-value began to increase from 18 hr onwards for all treatments. Treatments 7F3P and 7F3S indicated significant higher k-value than others at 36 hr and 54 hr while 10F0P0S, 6F4P and 6F4S showed significantly lower value. At 72 hr, treatments 6F4P and 7F3P showed significantly higher k-value than 6F4S.

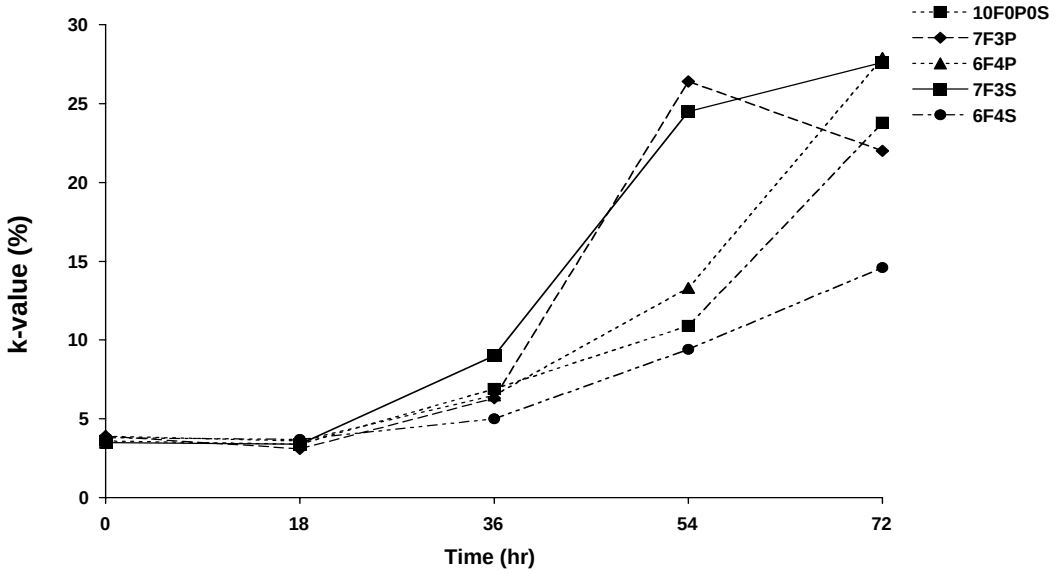


Fig. 13. Changes of k-value according to time series for muscle fillets of cultured red sea bream

In terms of k-value QDR (Fig. 13), deterioration ratio for all treatments increased with time. At 0 hr, 6F4P and 6F4S showed significantly highest deterioration ratio while lowest ratio was shown in 7F3P. Treatments 10F0P0S, 7F3P and 6F4P remained plateau at 36 hr, while 7F3S showed highest QDR and lowest in 6F4S. At 54 hr, 7F3P (0.49%) and 7F3S (0.45%) showed significantly higher QDR than other treatments. Ratio increased in 10F0P0S, 6F4P and 6F4S respectively at 72 hr while 7F3P and 7F3S decreased.

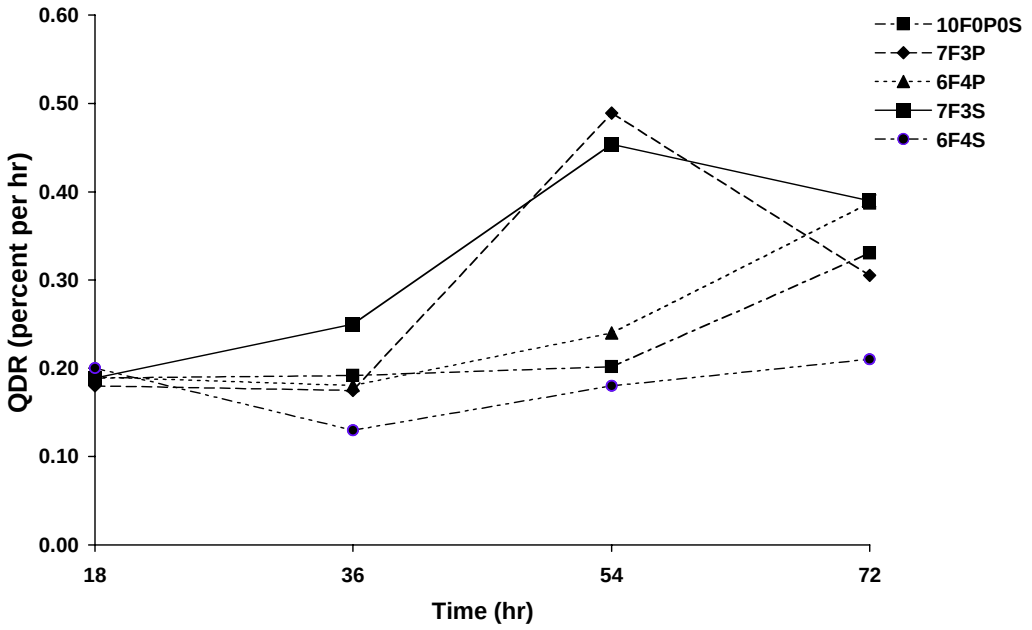


Fig. 14. Changes of quality deterioration ratio according to time series in muscle fillets of cultured red sea bream

6.4.2 Water-holding capacity (WHC)

WHC was represented by water loss (WL) and fat loss (FL) in muscle samples are illustrated in Fig. 14. The pattern of WL among treatments differed in terms of trend and time. WL value of all samples at 0 hr ranged from 11.5% to 17.8%. Treatment 6F4P indicated a gradual increase in WL while other treatments showed irregular pattern at each respective hours. No significant differences of WL were detected among treatments at 0 hr, 18 hr and 54 hr. At 36 hr, all samples except 6F4S showed significantly higher WL while 6F4P and 6F4S both showed higher WL at 72 hr. Comparatively, FL in all treatments did not fluctuate much as compared to WL according to time. However, significant differences were found among treatments at all sampling hours except at 18 hr. Treatment 6F4P consistently showed highest FL from 0 hr to 72 hr.

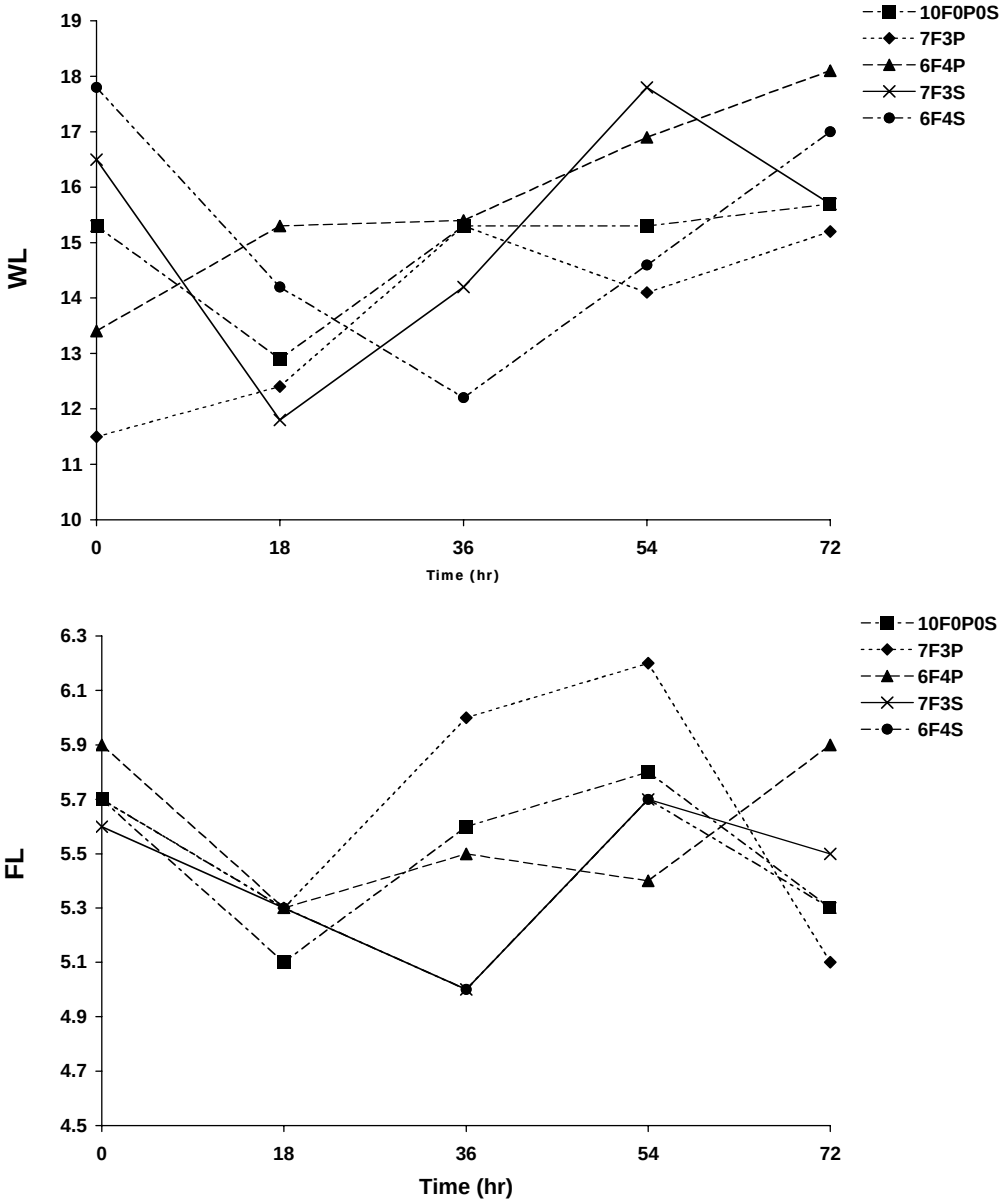


Fig. 15. Changes of water holding capacity according to time series in muscle fillets of cultured red sea bream

6.4.3 Rheological (Texture profiles) analysis

Fig. 16-20 illustrated maximum force (N), maximum hardness force (N), brittleness force (N), cohesiveness (N) and adhesiveness in cultured fish respectively. In Fig.16, significant differences were observed between all the treatments at 36 hr and 54 hr. At 36 hr, treatment

7F3P showed higher maximum force than 10F0P0S and 6F4P while 6F4P was lower than 7F3S and 6F4S. Maximum force at 54 hr observed that 10F0P0S, 7F3P and 7F3S required lower force than 6F4P.

Maximum hardness force (Fig.17) differed significantly at 18 hr, 36 hr and 54 hr for all treatments. Maximum hardness force at 18 hr demonstrated that 7F3S was significantly higher than 10F and 7F3P. At 36 hr, 7F3P was significantly higher than 6F4P; 7F3S higher than 6F4P and 6F4P higher than 6F4S respectively. In comparison, 6F4P shower higher maximum force than 7F3P and 7F3S while 6F4P higher than 6F4S.

Significant differences of brittleness force were observed among 10F0P0S, 7F3P and 6F4S at 36 hr (Fig.18). 6F4S demonstrated significantly higher brittleness force than 10F0P0S and 7F3P.

In comparison, results showed that cohesiveness (Fig.19) among treatments differed significantly at 54 hr. 6F4P was significantly less cohesive than 10F0P0S and 7F3P while 7F3P was more cohesive than 7F2S and 6F4S.

In adhesiveness criterion, all treatments differed at 0 hr and 18 hr (Fig. 20). 10F0P0S was significantly less adhesive than 7F3P and 7F3S at 0 hr while differed with 6F4P at both 0 hr and 18 hr. In comparison, 7F3P was significantly less adhesive than 7F3S at 0 hr. This treatment was also significantly less adhesive than 6F4P and 6F4S at 18 hr.

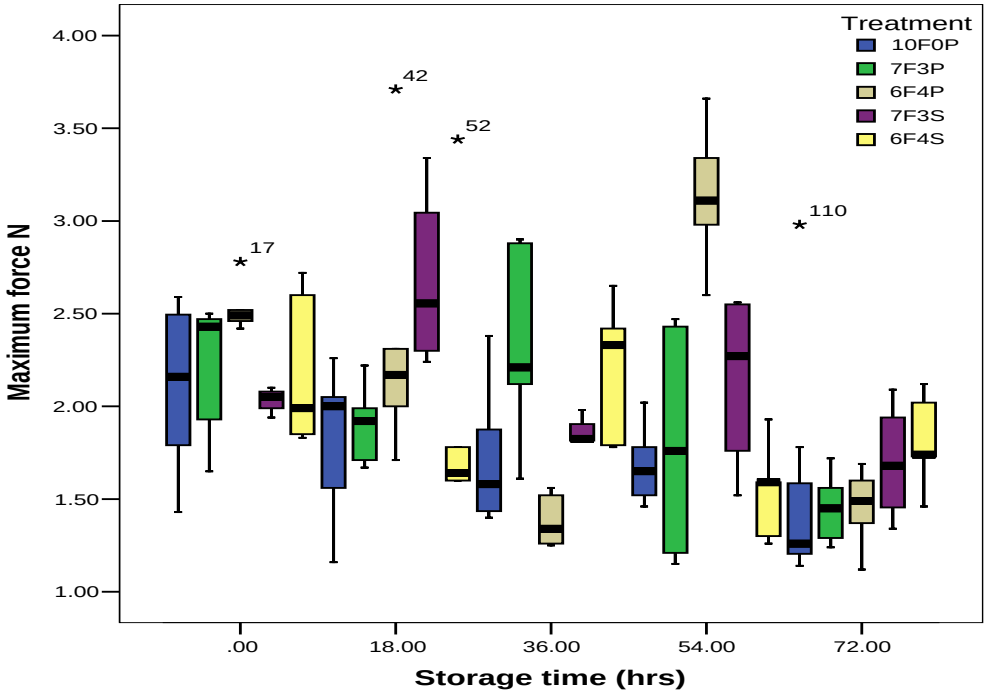


Fig. 16. Maximum force pattern in muscle fillets of cultured red sea bream

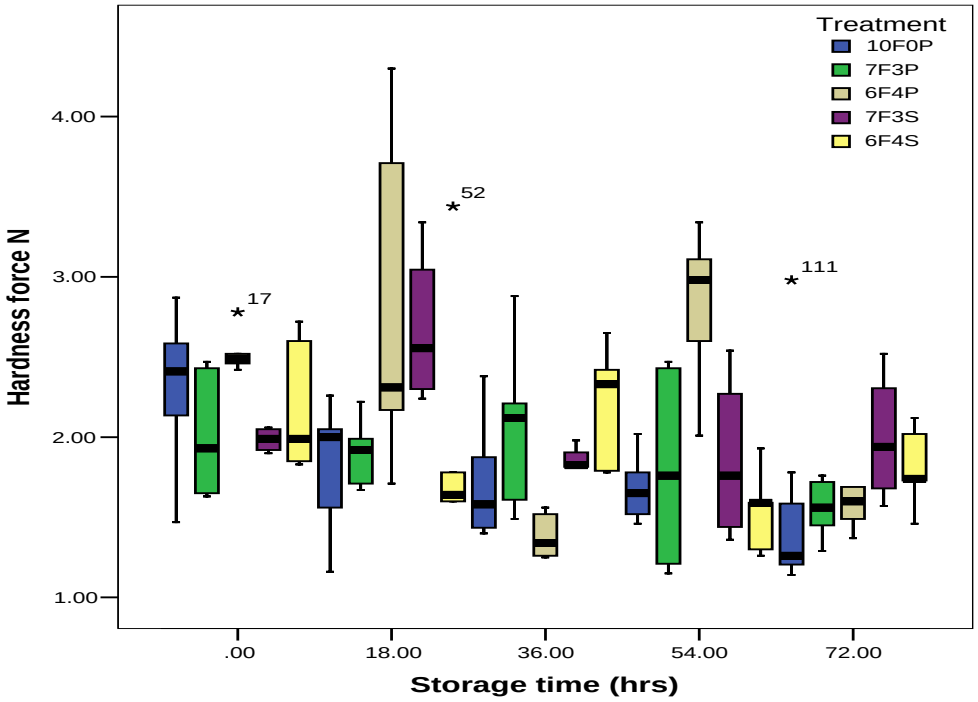


Fig. 17. Maximum hardness force pattern in muscle fillets of cultured red sea bream

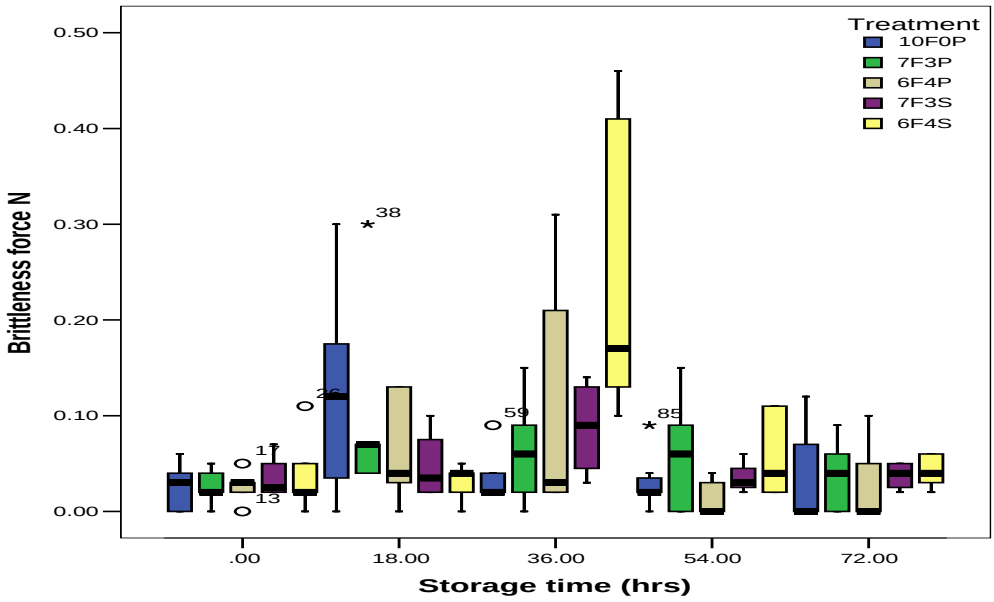


Fig. 18. Brittleness force pattern in muscle fillets of cultured red sea bream

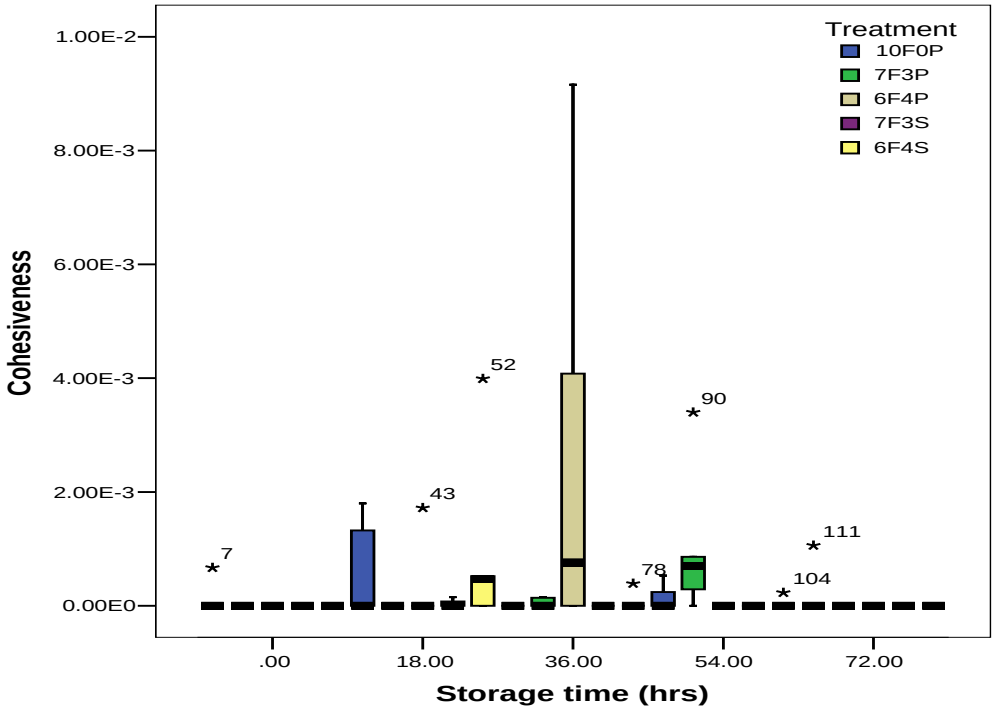


Fig. 19. Cohesiveness pattern in muscle fillets of cultured red sea bream

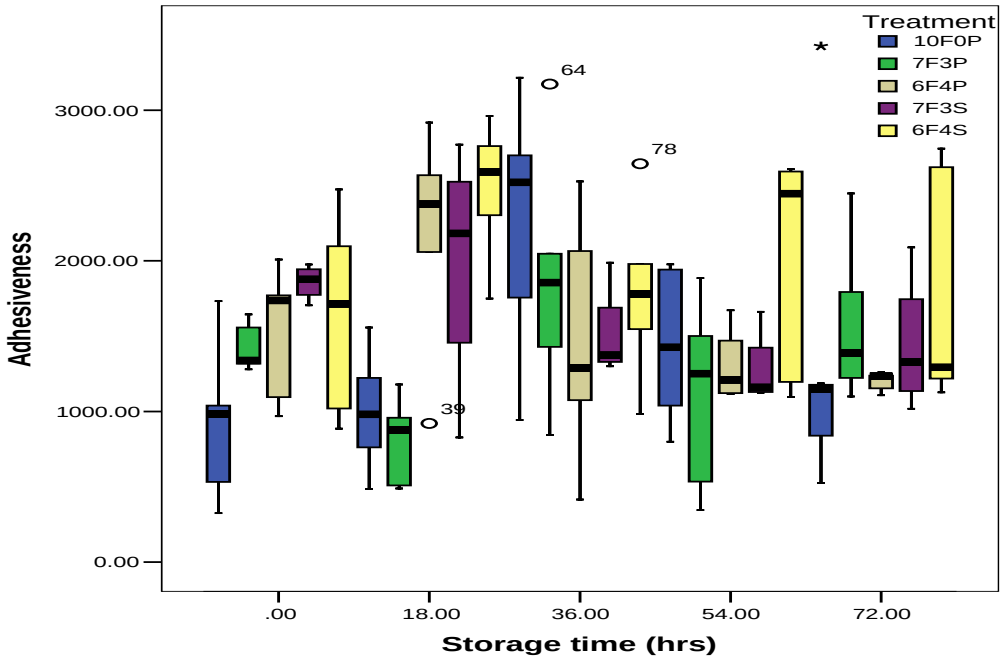


Fig. 20. Adhesiveness pattern in muscle fillets of cultured red sea bream

6.4.4 Sensory Analysis

The sensory scores of cultured red sea bream were demonstrated in Fig. 21. Freshness, taste, odor and texture scores were good in average and there were insignificant differences ($P>0.05$) among all treatments. Results indicated quality of both specimens was well accepted by panelists whereby they were not able to distinguish differences of sashimi in terms of freshness, taste, odor and texture obtained from fish fed with either palm oil or soya oil blended with fish oil at different ratio.

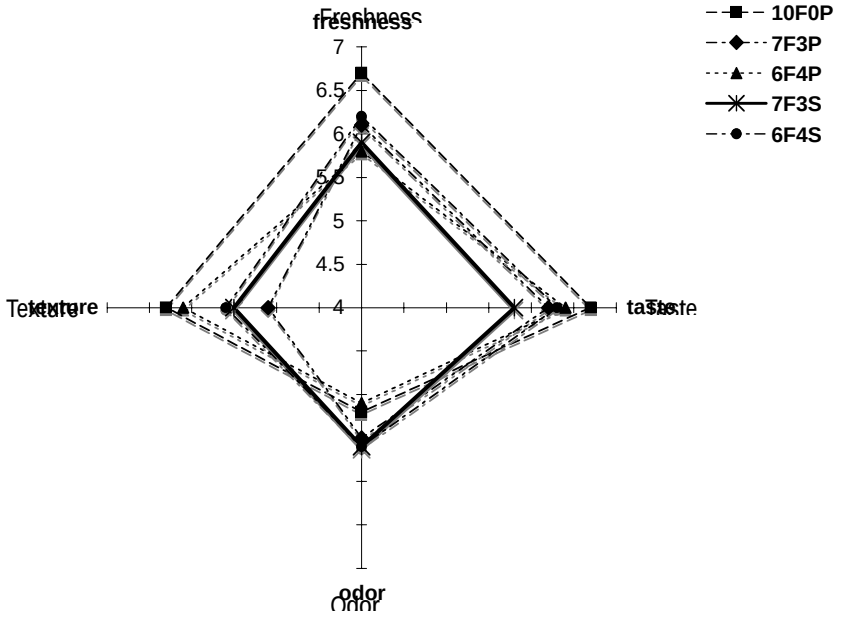


Fig. 21. Sensory analysis of cultured red sea bream sashimi

6.4.5 Fatty acid composition

Dietary profiles of fatty acid composition (Table 27) showed dominance of saturated, monounsaturated fatty acids (MONOs) and total poly-unsaturated fatty acids (PUFAs), ranging from 14.5% to 29.2% , 24.0% to 28.6% and 15.6% to 22.4% respectively. Higher inclusion of palm oil and soya oil increased concentration of saturates. Monoenes particularly C18:1n-9 too increased with inclusion of palm and soya oil. Incremental palm and soya oil decreased C20:4n-3, C20:5n-3 and C22:6n-3 respectively which decreased total PUFA and total n-3 HUFA.

Saturates, total PUFAs and monoenes were more prominent in muscle (Table 28) and and liver (Table 20). Dietary elevated palm oil and soya oil gradually increased n-6 and decreased total n-3 particularly EPA and DHA concentrations in muscles but not in dietary palm oil in liver.

Table 27. Fatty acid composition (% of total fatty acid) in experimental diets

Types of fatty acid[1]	Diets				
	10F0P0S	7F2P	6F4P	7F3S	6F4S
14:0	4.8	4.1	3.7	3.4	3.6
16:0	15.9	20.1	20.7	14.4	15.0
18:0	3.7	4.9	4.8	4.8	4.9
∑Saturated	24.4	29.1	29.2	22.6	23.5
18:1n-9	15.0	18.5	18.3	15.5	16.3
20:1n-9	6.5	4.3	3.8	4.6	3.8
22:1n-7	6.9	4.4	3.9	4.3	3.8
22:1n-9	0.2	0.2	0.1	0.1	0.2
∑Monoenes	28.6	27.4	26.1	24.5	24.1
18:2n-6	2.4	3.5	3.6	4.6	3.8
20:4n-6	1.0	0.8	0.8	0.8	0.8
22:4n-6	5.3	6.2	6.2	6.8	6.1
∑n-6fatty acids	8.7	10.5	10.6	12.2	10.7
18:3n-3	0.9	0.8	0.8	0.6	0.5
18:4n-3	1.9	1.3	1.2	1.4	1.3
20:4n-3	0.5	0.3	0.4	0.4	0.3
20:5n-3	9.5	7.0	6.7	7.0	6.5
22:5n-3	1.3	1.0	1.1	1.0	0.9
22:6n-3	8.3	6.6	6.4	6.6	6.1
∑n-3 fatty acids	22.4	17.0	16.6	17.0	15.6
∑PUFA[2]	31.1	27.5	27.2	29.2	26.3
∑n-3HUFA[3]	19.6	14.9	14.6	15.0	13.8
∑n-3/n-6 ratio[4]	2.6	1.6	1.6	1.4	1.4
∑EPA+DHA[5]	17.8	13.6	13.1	13.6	12.6

[1] Values are expressed as means ±S.E (n=2). Same superscripts are not significant different (P > 0.05).

[2] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[3] Total n-3HUFA is expressed as sum of n-3 fatty acids in carbons more than 20

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of Eicosapentaenoic Acid (C20:5n-3) and Docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 28. Fatty acid composition (% of total fatty acid) in muscle of red sea bream *Pagrus major* fed with different diets

Types of fatty acid[1]	muscle				
	10F0P0S	7F3P	6F4P	7F3S	6F4S
14:0	3.4±0.0^{b}	3.1±0.0^{a}	2.9±0.1^{a}	3.1±0.0^{a}	2.9±0.0^{a}
16:0	18.5±0.2^{a}	19.7±0.1^{b}	19.9±0.0^{b}	17.7±0.1^{a}	17.6±0.3^{a}
18:0	4.8±0.0^{a}	4.8±0.0^{a}	5.1.±0.1^{b}	5.1±0.0^{b}	5.2±0.0^{b}
∑saturated	26.7±0.1^{ab}	27.6±0.1^{bc}	27.9±0.1^{c}	25.9±0.1^{a}	25.7±0.4^{a}
16:1n-9	6.6±0.0^{c}	5.9±0.1^{b}	5.6±0.1^{a}	5.8±0.0^{ab}	5.6±0.0^{a}
18:1n-9	19.5±0.1^{a}	22.1±0.4^{d}	21.8±0.2^{cd}	20.3±0.1^{ab}	20.9±0.8^{bc}
20:1n-7	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0
22:1n-7	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
22:1n-9	0.3±0.0^{c}	0.3±0.0^{bc}	0.3±0.0^{ab}	0.2±0.0^{abc}	0.2±0.0^{a}
∑monoenes	26.8±0.7^{a}	28.7±0.4^{c}	27.9±0.1^{bc}	26.6±0.0^{a}	27.1±0.0^{ab}
18:2n-6	2.8±0.0^{a}	3.8±0.0^{b}	4.2±0.0^{c}	4.6±0.1^{d}	5.1±0.1^{c}
20:4n-6	1.1±0.0^{b}	1.1±0.0^{b}	1.0±0.0^{ab}	0.9±0.0^{a}	0.9±0.1^{a}
22:3n-6	0.3±0.0^{b}	0.3±0.0^{ab}	0.3±0.0^{ab}	0.3±0.0^{ab}	0.2±0.0^{a}
22:4n-6	6.3±0.1^{c}	5.0±0.0^{a}	5.8±0.1^{b}	4.9±0.0^{a}	5.2±0.1^{a}
22:5n-6	0.3±0.0^{c}	0.5±0.0^{b}	0.4±0.0^{ab}	0.3±0.0^{ab}	0.3±0.0^{ab}
∑n-6	10.7±0.1^{a}	10.6±0.1^{a}	11.6±0.1^{b}	10.9±0.0^{a}	11.7±0.1^{b}
18:3n-3	0.7±0.0^{a}	0.7±0.0^{a}	0.7±0.0^{a}	1.3±0.0^{b}	1.5±0.0^{c}
18:4n-3	1.0±0.0^{b}	0.9±0.0^{a}	0.9±0.0^{a}	0.9±0.0^{a}	0.9±0.0^{a}
20:4n-3	0.6±0.0^{c}	0.5±0.0^{b}	0.5±0.0^{ab}	0.5±0.0^{ab}	0.5±0.0^{a}
20:5n-3	6.6±0.1^{d}	5.8±0.0^{c}	5.4±0.1^{b}	5.7±0.1^{a}	5.1±0.0^{a}
22:5n-3	2.1±0.0^{b}	2.0±0.1^{ab}	2.0±0.0^{ab}	1.8±0.1^{a}	1.8±0.0^{a}
22:6n-3	10.8±0.0^{c}	10.2±0.3^{c}	9.4±0.4^{bc}	8.3±0.1^{ab}	7.4±0.4^{a}
∑n-3	21.8±0.1^{c}	20.1±0.4^{bc}	18.9±0.5^{ab}	18.5±0.1^{ab}	17.2±0.3^{a}
∑PUFA[3]	32.5±0.0^{c}	30.6±0.4^{b}	30.6±0.4^{b}	29.5±0.1^{ab}	28.9±0.2^{a}
∑n-3/n-6 ratio[4]	2.1±0.1^{d}	1.9±0.0^{cd}	1.7±0.1^{ab}	1.7±0.0^{bc}	1.5±0.1^{a}
∑EPA+DHA[5]	17.4±0.1^{d}	16.1±0.3^{cd}	14.8±0.4	14.0±0.0^{ab}	12.6±0.4^{a}

[1] Values are expressed as means ±S.E (n=2). Same superscripts are not significant different (P > 0.05).

[2] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[3] Total n-3HUFA is expressed as sum of n-3 fatty acids in carbons more than 20

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of Eicosapentaenoic Acid (C20:5n-3) and Docosahexanoic acid (C22:6n-3) as essential fatty acids

Table 29. Fatty acid composition (% of total fatty acid) in liver of red sea bream *Pagrus major* fed with different diets

Types of fatty acid[1]	liver				
	10F0P0S	8F2P	6F4P	4F6P	6F4S
14:0	2.3±0.1^{c}	2.3±0.1^{c}	2.3±0.0^{c}	1.5±0.0^{a}	1.9±0.0^{b}
16:0	17.6±0.0^{b}	18.1±0.0^{b}	20.5±0.2^{c}	15.8±0.1^{a}	16.3±0.2^{a}
18:0	5.9±0.0^{b}	5.4±0.1^{b}	4.5±0.0^{a}	6.7±01^{c}	7.4±0.2^{d}
∑Saturated	25.9±0.2^{c}	25.8±0.1^{b}	27.4±0.2^{c}	24.0±0.4^{a}	25.6±0.4^{b}
16:1n-9	7.4±0.1^{c}	6.4±0.0^{b}	6.7±0.1^{bc}	6.5±0.2^{b}	4.7±0.1^{a}
18:1n-9	25.1±0.4^{bc}	26.1±0.3^{c}	23.6±0.0^{b}	24.5±0.2^{b}	21.6±0.3^{a}
20:1n-7	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0
22:1n-9	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
∑Monoenes	33.0±0.3^{c}	33.1±0.2^{c}	30.8±0.1^{b}	31.5±0.5^{bc}	26.8±0.1^{a}
18:2n-6	1.8±0.0^{a}	3.6±0.1^{b}	4.3±0.1^{c}	7.2±0.0^{d}	7.7±0.2^{d}
20:4n-6	1.3±0.0^{ab}	1.3±0.1^{ab}	1.6±0.0^{c}	1.5±0.1^{bc}	1.2±0.0^{a}
22:3n-6	0.2±0.0^{ab}	0.2±0.0^{b}	0.2±0.0^{b}	0.1±0.0^{a}	0.1±0.0^{a}
22:5n-6	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
∑n-6	3.6±0.0^{a}	5.4±0.0^{b}	6.4±0.1^{c}	9.2±0.1^{d}	9.3±0.3^{d}
18:3n-3	0.2±0.0^{bc}	0.2±0.0^{bc}	0.3±0.0^{c}	0.2±0.0^{ab}	0.1±0.0^{a}
18:4n-3	0.5±0.0^{c}	0.5±0.0^{c}	0.4±0.0^{bc}	0.3±0.0^{a}	0.4±0.0^{ab}
20:4n-3	0.5±0.0^{ab}	0.6±0.0^{b}	0.6±0.0^{ab}	0.5±0.0^{ab}	0.5±0.0^{a}
20:5n-3	5.2±0.1^{b}	5.3±0.0^{b}	5.5±0.1^{b}	4.2±0.0^{a}	4.3±0.1^{a}
22:5n-3	1.7±0.1^{a}	2.1±0.0^{b}	2.1±0.0^{b}	2.0±0.0^{b}	1.6±0.0^{a}
22:6n-3	8.4±0.4^{a}	8.3±0.1^{b}	8.5±0.2^{b}	8.4±0.2^{b}	7.4±0.2^{a}
∑ n-3	16.6±0.1^{c}	17.1±0.1^{cd}	17.5±0.1^{d}	15.6±0.1^{b}	14.3±0.0^{a}
∑PUFA[3]	20.2±0.1^{a}	22.6±0.1^{b}	23.8±0.0^{c}	24.8±0.1^{d}	23.7±0.3^{c}
∑n-3/n-6 ratio[4]	4.7±0.0^{d}	3.2±0.1^{c}	2.8±0.1^{b}	1.7±0.0^{a}	1.6±0.1^{a}
∑EPA+DHA[5]	13.7±0.2^{c}	13.7±0.1^{c}	14.1±0.1^{c}	12.6±0.2^{b}	11.7±0.1^{a}

[1] Values are expressed as means ±S.E (n=2). Same superscripts are not significant different (P > 0.05).

[2] Total PUFA is expressed as sum of total n-3 fatty acids and total n-6 fatty acids

[3] Total n-3HUFA is expressed as sum of n-3 fatty acids in carbons more than 20

[4] n-3/n-6 ratio is expressed as total n-3 PUFA divided by total n-6 PUFA

[5] Sum of Eicosapentaenoic Acid (C20:5n-3) and Docosahexanoic acid (C22:6n-3) as essential fatty acids

6.4.6. Proximate analysis

Proximate composition was presented in Table 30. There were significant differences ($P<0.05$) in crude protein and ash among treatments. Protein content significantly increased in 6F4P, 7F3S and 6F4S while ash was significantly lower in treatments using soya oil.

Moisture observed no significant differences among treatments. Lipid content in muscle showed higher accumulation of lipid with incremental palm oil and soya oil. However, inverse results in liver showed that higher inclusion of both palm oil and soya oil decreased lipid content.

Table 30. Lipid, protein, ash, and moisture contents of muscles and liver in red sea bream *Pagrus major* fed with different diets

Parameters	10F0P	7F2P	6F4P	7F3S	6F4S
Lipid (%)[1]					
Muscle	13.9±0.8^{a}	15.2±0.6^{ab}	20.3±1.5^{cd}	19.1±0.1^{bc}	24.9±0.9^{d}
Liver	10.6±0.1^{c}	10.6±0.1^{c}	9.5±0.3^{b}	9.2±0.0^{b}	7.7±0.1^{a}
Protein (% whole body)[2]	19.2±0.0^{a}	19.1±0.8^{a}	24.9±0.6^{b}	26.6±0.0^{c}	25.5±0.1^{bc}
Ash (% whole body)[3]	5.8±0.0^{b}	5.8±0.1^{b}	5.9±0.0^{b}	5.1±0.1^{a}	4.8±0.0^{a}
Moisture (% whole body)	70.5±0.1	68.1±0.0	69.2±0.2	69.0±0.2	68.7±0.1

[1] Values are expressed as mean ± SE(n=2). Data with same alphabets are not significantly different ($P>0.05$).
[2] Dry weight basis
[3] Wet weight basis

6.5 Discussion

Results from this study affirm potential ability of red sea bream in using both palm oil and soya oil at limited amount to attain sashimi shelf-life. Changes in k-value, pH and water binding capacity by storage time generate high impact to fish shelf-life. Results showed that pH declined significantly 72 hr with highest pH in higher inclusion of fish oil except in 7F3P. Treatment 6F4P indicated a gradual increase in WL while other treatments showed irregular pattern at each respective hours. Although no significant differences of WL were detected among treatments at 0 hr, 18 hr and 54 hr, 6F4P and 6F4S both showed higher WL at 72 hr. In comparison, FL in all treatments did not fluctuate much as compared to WL according to time except 6F4P which observed highest FL from 0 hr to 72 hr. The present study showed that fish fed with not less than 70% fish oil inclusion has better WHC as compared to those fed with 40% palm oil or 40% soya oil. The former treatment group had demonstrated significantly lower water loss. In general, water loss and pH have an inverse relationship (Olsson *et al.,* 2003b). In this present study, pH began to degrade beginning from 36 hr for all treatments and decreased significantly at 72 hr which influenced a pronounced WL which at 72 hr. This observation corresponds to Olsson *et al* (2003b) that liquid loss (LL) increased with decreasing pH lower than 6.3, whereas at higher pH values, LL was independent on pH.

The k-value relates to fish freshness and it emphasizes on degradation of energy metabolites from ATP to hypoxanthine and inosine in fish products and normally demonstrates a positive relationship between degradation of fish quality and storage time. In this study, fish fed with 30% inclusion of either palm oil or soya oil showed higher k-value than others at 36 hr and 54 hr. Both treatments also showed significantly higher QDR than other treatments. This demonstrates that fish fed with minimal 30% vegetable oil inclusion starts accumulating more lipids in its muscles. Due to incremental lipid accumulation, k-value and QDR increased

showing signs of gradient degradation in fish freshness. The k-value of 7F3P and 7F3S exceeded acceptable k-value for "sashimi" at 54 hr whereas slower elevation rate was observed in other treatments including those with highest inclusion of both palm and soya oil. Thus, these elevations may be due to synergism impact between rancidity and autolysis of ATP (Erikson *et al.,* 1997) which resulted undesirable k-value as reported by Aubourg *et al* (2007).

The present results revealed that fish fed with 30% inclusion palm oil and soya oil has tougher muscle structure even compared to 10F0P0S. Tougher or firmness defines good muscle texture in fish, conforming to Dunajski (1979) and Johnston *et al* (2000), in which firmness in fish texture is influenced by high fiber density. Inclusion of palm oil particularly at 30% concentration developed a more cohesive and adhesive muscle structure. This could be related to muscle WHC which enable muscles to retain water longer in structure. Higher lipid deposition in fish too may have resulted structural weakening of the muscle, leading to a softer meat texture as observed by Prasad Thakur *et al* (2003) on yellowtail.

Sensory analysis showed that panelists were unable to differentiate between sashimi obtained from all fish fed with either palm oil or soya oil. High scores of freshness, taste, odor and texture of the sashimi indicated well acceptance by panelists. Scores showed that in average, panelists graded all cultured fish in experiment as acceptable sashimi. Acceptability of these samples was most probably due to the intact water content in all samples as samples only begun to deteriorate at 54 hr. However, texture becomes tenderer with storage time (Skjervold *et al.,* 2001) due to reduced content and strength of the connective tissue (Sato *et al.,* 1986). At this point of time, sashimi quality may deteriorate and unsuitable for consumption.

Replacing fish oil by either feeding palm oil or soya oil to fish reduced EPA and DHA contents in muscles and this conforms to Ghioni *et al* (1999) study on turbot, Mourente and Bell (2006) on European seabass, Bell *et al* (2002) on Atlantic salmon and Ng *et al* (2003) on African catfish. Gradient reduction of fatty acid compositions particularly DHA and EPA

in both diet and muscles suggest inherent complexity due to interactions between various fatty acids and catabolism mechanism (Robin and Skalli 2006), and Stubhaug *et al* (2005) also observed similar complexity in Atlantic salmon fed with rapeseed oil. Excessive palmitic (C16:0) and linoleic (C18:2n-6) acids due to incremental palm oil may have interrupted the β-oxidation metabolism which caused inherent complexity. In addition, red sea bream may also have lower ability to synthesize these fatty acids *de novo* from palm oil and soya oil based diet. Izquierdo *et al* (2005) has reported similar observation on gilthead sea bream and Regost *et al* (2003) on turbot regarding inability of these fish in synthesizing C18:2n-6 into C20:4n-6. Although it was reported that other vegetable oils too like linseed oil and rapeseed oil may substitute up to 60% of fish oil (Izquierdo *et al.,* 2005) for gilthead sea bream and linseed oil, rapeseed oil and olive oil (Mourente *et al.,* 2005) for European seabass, result indicated that EPA and DHA were lowest at 40% inclusion of both palm oil and soya oil in muscles. Similar gradient degradation in n-3/n-6 ratio was also demonstrated in muscles which conform to reports by Torstensen *et al* (2004) on salmon. According to Takeuchi *et al* (1991), juvenile red sea bream requires EFA comprising 1% EPA and 0.5% DHA. As all test diets used in this study met the 0.5% DHA with lower EPA with n-3 HUFA in the test diets of this study ranging from 1.39% to 1.96%, it can considered that all these formulated diets are still within the requirement of most marine fish species (0.5% to 1.9%) as reported by Xue *et al* (2006).

It can be concluded that sashimi of fish fed with diets containing not more than 40% palm oil or soya oil blended with 60% fish oil are consumable within 24 hr without deterioration of k-value, high pH and high WHC and contained acceptable beneficial DHA and EPA.

Chapter 7

7. General discussion and conclusion

7. General discussion and conclusion

Inclusion of palm oil and soya oil as replacement for fish oil significantly affects some fish growth performance and quality. Results showed that inclusion of both palm oil and soya oil not exceeding 40% indicated promising growth performance. Inclusion of these two vegetable oils in diets could not increase EPA and DHA concentrations; but instead increased saturates and monoenes in particularly C18:2n-6. The current study confirmed expected degradation of n-3 EFAs with higher inclusion of vegetable oil in diets. However, red sea bream seemed to express low ability in converting C18:2n-6. This state could probably prohibit further elongation of C18:3n-3 to C20:5n-3; then further elongation to C22:6n-3. This conditions fish fed with higher inclusion of vegetable oil to develop high resistance to β-oxidation. Liver which functions as a regulator in fat metabolism therefore fails to function normally in lipid esterification; instead liver need to reposition its function at a more vigorous manner to β-oxidation metabolism instead of elongating C18:3n-3. In other words, fatty acid composition in fish muscles and also liver depend on each respective fish metabolism pathway in diluting incorporated dietary fatty acids. Result also suggested that excessive inclusion of vegetable oil to fish may introduce excessive C18:2n-6 will then further depress desaturation and elongation of fatty acid.

Conditions of fish fed with vegetable oils notably illustrated negative effects due to excessive feeding of either palm oil or soya oil. HSI increased significantly at 60% vegetable ratio. This implies that excessive vegetable oil may deteriorate fish health condition with time. Nevertheless, results also demonstrated that inclusion of palm oil at maximum 50% could improve fish ability in building up its biological antioxidant potentials with lower oxidative metabolites. However, mixing palm oil with oxidized fish oil may not be effective in improving fish potential to antioxidants and ability to withstand excessive oxidative metabolites.

Gradient deposition of lipid particularly in ventral muscles of cultured fish fed with either palm oil or soya oil blend demonstrated that higher deposition correlates linearly with

incremental palm oil or soya oil. Melting points of vegetable used as replacement for fish oil should also be considered prior to usage because vegetable oils with low melting points could promote high amount of fat deposition in fish adipose particularly in ventral muscle. Existence of lipid in fish tissues therefore influences shelf-life and sensorial preferences. Physico-chemical differences between wild and cultured fish obviously showed a lower shelf-life in cultured fish. Formation of lipid in the adipose area-surrounding belly of ventral muscle promotes more rapid lipid hydrolysis and fish deterioration Substitution of either palm oil, soya oil or even blend with oxidized fish oils as lipid source may also have created a temporary shocking condition for fish and therefore causes impaired enzyme to meet liver's aggressive efforts in β-oxidation metabolic. This condition may prolonged high intake of vegetable oil to induce these functional organs to deteriorate and subsequently confers poor health to fish. Based on these finding, this research concludes that

a. Sashimi of both wild and cultured red sea bream is best consumed at approximately 24 hr after slaughter due to low k-value, high pH, high WHC and good texture chilled under low designated temperature 0 to 4°C.

b. Wild and cultured red sea bream also demonstrated similar quality characteristics within 48 hrs after slaughter.

c. 40% palm oil blended with 60% fish oil may be a suitable ratio for red sea bream.

d. 40% soya oil blended with 60% fish oil may be a suitable ratio for red sea bream.

e. 40% palm oil blended with 60% oxidized fish oil may be a suitable ratio for red sea bream.

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