Establishment of protocol for producing high yield of antioxidant active flavonoids from *Mimosa pigra*

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ABSTRACT

A study was carried out to determine a procedure for producing high yield of bioactive flavonoids from *Mimosa pigra* as antioxidant. Various parts of *Mimosa pigra* were extracted using various solvents such as methanol, 80% methanol, ethanol, 70% ethanol and ethyl acetate in two ways: (1) eluted in the solvents overnight; (2) boiled the *Mimosa pigra* in the solvents for 1-2 hours and left it overnight. An *in-vitro* antioxidant test was conducted using FTC (Ferric thiocyanate) and TBA (Thiobarbituric acid) methods. A total of 30 samples were screened for their antioxidant activities. The non-heated extract of the stem using 70% ethanol showed the best antioxidant activity. The extract was then tested in fishmeal and fish feeds by conducting feeding trials using tiliapia as a model. The results indicated the potential value of the extract in maintaining the quality of fish muscle and protection of fish flash. The determination of the phenolic compounds was conducted based on the Rf values, UV spectrum, mass spectrum and HPLC (High performance liquid chromatography) retention times. Four acylated flavonols are the major constituents of the extract and were tentatively identified as myricetin 3-*O* (4"-acetyl)-β-D-xyloside, quercetin 3-*O*-(4"-acetyl)-α-L-arabinoside, quercetin 3-*O*-(6"-acetyl)-β-D-galactoside and kaempferol 3-*O*-(2"-cinnamyl)-(β1→2)-sophoroside.

INTRODUCTION

Much interest has been focused on the use of anti-free radical or antioxidant supplements as a form of protection against various diseases. Nutrients such as flavonoids, beta-carotene, vitamins C and E and zinc have the ability to neutralize the damaging effects of free radicals (Velioglu *et al.* 1998). Each of these nutrients can block the conversion of free radicals into damaging chemical compounds within the body, preventing oxidative damage to biomolecules such as proteins, lipids and DNA (Salvi *et al.*, 2001). Among the sources of antioxidants are fruits, fruit juices, vegetables and legumes.

Flavonoids are found in abundance in species from the Leguminosae family (Heqnauer and Grayer-Barkmeijer, 1993). *Mimosa pigra,* a spiny shrub from the Leguminosae family is selected in this study. It is a noxious and ubiquitously distributed weed in the tropics. The goal of this research is to determine a procedure for producing a high yield of antioxidant active flavonoids from *Mimosa pigra.* Several reputed antioxidants compounds, which belong to flavonoids group, are quercetin, rutin, kaempferol, cathecin, fisetin, apigenin, daidzein and petunidin(Dewick 1997). The result will assist in the development of the potential of *Mimosa pigra* and change its current position from weed to economic importance crop. It is also expected that the optimization of extraction and production of antioxidant active flavonoids from *Mimosa pigra* will benefit the aquaculture industry to be more profitable.

Several scientific publications have proved the antioxidant properties of flavonoids against the fishes without side effect. For instance, Plakas *et al.* (1985) confirmed the absence of overt toxicity from feeding the flavonoids to rainbow trout. Hsieh *et al* (1988) found quercetin to be the most potent inhibitors 12-lipoxygenase of the fish gill compared to other nine tested flavonoids. Furthermore, the antioxidant capacity of flavonoids in the fish blood plasma is examined by Arts *et al* (2001) using Trolox equivalent assay.

MATERIALS AND METHODS

Plant materials

Mimosa pigra was collected from various places in Penang island. The voucher specimens are deposited at the Hebarium of The School of Biological Sciences, University Sains Malaysia.

Crude extracts

Various parts of *Mimosa pigra* i.e. stems, leaves and the mixture of the whole plants' organs were extracted using five different solvents i.e. methanol, 80% methanol, ethanol, 70% ethanol and ethyl acetate in two ways: (1) eluted in the solvents overnight; (2) boiled in the solvents for 1-2 hour and left it overnight.

Antioxidant assays

Ferric Thiocyanate (FTC) method

The autoxidation assay was performed based on the method of Osawa and Namiki (1981) with slight modification. A sample solution containing 4 mg plant extract in 4 ml 99.5% ethanol, 4.1 ml 2.5% linoleic acid in 99.5% ethanol, 8 ml 0.02 M phosphate buffer (pH 7.0) and 3.9 distilled water was placed in a columnar vial with a screw cap and incubated in the dark at 40° C for 11 days. To 0.1 ml of this sample solution, 9.7 ml 75% ethanol and 0.1 ml 30% ammonium thiocyanate were added. Precisely 3 min after the addition of 0.1 ml 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, absorbance of the red colour was measured at 500 nm. BHT (4 mg) was used as a positive control.

Thiobarbituric acid (TBA) method

The sample solution was prepared and incubated as describe above. The assay was based upon the reaction of TBA with Malonaldehyde, one of the aldehyde products of lipid peroxidation. The sample was heated with TBA under acidic conditions (add 2.0 ml 0.67% trichloroacetic acid), and the formation of malonaldehyde was measured by reading the absorbance at 532 nm (Ottolenghi, 1959).

Purification and identification of the flavonoids in the best fraction

The concentrated extract was applied as a streak on 12-15 sheets of Whatman no 3 paper (46 x 57 cm) and run in solvent BAW overnight. The dried papers were viewed under UV and all the flavonoid glycoside bands were cut out and eluted in 80% methanol overnight. The eluates were concentrated and again streaked and rerun on Whatman no 3 paper in the solvent 15% HOAc. Then the separated glycoside bands were cut out and eluted in 80% methanol. To test their purity, they were spotted on small TLC plates and rerun with the following solvents : BAW, 15% HOAc, Phenol and water.

For further purification, the solvent with the greatest separation was chosen to rerun them again. The R_f values of the separated individual compounds were determined for all the four solvents to test the polarity of the compounds. The acylated glycosides produce obvious differences of R_f values compared with normal glycosides.

Acid hydrolysis

An aliquot of each of the pure extracts was hydrolysed with an equal volume of 2M HCl for 30-40 minutes or four hours. Hydrolysed samples were allowed to cool and the flavonoid aglycones were extracted with ethyl acetate. The extract was evaporated to dryness and was redissolved with a few drops of 100% ethanol. The aglycones were identified. This method involved the acid hydrolysis treatment where the flavonoid glycosides were separated to flavonoid aglycones and sugars.

Identification of the sugars

Sugars occurred in the aqueous residues of the acid hydrolysed samples. The acid was removed by drying in the rotary evaporator. The sugars were then redissolved with a few drops of water and were identified by chromatographic comparison with an authentic sugar mixture, in solvents TPBW (toluene: pyridine: butanol: water = 1: 3: 5: 3), Phenol (PhOH: H_2O = 4: 1) and BAW. They were spotted on

Whatman no 1 papers. After 48 hours, the chromatograms were developed and dried in the fume cupboard. To visualise the sugar, the dried chromatograms were dipped in aniline hydrogen phthalate solution and were heated in an oven for 10-15 minutes at 100°C. The papers were viewed under UV light to detect the clear sugar spots. The increasing order of the sugar mobility in the solvents was as follows:-

• TPBW and BAW	galactose, glucose, arabinose, xylose, rhamnose
• Phenol	glucose, galactose, xylose, arabinose, rhamnose

- Marker sugars were run on all chromatograms.

UV- Visible Spectrophotometry

A Philips PU 8700 UV spectrophotometer with shift reagents was used to determine the position of the hydroxylation, methylation and glycosylation in the flavonoid nucleus and to confirm the type of flavonoid. The spectrum of a flavonoid usually consists of two major absorption maxima in the ranges 240-285nm (band 1) and 300-550nm (band 2).

Only a few drops of the pure flavonoid glycoside were added to a blank solution (80% methanol) and the spectrum was observed

Co-chromatographic comparison

Co-chromatographic comparison is used for the confirmation of the identity of the pure compound. The compounds were co-chromatographed with authentic markers that were suspected to be the compounds and run in the four solvents: BAW,15% HOAc, Phenol and Water. If the mobility of the pure compounds is similar to the markers in these four solvents, this confirms that the compounds are the same as the markers.

High Performance Liquid Chromatography (HPLC)

HPLC was carried out using a 3.9 x 300 mm reverse phase column with C18 phenyl packing material. Two different solvent gradient programmes were used: (1) 40% A and 60% B, changing linearly to 0% A and 100% B in 20 minutes (for confirming the aglycones); (2) from 75%A / 25%B to 35% A, 65% B over 23 minutes in linear mode at room temperature with the detector set at 350nm. (composition of solvent A: 2% HOAc in H_2O, solvent B: methanol: HOAc: water (18 : 1 : 1). Flow rate was 1.00 ml min^{-1} and the pressure 3000 PSI.

Removing and identifying the Acyl groups

At least 0.5 mg of the dry acylated flavonol glycosides were saponified at room temperature with 2N NaOH under nitrogen for 2 hours. Then an acidic resin was used to neutralise the samples and they were concentrated to dryness and were extracted with dry ether. The ether extracts were spotted on the TLC plates and were run in:

1) BAW (4: 1: 5)
2) EtOAC: HOAc: H_2O (3: 1: 1)
3) EtOH: H_2O: NH_4OH (16: 3: 1) against markers.

They were then sprayed with glucose aniline spray and heated for 5-10 minutes at 125°C.

Mass Spectrometry

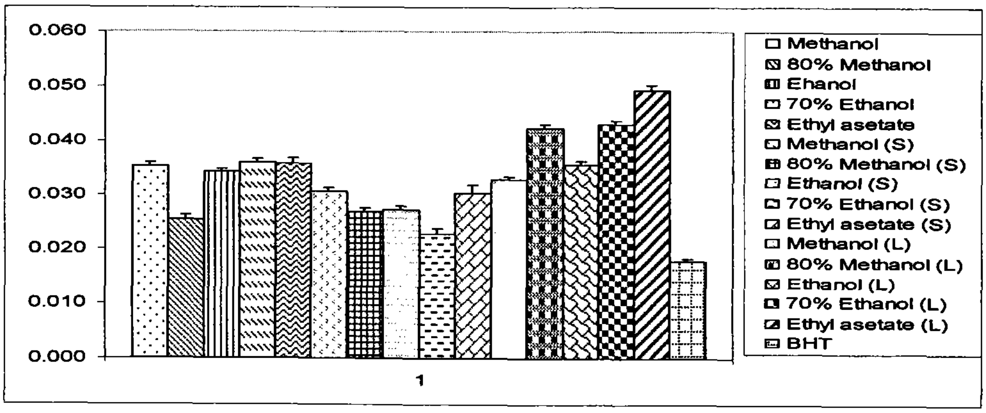
The purified compounds were sent for the Fast Atom Bombardment-Mass Spectrometry (FAB-MS) to determine the molecular weight of the compounds and to confirm the identification.

RESULTS AND DISCUSSION

For determining a procedure to increase the yield of bioactive flavonoids from *Mimosa pigra* as antioxidant, five different solvents systems were used to extract the plant samples. The antioxidant activities of a total of 30 extracts of *Mimosa pigra* were determined by FTC and TBA method. The results of FTC are indicated in Figure 1 and the results of TBA are shown in Figure 2. According to the polarity, 70% ethanol is the most polar solvent follows by 80% methanol, methanol and ethanol. Ethyl acetate is the most non polar solvent used in this evaluation.

Among all the tested samples, the extraction of stems parts using 70% ethanol showed the strongest antioxidant activity for the FTC method (Figure 1 (a)). The extract was selected for further analysis. The major flavonoid constituents of the extract were purified and identified. FTC method measures the amount of peroxide produced during the initial stages of lipid oxidation; while the TBA method measures the peroxide decomposes to form carbonyl compounds at a later stage of lipid oxidation (Mackeen *et. al.* 2000).

(a)



(b)

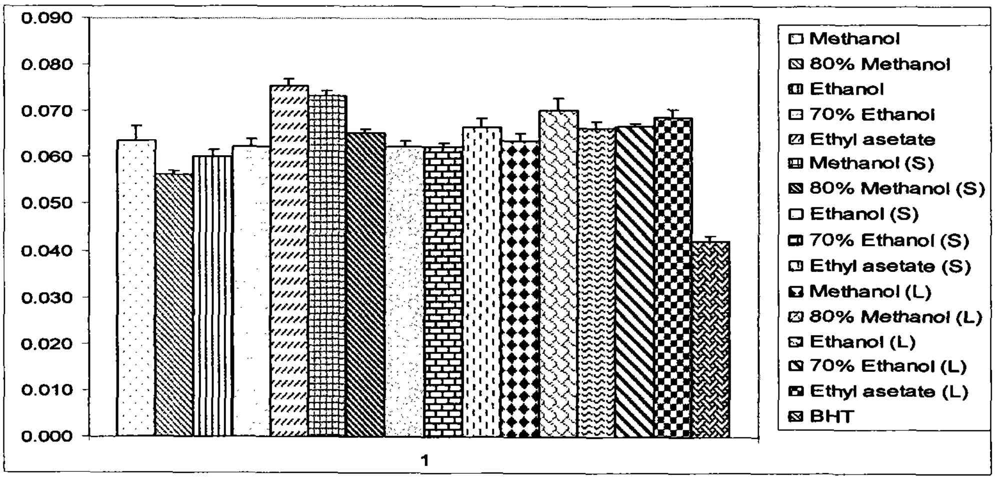
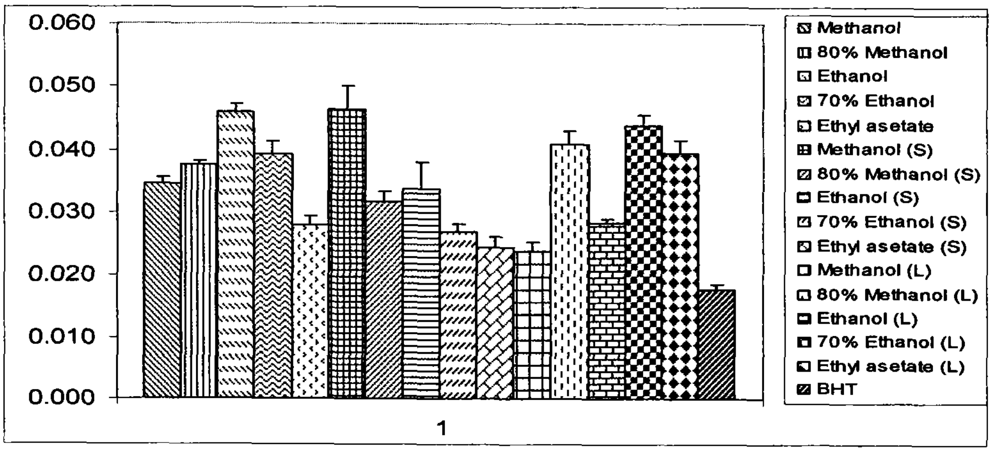


Fig. 1. Antioxidant activities for FTC method on the 10th day: (a) Non-Heat (b) Heat.

(c)



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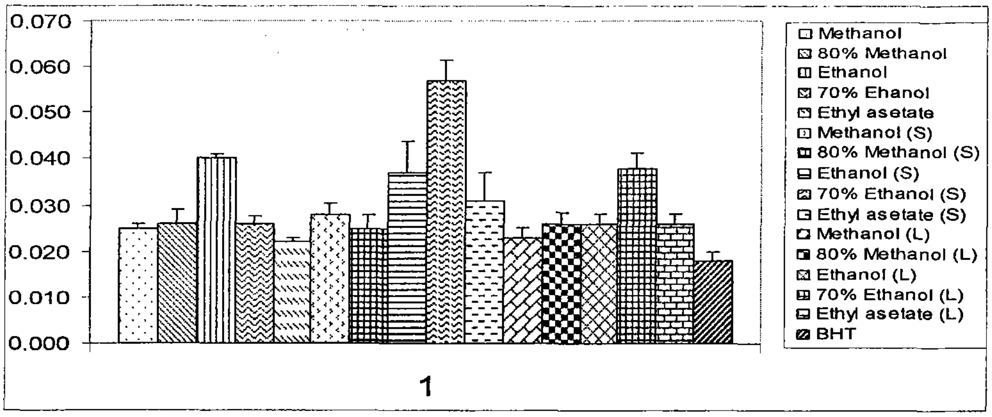


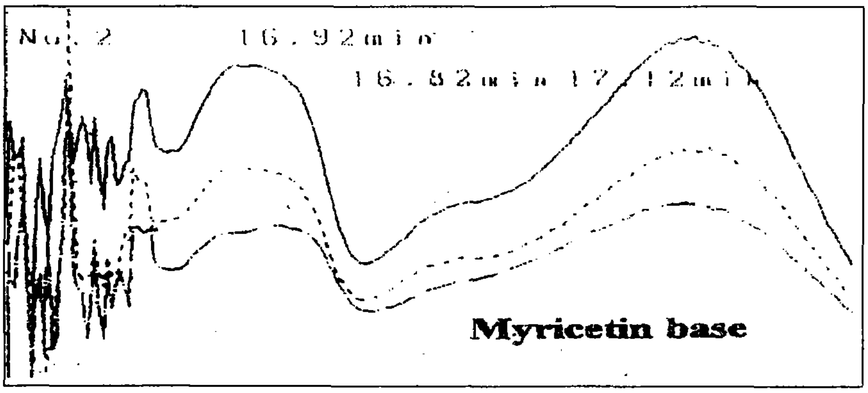
Fig. 2. Antioxidant activities for TBA method: (c) Non-Heat (d) Heat. These data are expressed as mean ± S.E.M. in nine replicates. BHT was used as a positive control.

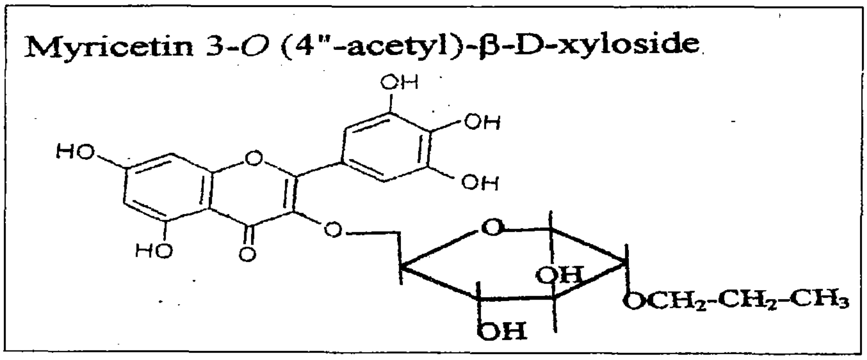
*** S-Stem ; L-Leaf**

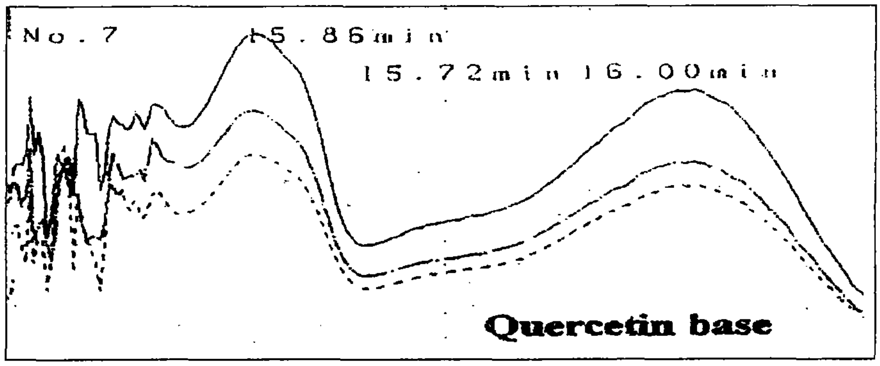
An acylated myricetin and kaempferol as well as two acylated quercetin were isolated from the 70% ethanol extract of the stems of *Mimosa pigra* (Table 1). Myricetin, quercetin, kaempferol and their sugars were found after acid hydrolysis. The identity of the separate aglycones and glycosides were confirmed by co-chromatographic comparison with authentic markers. The acylated groups were removed and identified as previously mentioned. No spots were observed after the glucose-aniline treatment of the acylated myrcetin and quercetin. This finding suggested the presence of acetyl group. For the acylated kaempferol, a spot of cinnamic acid was observed after the treatment. The positive NaOAc shift suggested that all of the acylated compounds and the glycosides were attached at the 3-position. The compounds were then purified on a Sephadex column for mass spectral analysis. The results of FAB-MS in the negative mode showed a strong molecular ion that corresponds to the structure of flavonoids. Insufficient material was available to determine the position of substitution of the acylated groups on the sugar moiety.

Table 1. Identification of flavonoid glycosides found in *Mimosa pigra*

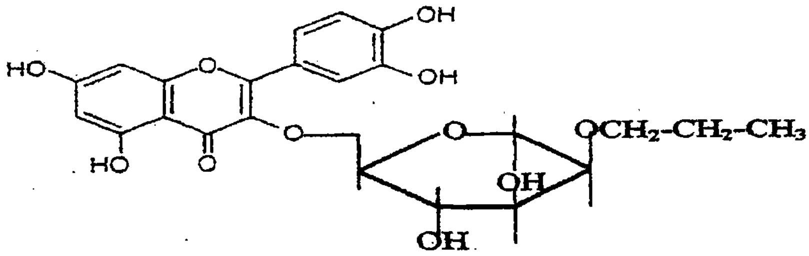
Flavonoids	MS	R_f Values				λ max (UV absorption spectrum data)/nm				HPLC Retention time	Colour (UV)
						Max MeoH		+ NaoAc			
		BAW	15% HOAc	H_2O	PhOH	Band I	Band II	Band I	Band II		
myricetin 3-*O*-acetylxyloside	492	80	40	10	80	263	358	268	394	20.45	Dark brown
quercetin 3-*O*-acetylgalactoside	506	80	15	50	80	267	359	270	367	21.14	Deep purple
quercetin 3-*O*-acetylarabinoside	476	90	10	40	90	267	360	272	367	21.80	Deep purple
kaempferol 3-*O*-cinnamylsophoroside	740	45	75	90	60	266	353	269	397	7.31	Deep purple

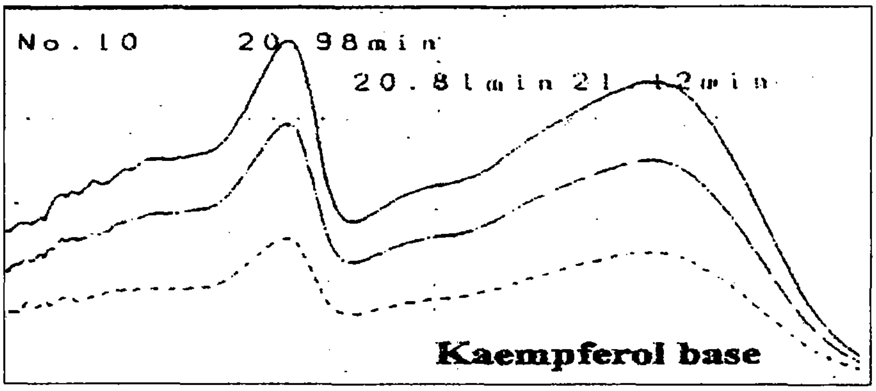



Myricetin 3-*O* (4"-acetyl)-β-D-xyloside



Quercetin 3-*O*- (4"-acetyl)-α-L-arabinoside





Kaempferol 3-*O*- (2"-cinnamyl)-(β1→2)-sophoroside

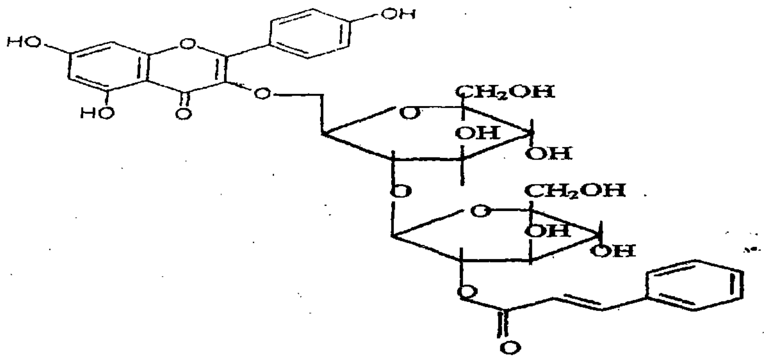


Fig. 3. UV spectra and new flavonoids structures

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Application of the best antioxidant extracts to improve the aquaculture products

1. INTRODUCTION

Much interest has been focused on the use of anti-free radical or antioxidant supplements as a form of protection against various diseases. Nutrients such as flavonoids, beta-carotene, vitamins C and E and zinc have the ability to neutralize the damaging effects of free radicals. Each of these nutrients can block the conversion of free radicals into damaging chemical compounds within the body, preventing oxidative damage to biomolecules such as proteins, lipids and DNA (Salvi *et. al.*, 2001). Among the sources of antioxidants are fruits, fruit juices, vegetables and legumes.

Several reputed antioxidants compounds, which belong to flavanoids group, are quercetin, rutin, kaempferol, cathecin, fisetin, apigenin, daidzein and petunidin. Among them, quercetin is found to be the most effective one (Dewick, 1997). Quercetin belong to the flavonol class with fifteen carbon atom in their basic nucleus and these are arranged in C_6-C_3-C_6 configuration. Simple quercetin with two hydroxyl groups in the B ring can be extensively modified glycosylation, methylation and acylation (sulphation). The possible mechanisme and relationships between the quercetin structure and its antioxidant potency are still obscured.

Several scientific publications have shown no side effects to fish when they were fed with flavonoids. For instance, Plakas *et al.* (1985) confirmed the absence of overt toxicity from feeding the flavonoids to rainbow trout. Hsieh *et al* (1988) found quercetin to be the most potent inhibitor of 12-lipoxygenase of the fish gill compared to other nine tested flavonoids and Pelissero *et al.* (1996) found the potential value of quercetin as the inhibitor of the ovarian aromatase activity in the rainbow trout. Furthermore, the antioxidant capacity of various flavonoids types in the fish blood plasma has been examined by Arts *et al* (2001) using Trolox equivalent assay.

To date various scientific evaluation have been conducted to verify the antioxidant potential of legume plants such as *Pisum sativum* L. (Lopez-Amoros *et al.*, 2004) and *Phaseolus vulgaris* (Nuria *et al.,* 2006). High concentration of proteins, carbohydrates and dietary fiber can be found in Legumes yet make an important contribution to human diet in whole over the countries (Lopez-Amoros *et al.*, 2004). Flavonoid has been found in abundance in species from the Leguminosae family (Rao & Deosthale, 1987; Elias, *et al.*, 1979; Heqnauer and Grayer-Barkmeijer, 1993; Jiratavan & Liu, 2004.).

Mimosa pigra, a spiny shrub from the Leguminosae family is selected in this study since it is a noxious and ubiquitously distributed weed in the tropics. Sulaiman (1997), has isolated three types of quercetin, *i.e.* quercetin glucoside, quercetin acetylarabinoside and quercetin acetylarabinoside from *Mimosa pigra*. This plant also contains other types of flavonoids, *i.e.* myrcetin glycoside, kaempferol glycoside and luteolin.

Flavonoids, 2-phenyl-benzo-α-pyrones, are polyphenolic compounds that exist commonly in foods of plant origin (Hollman, *et al.*, 1996). Some of these have oxidation inhibiting properties (antioxidants) (Emanuel & Lyaskovskaya, 1967) that can delay the oxidation of lipids by inhibiting the initiation of oxidizing chain reactions (Velioglu *et al.*, 1998). Since the Mimosa is a tremendous threat to agriculture, therefore, a further study have been proceeded in determination flavonoids constituents to increase the chemical knowledge of this species as well as to change its current position from weed to economic important crop in aqua feed and fish processing industries. There is a need to increase harvests as well as improve aquaculture products in terms of maintaining muscle quality during storage or after cooking by using a natural antioxidant.

The goals of this project is to find something more active and powerful, probably mixture of flavonoids or pure compounds from Mimosa pigra, and to study the effect of these bioactive flavonoids supplementation in fish diet on the stability of fish muscle/fillet pre and post processing. To date the demand of flavonoids has been extensive due to its inadequate supply. It is expected that the optimization of activity-guided fractionation from *Mimosa pigra* will assist the aquaculture industry to be more profitable.

2. MATERIAL AND METHODS

2.1 Plant material

Mimosa pigra was collected from various places in Penang Island. The samples were air-dried and a voucher specimen for the species has been deposited in the Herbarium of The School of Biological Sciences, University Sains Malaysia.

2.2 Extraction

Mimosa pigra was divided into three different parts, namely leaf and stem or crude (whole part of the species), leaf and stem, for the extraction. Each part of the species were extracted with six different solvents which were methanol, 80% methanol, ethanol, 70% ethanol, ethyl acetate and distilled water, with boiling water (300 ml) for 1 hour, and soaked for overnight. Those extracts were then filtered, evaporated to dryness and used for the assessment of antioxidant activity. For the isolation of flavonoids, extract was prepared by using the above procedure with its own solvent whether it is boiling or soaking.

2.3 Determination of antioxidant activity (in-vitro)

2.3.1 Ferric thiocyanate (FTC) method

The FTC method was adapted from the method of Osawa and Namiki (1981). 4 mg of sample dissolved in 4 ml of 99.5% (w/v) ethanol were mixed with linoleic acid (2.51% v/v) in 99.5% (w/v) ethanol (4.1 ml), 0.05 M phosphate buffer pH 7.0 (8 ml) and distilled water (3.9 ml) and kept in screw-cap container in the dark at 40°C. To 0.1 ml of this solution was then added 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance of the resulting red colour was measured at 500 nm every 24 h until the day after the absorbance of the control reached maximum value.

2.3.2 Thiobarbituric acid (TBA) test

The TBA test was conducted according to the combined method of Kikuzaki and Nakatani (1993) and Ottolenghi (1959). 1 mg of sample from the FTC method was added to trichloroacetic acid (2 ml) and thiobarbituric acid solution (2 ml). This mixture was then placed in a boiling water bath at 100°C for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and absorbance of the supernatant was then measured at 532 nm.

2.4 Isolation of flavonoids

The sample with the best antioxidant activity was used to determine its flavonoids. Crude extract was first separated by preparative paper chromatography (PC) using BAW (*n*-butanol, HOAc, H_2O, 4:1:5, upper layer) and 15% HOAc as subsequent solvents. Fractions were cut out based on separated band observed under long wave UV light and eluted in 80% methanol overnight. Eluates were then filtered and concentrated to dryness.

2.5 Fish and experimental design

The experiment was started on 7 April 2005. Red tilapia (*Oreochromis spp.*) (Plate 1.2) were obtained from Aquaculture Research Centre, Department of Fishery, Jitra, Kedah and held in acclimatized to laboratory condition for two weeks in 1000-l fiberglass tanks upon arrival and fed with commercial fish pellet (32% protein). Fish with a mean weight of ±10g were selected and randomly assigned 18 aquaria (30.5 width x 60 length x 29.6 height). Each aquarium was stocked with 20 fish. An air stone continuously aerated each aquarium. All aquaria were cleaned daily in the morning by siphoning off accumulated waste materials. Approximately 1/3 of water in each aquarium was replaced with aerated fresh water daily. Water temperature was between 29 and 30°C throughout the experiment. Each group of fish was weighed every two weeks and the amount of diet fed was adjusted accordingly. Meanwhile, water quality (ammonia, nitrite, phosphate, phosphorus) was also measured in every sampling.

2.6 Diet preparation and feeding

The formulation and proximate composition of the pelleted feed is shown in Table 1. The feed was prepared and stored in a freezer at -20°C until utilized. Fish were fed *ad libitum* 2 times a day at 0900 and 1700. Fish were fed diets containing five different doses of *Mimosa pigra* (10, 15, 20, 25, 30 mg) and 4 mg of BHT as a control diet for 8 weeks.

Table 1. Composition of the experiment diet

Ingredients[1]	**BHT**	**1**	**2**	**3**	**4**	**5**
Fish meal	13.93	13.93	13.93	13.93	13.93	13.93
Soybean meal	40.54	40.54	40.54	40.54	40.54	40.54
Corn starch	34.30	34.30	34.30	34.30	34.30	34.30
Fish oil	4.75	4.75	4.75	4.75	4.75	4.75
Corn oil	2.88	2.88	2.88	2.88	2.88	2.88
Vitamin mix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Antioxidant extract	0.004	0.010	0.015	0.020	0.025	0.030
Cellulose	1.61	1.60	1.60	1.59	1.59	1.61

[1] **All purified diets were obtained from Asia Veterinary (Penang, Malaysia). Lipid source: fish oil was kindly supplied by National Prawn Fry Production and Research Center, Pulau Sayak, Malaysia. Corn oil and soybean oil were purchased from local shop.**

[2] Vitamin mix from Hoffman-La Roche, Basel, Switzerland. Contained (as g/kg): thiamine hydrochloride, 0.92g; pyridoxine. HCl, 1.00g; vitamin B12, 1.35g; niacin, 40.1; calcium *d*-pantothenate 3.00g; folic acid, 90mg; biotin, 20mg; vitamin A, 1.8 MIU; vitamin D3, 3.32 MIU; menadiaone sodium bisulphate, 1.67g; cellulose, 990G.

[3] Mineral: reagent grade mineral premix comprises (per kg) calcium phosphate monobasic 397.65g; calcium lactate 327g; ferrous sulphate 25g; magnesium sulphate 137g; potassium chloride 50g; carbonate 0.1g; zinc oxide 1.5g and sodium selenite 0.02g.

Table 2 Proximate composition of the experiment diet[1]

Ingredients	**Moisture**	**Protein**	**Lipid**	**Ash**	**Fiber**
M.pigra extract	0.44±0.601	9.55±0.211	0.08±0.100	6.37±0.396	0.12±0.202
Fish meal	5.57±1.000	88.16±1.493	13.57±2.331	15.0±2.108	0
Soybean meal	8.72±0.477	47.68±3.076	2.15±0.220	6.93±0.172	24.51±8.748

[1] Values are the mean of triplicate of each sample.

2.7 Sample collection and analysis

Three replicates of samples with 10 fishes per replicate were taken at the beginning of the experiment, and these were kept frozen at -20°C for analysis of whole body proximate analysis.

On completion of the feeding trial, all fish were starved for 48 h (to empty the digestive tract), killed and weighed. 3 fishes were randomly sampled from each tank, dissected and their livers and viscera were weighed for estimation of hepato- (HSI) and viscerosomatic indices (VSI). These indices were calculated as a percentage of organ or tissue to the whole body weight of individual fish.

HIS = [100 x liver weight (g) / bodyweight (g)]

VSI = [100 x visceral weight (g) / bodyweight (g)]

The remaining fish carcasses were ground for whole body proximate determination. Moisture, crude, lipid, fiber and ash were determined following methods of Association of Office Analytical Chemists (AOAC) (1984).

2.7.1 Data collecting and statistical analysis

The experiment was terminated after 32 days. The fish were weighed individually and monitored. Growth performance indicators measured were weight gain (WG), relative growth rate (RGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). These indicators were calculated as:

WG (%) = 100 (final weight – initial weight)

RGR (%) = 100 [final weight – initial weight / initial weight]

SGR (%) = 100 [In final weight – in initial weight / days of experiment]

FCR (%) = feed intake (g) / weight gain (g)

PER = weight gain (g) / crude protein in diet (g)

2.7.2 Sampling procedure and storage condition

At the end of the feeding period, 10 fish from each treatment were randomly selected, sacrificed and pooled for fatty acid analysis following the method of Bligh and Dyer (1985). An additional 30 fish per treatment were sacrificed, eviscerated, skinned and filleted. Each fillet was carefully wrapped and immediately stored in freezer at -20°C. Ten fish of each treatment were analyzed for oxidation stability (TBA) following the method of Csallany *et al.* (1984) with slightly modification and electrophoresis method (SDS-PAGE) after 1, 2, 3, 4, 5, 6, 7 and 8 hours.

2.8 Statistical analysis

Experimental data of TLC and TBA test were analyzed using analysis of variance (ANOVA) and significant difference among means from triplicate analysis ($P<0.05$) were determined by One-way ANOVA test using the Graphpad prism 3.02.

One-way analysis of variance (ANOVA) was also carried out to determine the fatty acid composition of diets and fish carcasses. Differences between means were assessed by Duncan's Multiple Range Test ($P>0.05$). Effects with a probability of $P<0.05$ were considered significant. Differences due to diet and storage time and their interaction were determined by two-way analysis of variance. Differences were regarded as significant when $P<0.05$.

3. RESULT

3.1 FTC and TBA

Table 1 shows the absorbance value of the extracts using FTC method. Meanwhile Table 2 indicates the results of TBA and the data were summarized in Table 3. The comparative evaluation of the antioxidants activities of the extracts is revealed in Fig. 1, 2, 3 and 4. Two different parts of the plant (leaf; L and stem; S) and their mixtures (W) were either soaked or boiled in six different solvents separately. All accessions tested showed high activities with significant variation between species, with distilled water (L) (soaking) having the highest activity in FTC method and ethyl acetate (W) (boiling) shows the best activity in TBA method. Based on the results obtained from the two methodologies used, the mixture of both organs extracted using boiling distilled water exhibits the highest antioxidative activity. Extraction method plays an important role in determination antioxidants activities for a sample. Solvents with the high polarities are usually used for an antioxidant test such as water, methanol and ethanol (Ivanova *et al.*, 2005; Duh & Yen, 1997; Zainol *et al.*, 2003; Heneidak *et al.*, 2006). Siriwardhana *et al.* (2003) have reported that, by using the water and methanol as a solvent system in the antioxidant activity test of *Hizikia fusiformis*, it was much higher than using the ethanol, chloroform and ethyl acetate as the solvent system. For the reason of that, distilled water (W) (boiling) sample was used as a supplementation in fish feeds and oxidative stability of fish muscle (tests).

Table 1. Absorbance value of different samples using FTC method. The data are expressed as mean ±S.E.M. in nine replicates. Values with the same letter are not significantly different ($P<0.05$) between samples

No.	**Soaking samples**	**Absorbance value (OD)**	**Position**
1	BHT	0.018 ± 0.001^{a}	Not counted
2	Methanol (W)	0.035 ± 0.001^{no}	17
3	80% Methanol (W)	0.025 ± 0.001^{def}	7
4	Ethanol (W)	0.034 ± 0.000^{mno}	16
5	70% Ethanol (W)	0.036 ± 0.001^{o}	18
6	Ethyl acetate (W)	0.036 ± 0.001^{o}	18
7	Distilled water (W)	0.017 ± 0.000^{a}	2
8	Methanol (S)	0.031 ± 0.001^{jkl}	13
9	80% Methanol (S)	0.027 ± 0.001^{fgh}	9
10	Ethanol (S)	0.027 ± 0.001^{fgh}	9
11	70% Ethanol (S)	0.023 ± 0.001^{bcd}	5
12	Ethyl acetate (S)	0.030 ± 0.001^{ijkl}	12
13	Distilled water (S)	0.018 ± 0.000^{a}	3
14	Methanol (L)	0.033 ± 0.001^{lmn}	15
15	80% Methanol (L)	0.042 ± 0.001^{p}	19
16	Ethanol (L)	0.036 ± 0.001^{no}	18
17	70% Ethanol (L)	0.043 ± 0.001^{p}	20
18	Ethyl acetate (L)	0.049 ± 0.001^{q}	21
19	Distilled water (L)	0.016 ± 0.000^{a}	1
	Boiling samples	**Absorbance value (OD)**	
20	Methanol (W)	0.027 ± 0.002^{fgh}	9
21	80% Methanol (W)	0.024 ± 0.001^{cde}	6
22	Ethanol (W)	0.026 ± 0.001^{def}	8
23	70% Ethanol (W)	0.027 ± 0.001^{efg}	9
24	Ethyl acetate (W)	0.032 ± 0.001^{klm}	14
25	Distilled water (W)	0.017 ± 0.001^{a}	2
26	Methanol (S)	0.031 ± 0.001^{jklm}	13
27	80% Methanol (S)	0.028 ± 0.001^{fghi}	10
28	Ethanol (S)	0.027 ± 0.001^{efg}	9
29	70% Ethanol (S)	0.027 ± 0.001^{efg}	9
30	Ethyl acetate (S)	0.029 ± 0.001^{fghij}	11
31	Distilled water (S)	0.021 ± 0.001^{b}	4
32	Methanol (L)	0.027 ± 0.001^{fgh}	9
33	80% Methanol (L)	0.030 ± 0.002^{hijk}	12
34	Ethanol (L)	0.028 ± 0.001^{fghij}	10
35	70% Ethanol (L)	0.029 ± 0.001^{fghij}	11
36	Ethyl acetate (L)	0.030 ± 0.001^{ghij}	12
37	Distilled water (L)	0.023 ± 0.001^{bc}	5

(W) – Whole part of the plant; (S) – Stem; (L) – Leaf

Table 2. Absorbance values of different samples using TBA method. The data are expressed as mean ±S.E.M. in nine replicates. Values with the same letter are not significantly different ($P<0.05$) between samples

No.	Soaking samples	Absorbance value (OD)	Position
1	BHT	0.018 ± 0.001^{a}	Not counted
2	Methanol (W)	0.035 ± 0.001^{no}	11
3	80% Methanol (W)	0.038 ± 0.001^{def}	13
4	Ethanol (W)	0.046 ± 0.000^{mno}	18
5	70% Ethanol (W)	0.039 ± 0.002^{o}	14
6	Ethyl acetate (W)	0.028 ± 0.001^{o}	7
7	Distilled water (W)	0.049 ± 0.002^{a}	19
8	Methanol (S)	0.046 ± 0.004^{jkl}	18
9	80% Methanol (S)	0.032 ± 0.002^{fgh}	9
10	Ethanol (S)	0.034 ± 0.004^{fgh}	10
11	70% Ethanol (S)	0.027 ± 0.001^{bcd}	6
12	Ethyl acetate (S)	0.024 ± 0.002^{ijkl}	3
13	Distilled water (S)	0.081 ± 0.005^{a}	22
14	Methanol (L)	0.024 ± 0.002^{lmn}	3
15	80% Methanol (L)	0.041 ± 0.002^{p}	16
16	Ethanol (L)	0.028 ± 0.005^{no}	7
17	70% Ethanol (L)	0.044 ± 0.002^{p}	17
18	Ethyl acetate (L)	0.039 ± 0.002^{q}	13
19	Distilled water (L)	0.078 ± 0.006^{a}	21
	Boiling samples	**Absorbance value (OD)**	
20	Methanol (W)	0.025 ± 0.001^{fgh}	4
21	80% Methanol (W)	0.026 ± 0.003^{cde}	5
22	Ethanol (W)	0.040 ± 0.001^{def}	15
23	70% Ethanol (W)	0.026 ± 0.002^{efg}	5
24	Ethyl acetate (W)	0.022 ± 0.001^{klm}	1
25	Distilled water (W)	0.023 ± 0.002^{a}	2
26	Methanol (S)	0.028 ± 0.002^{jklm}	7
27	80% Methanol (S)	0.025 ± 0.003^{fghi}	4
28	Ethanol (S)	0.037 ± 0.006^{efg}	12
29	70% Ethanol (S)	0.057 ± 0.005^{efg}	20
30	Ethyl acetate (S)	0.031 ± 0.006^{fghij}	8
31	Distilled water (S)	0.031 ± 0.002^{b}	8
32	Methanol (L)	0.023 ± 0.002^{fgh}	2
33	80% Methanol (L)	0.026 ± 0.003^{hijk}	5
34	Ethanol (L)	0.026 ± 0.002^{fghij}	5
35	70% Ethanol (L)	0.038 ± 0.003^{fghij}	13
36	Ethyl acetate (L)	0.026 ± 0.002^{ghij}	5
37	Distilled water (L)	0.039 ± 0.005^{bc}	14

Table 3. Number position according to the activity in both FTC and TBA test

No.	**Soaking samples**	**Reposition**
1	BHT	Not counted
2	Methanol (S)	17+11=28
3	80% Methanol (S)	7+13=20
4	Ethanol (S)	16+18=34
5	70% Ethanol (S)	18+14=32
6	Ethyl acetate (S)	18+7=25
7	Distilled water (S)	2+19=21
8	Methanol (B)	13+18=31
9	80% Methanol (B)	9+9=18
10	Ethanol (B)	9+10=19
11	70% Ethanol (B)	5+6=11
12	Ethyl acetate (B)	12+3=25
13	Distilled water (B)	3+22=25
14	Methanol (D)	15+3=18
15	80% Methanol (D)	19+16=35
16	Ethanol (D)	18+7=25
17	70% Ethanol (D)	20+17=37
18	Ethyl acetate (D)	21+13=34
19	Distilled water (D)	1+21=22
	Boiling samples	
20	Methanol (S)	9+4=13
21	80% Methanol (S)	6+5=11
22	Ethanol (S)	8+15=23
23	70% Ethanol (S)	9+5=14
24	Ethyl acetate (S)	14+1=15
25	Distilled water (S)	2+2=4
26	Methanol (B)	13+7=20
27	80% Methanol (B)	10+4=14
28	Ethanol (B)	9+12=21
29	70% Ethanol (B)	9+20=29
30	Ethyl acetate (B)	11+8=19
31	Distilled water (B)	8+4=12
32	Methanol (D)	9+2=11
33	80% Methanol (D)	12+5=17
34	Ethanol (D)	10+5=15
35	70% Ethanol (D)	11+13=23
36	Ethyl acetate (D)	12+5=17
37	Distilled water (D)	5+14=19

(W) – Whole part of plant; (S) – Stem; (L) – Leaf

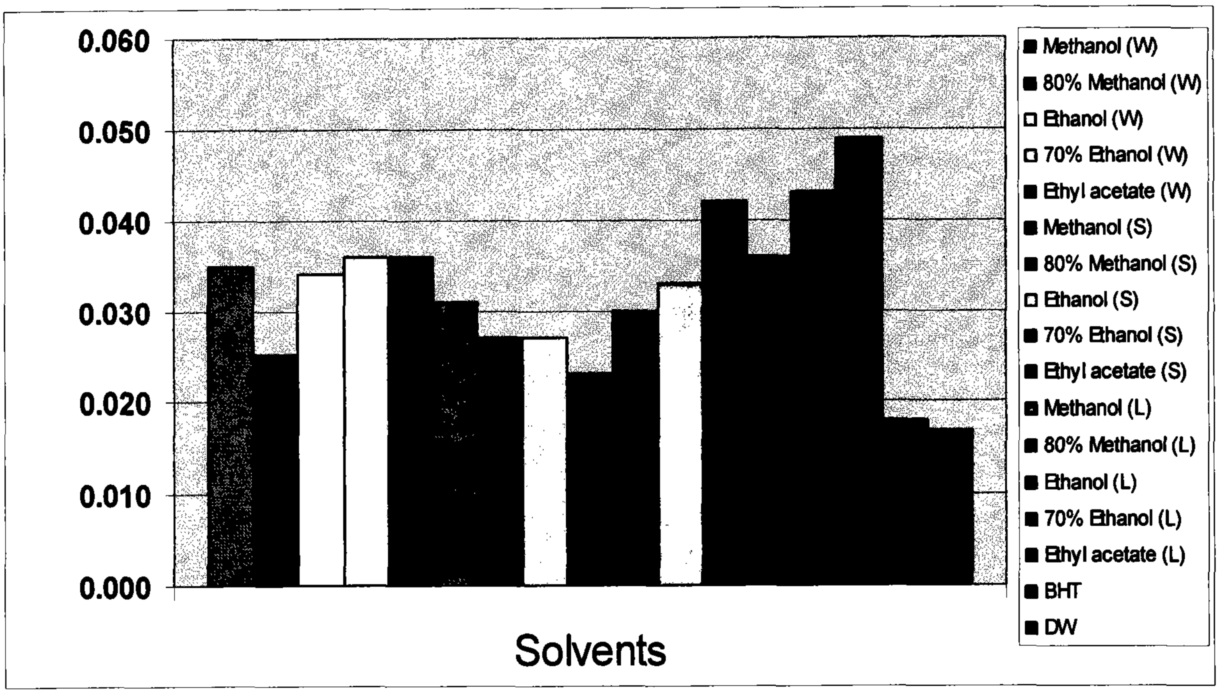

Fig. 1 Antioxidant activities of soaking samples for FTC method

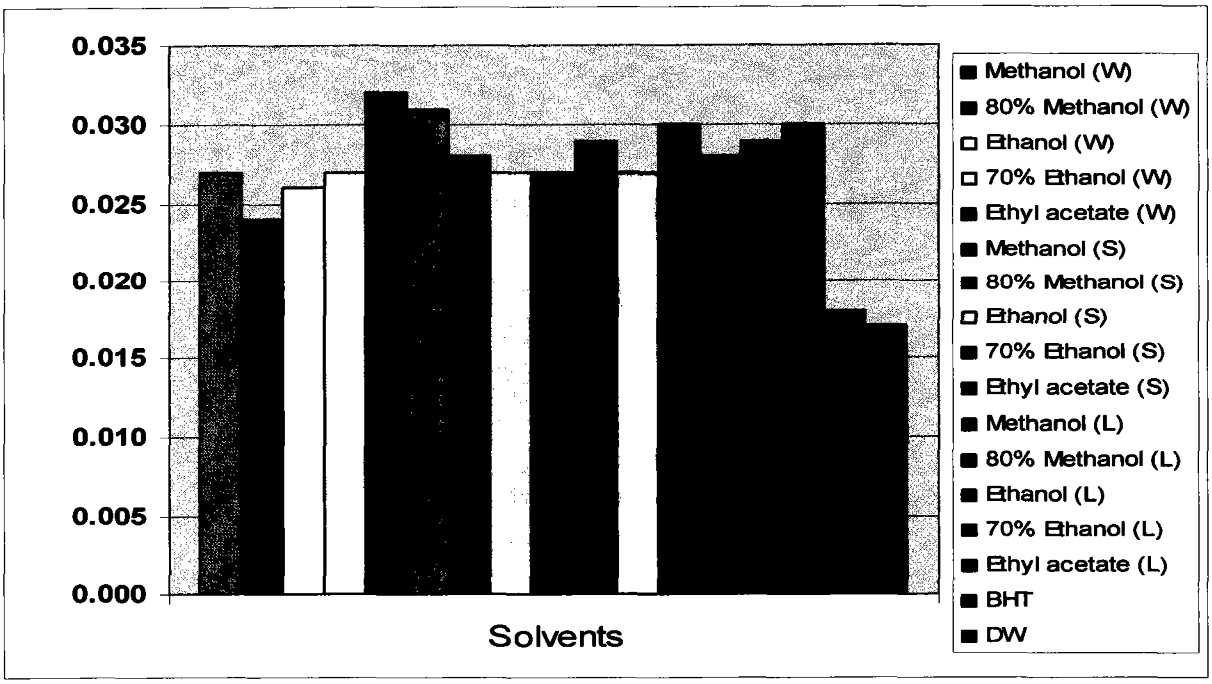

Fig. 2 Antioxidant activities of boiling samples for FTC method

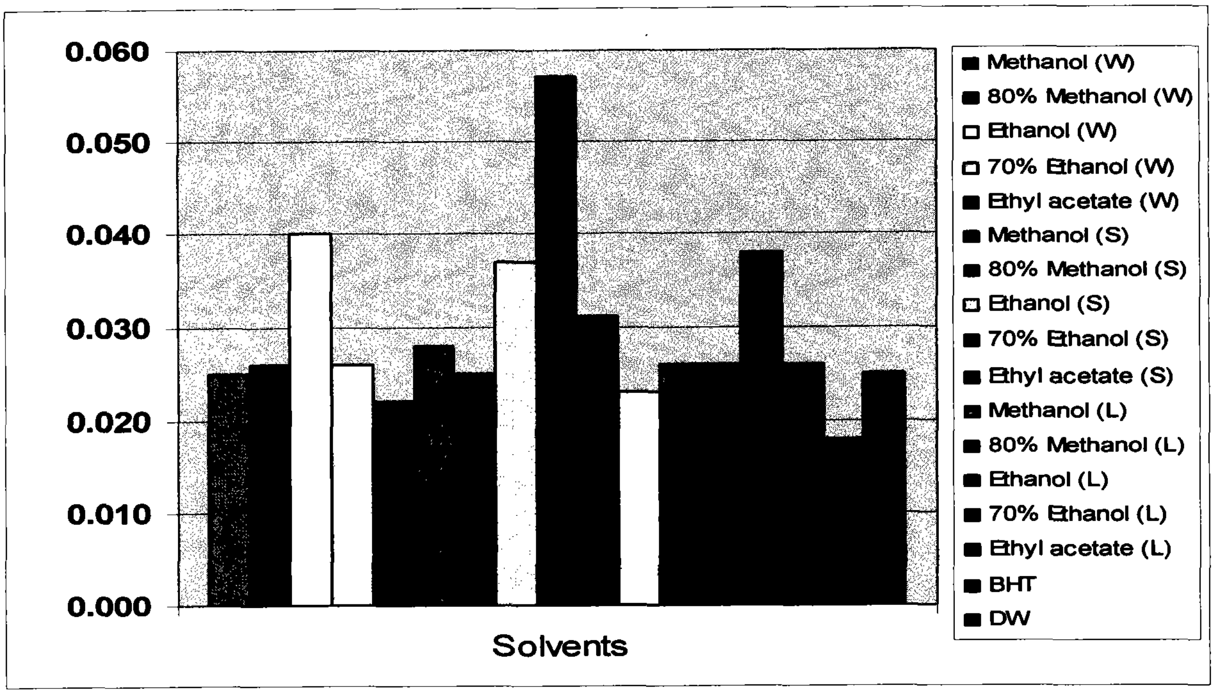

Fig. 3 Antioxidant activities soaking samples for TBA method

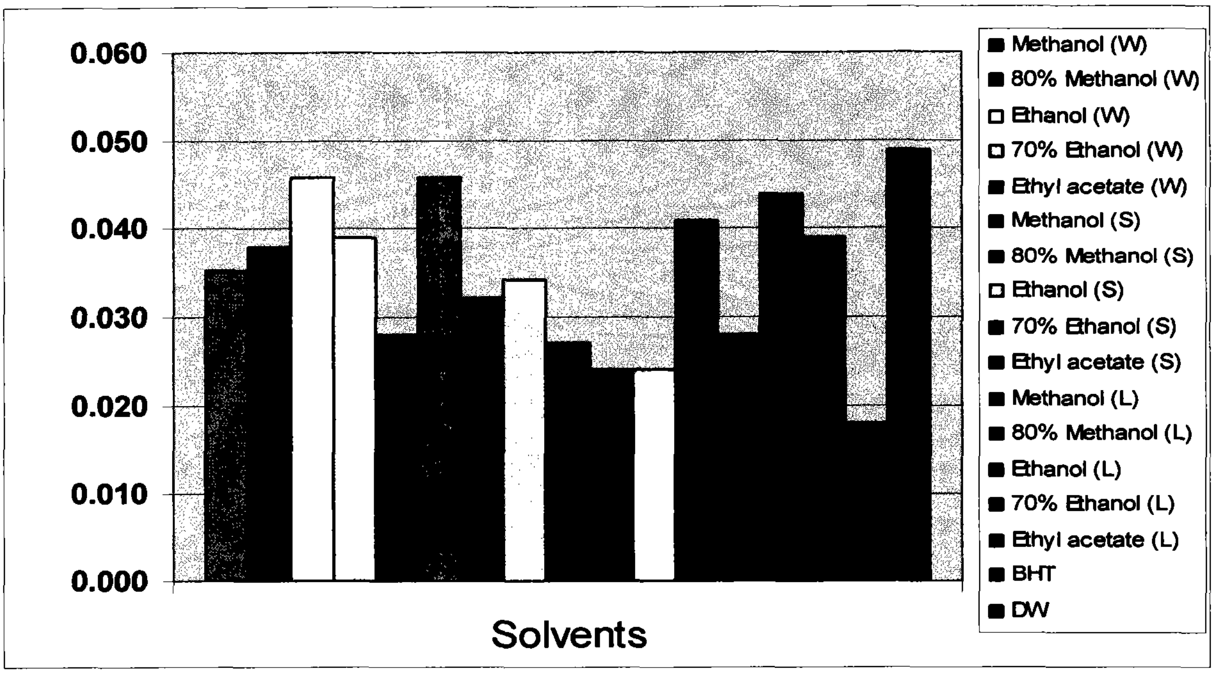

Fig. 4 Antioxidant activities boiling samples for TBA method

3.2 Growth performance and body composition of tilapia

Survival ranged from 86.67 to 95.56%. RGR, SGR, FCR, PER and survival are shown in Table 4. The comparative bar chart are indicated in Fig. 5 to 8. Percent weight gain or relative growth rate (RGR) of tilapia increased linearly from diet control till diet 3 and but no further increment ($P>0.05$) was observed when feeding rate increased beyond diet 3. Control diet showed the lowest SGR followed by diet 5, 1, 2, 4 and 3 ($P>0.05$). Feed conversion ratio (FCR) did not differ significantly among diet 2, 3, and 5, but significantly differ between diet 4 and 1 where diet 4 had the best FCR in the experiment. Protein efficiency ratio (PER) was highest in fish fed diet 3 followed by diet 4, control, 2, 1, and 5 ($P>0.05$). HSI, VSI and IPF of tilapia were not significantly different.

Table 4. Growth performance and feed utilization of tilapia, fed different dietary sources[1]

	Diet					
	C	**1**	**2**	**3**	**4**	**5**
Initial weight (g)	10.03 ±0.17^a	10.02 ±0.31^a	9.89 ±0.11^a	9.73 ±0.29^a	9.92 ±0.54^a	9.69 ±0.32^a
Final weight (g)	43.13 ±6.22^a	46.94 ±7.69^a	50.58 ±3.94^a	50.83 ±6.51^a	48.67 ±6.12^a	46.90 ±5.21^a
% Survival	93.33	91.11	93.33	86.67	95.56	95.56
RGR	329.82 ±62.00^a	368.38 ±76.77^a	411.44 ±39.85^a	422.16 ±66.93^a	390.45 ±61.74^a	384.14 ±53.75^a
SGR	1.15±0.85^a	1.49±1.17^a	1.62±0.56^a	1.98±0.95^a	1.79±0.87^a	1.33±0.81^a
FCR	1.25±0.26^{ab}	1.45±0.24^b	1.33±0.11^{ab}	1.34±0.20^{ab}	1.12±0.90^a	1.44±0.22^{ab}
PER	1.81±1.35^a	1.59±1.33^a	1.65±0.63^a	1.98±1.02^a	1.82±1.01^a	1.32±0.85^a

[1] Values are the mean of triplicate of 20 fish. Mean values in rows with different superscripts are significantly different ($P<0.05$).

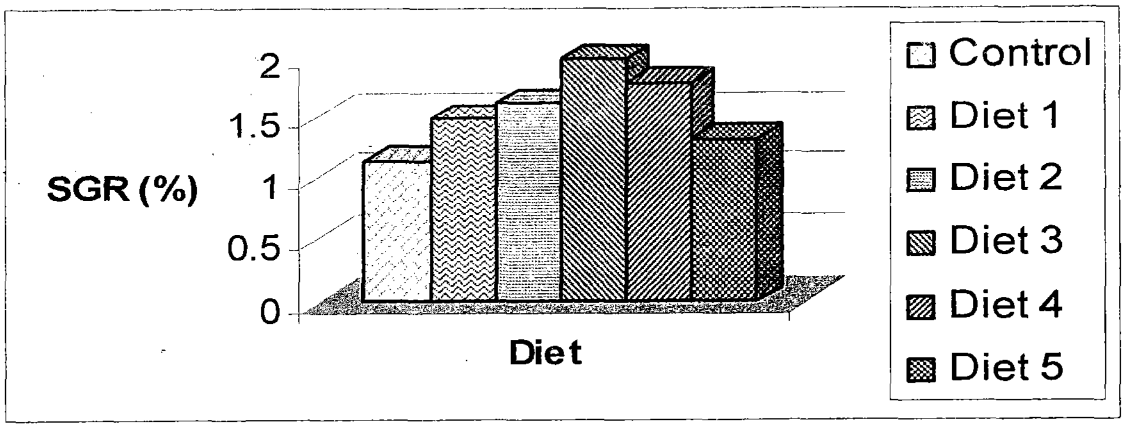

Fig. 5 SGR (%) bar chart of tilapia fed different dietary sources

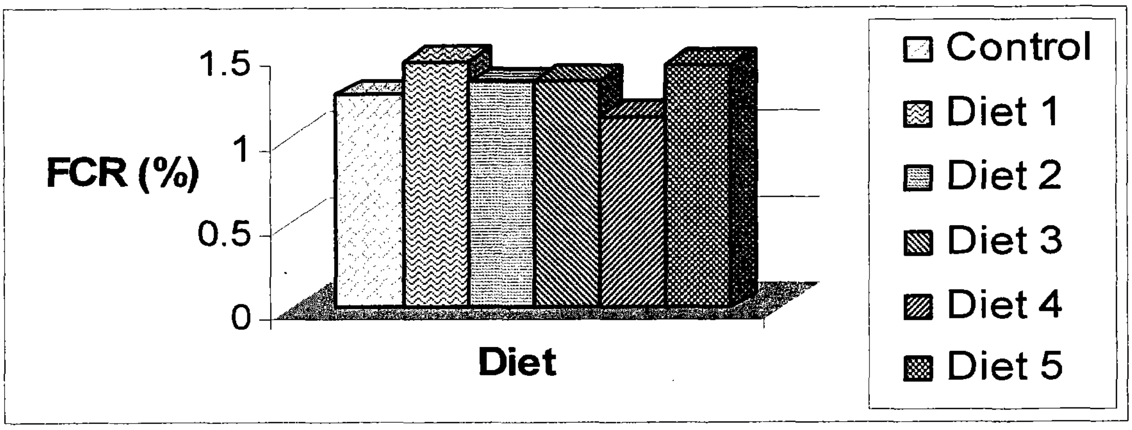

Fig. 6 FCR (%) bar chart of tilapia fed different dietary sources

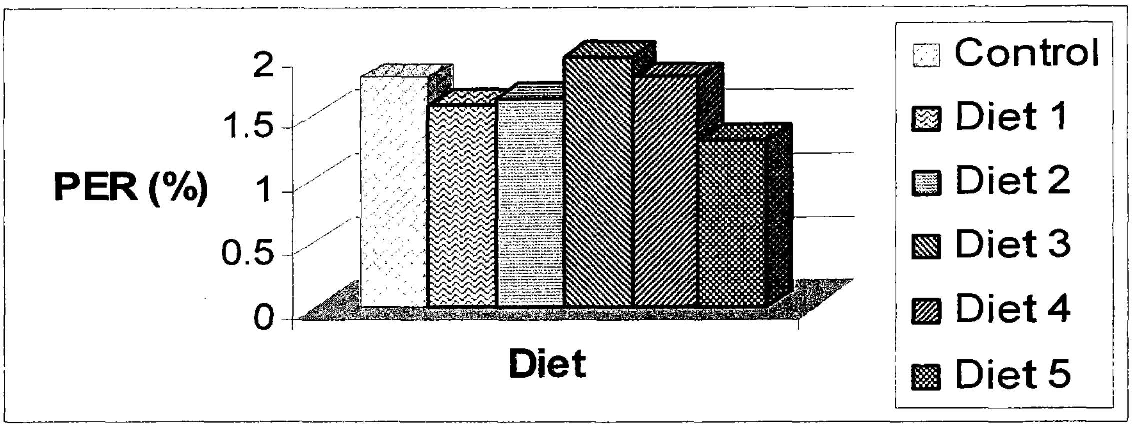

Fig. 7 PER (%) bar chart of tilapia fed different dietary sources

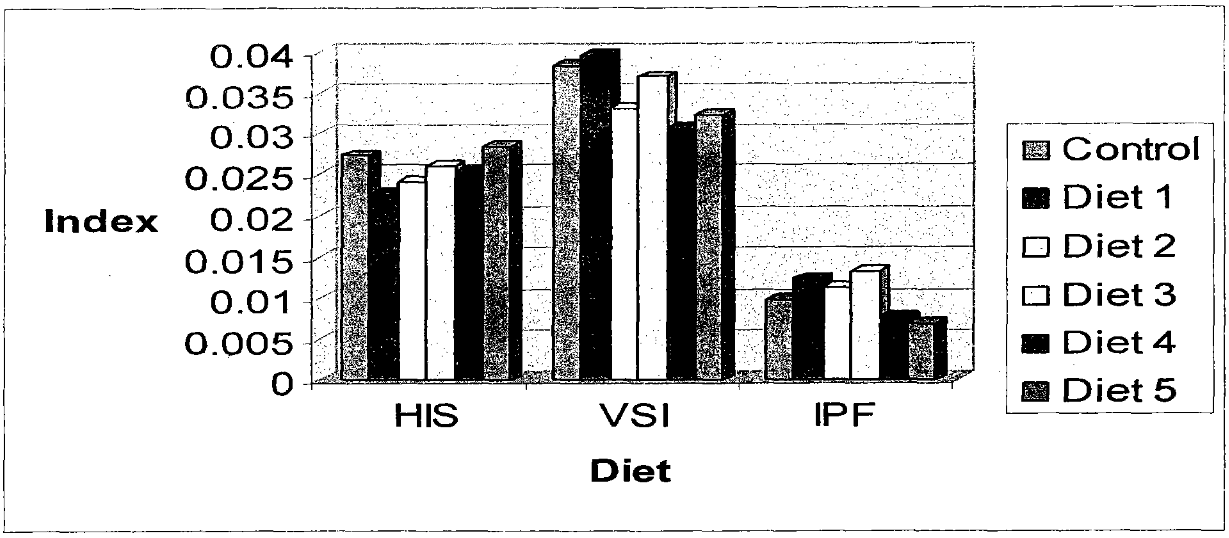

Fig. 8 Hepatosomatic, viscerosomatic and intraperitoneal index of tilapia fed different dietary sources

3.3 Proximate composition

The proximate compositions of the final experiment fish carcass are given in Table 5. Lipid was highest in fish fed diet 3, followed by diet 1, 2, control, 5 and 4. Moisture content of the fish was highest in fish fed diet 4 and no significant different among the other diet. Meanwhile the protein content increased with increasing dietary extract up to diet 5.

Table 5. Proximate composition of tilapia fed different dietary[1]

	Diet					
	C	**1**	**2**	**3**	**4**	**5**
Moisture	88.09 ±1.83^{a}	87.99 ±4.06^{a}	88.67 ±1.02^{a}	86.19 ±2.92^{a}	89.76 ±1.51^{a}	88.98 ±2.63^{a}
Protein	54.66 ±1.17^{a}	55.64 ±2.63^{ab}	55.74 ±1.44^{ab}	59.11 ±0.67^{bc}	59.51 ±3.49^{bc}	59.90 ±2.09^{c}
Lipid	17.29 ±3.21^{ab}	20.63 ±2.14^{b}	20.46 ±1.75^{b}	20.97 ±0.92^{b}	15.04 ±3.27^{a}	17.18 ±2.18^{ab}
Ash	6.07 ±1.97^{a}	4.58 ±0.61^{a}	4.74 ±0.93^{a}	4.62 ±0.40^{a}	4.91 ±1.03^{a}	4.54 ±1.27^{a}

[1] Values are the mean of triplicate group of five fish.

3.4 Water quality

The mean water temperature for the month of April was in a range of 27.4 – 30.7°C. But when it came to the 5th week (16th-18th, May), the water temperature dropped till the range of 26.3 – 28.6°C. For the following days till the end of the experiment (19th May – 2nd June), the water temperature was in a range of 27.7 – 30.0°C (Table 6). DO concentrations was always above 26.0 mg/l and pH was also maintained at the range of 6.13 – 6.49 (Table 7). Mean concentrations of phosphorus, ammonia, nitrate and nitrite varied from 0.08-0.26 mg/l, 0.09-0.34 mg/l, 0.008-0.010 mg/l and 8.00-12.2 mg/l, respectively, and were not significantly different (Table 8).

Table 6. Water temperature throughout the experiment from 7th April till 2nd June.

Sample	**7th/4** (1st day)	**21st/4** (1st sampling)	**5th/5** (2nd sampling)	**16th/5**	**17th/5**	**18th/5**
C1	30.2	30.6	30.5	27.0	26.7	27.5
C2	30.2	30.5	30.6	27.3	26.8	27.5
C2	30.3	30.4	30.6	27.1	26.3	27.8
1A	30.4	30.3	30.4	27.2	26.2	28.5
1B	30.4	30.2	30.4	26.9	26.7	26.9
1C	30.5	30.2	30.4	26.9	26.7	28.4
2A	30.5	30.4	30.3	27.1	26.7	27.5
2B	30.6	30.4	30.3	26.8	26.5	27.6
2C	30.4	30.5	30.1	27.2	26.9	27.4
3A	30.2	30.6	30.5	27.1	26.7	28.1
3B	30.2	30.5	30.5	26.6	26.9	28.3
3C	30.3	30.1	30.5	26.9	27.1	27.6
4A	30.4	30.7	30.6	27.0	27.1	27.8
4B	30.6	30.7	30.7	27.0	26.7	27.9
4C	30.5	30.7	30.4	27.1	26.6	27.5
5A	30.4	30.7	30.4	27.2	26.6	28.4
5B	30.5	30.6	30.3	27.2	26.3	28.6
5C	30.6	30.5	30.3	27.1	26.6	27.9

		19th/5 (3rd sampling)	**2nd/6** (4th sampling)
C1		29.9	29.9
C2		30.0	29.5
C3		29.9	29.6
1A		29.8	29.8
1B		29.7	29.8
1C		29.9	29.8
2A		29.9	29.7
2B		29.9	29.8
2C		29.9	29.6
3A		29.8	29.9
3B		29.8	29.4
3C		29.8	29.5
4A		29.8	30.0
4B		29.8	30.0
4C		29.8	29.9
5A		29.7	29.6
5B		29.9	29.6
5C		29.9	29.5

Table 7. Mean values[1] water physical factor throughout the experiment

Aquarium	**Physical factor**	
	DO (mg/l)	**pH**
C1	3.6±0.00	6.49±0.01
C2	3.6±0.00	6.28±0.01
C3	3.6±0.00	6.27±0.01
1A	3.2±0.00	6.22±0.00
1B	3.0±0.00	6.19±0.01
1C	3.1±0.00	6.13±0.01
2A	4.1±0.00	6.18±0.00
2B	4.1±0.00	6.21±0.01
2C	4.0±0.00	6.14±0.00
3A	3.8±0.00	6.28±0.00
3B	2.8±0.00	6.16±0.01
3C	3.7±0.00	6.28±0.00
4A	2.8±0.00	6.35±0.01
4B	3.7±0.00	6.25±0.00
4C	3.5±0.00	6.24±0.01
5A	3.6±0.00	6.25±0.01
5B	2.7±0.00	6.24±0.01
5C	2.7±0.00	6.27±0.01

[1] Values are mean in triplicate

Table 8. Water quality parameters for the experiment period in aquarium

Parameter (mg/L)	**Diet**					
	C	**1**	**2**	**3**	**4**	**5**
Ammonia	2.20±0.26^a	2.20±0.47^a	2.23±0.26^a	2.15±0.18^a	2.09±0.38^a	2.34±0.27^a
Nitrite	8.30±2.12^a	8.00±0.58^a	10.1±0.96^a	11.2±0.95^a	11.6±0.52^a	12.2±1.47^a
Nitrate	0.010±0.00^a	0.008±0.00^a	0.010±0.00^a	0.009±0.00^a	0.008±0.00^a	0.017±0.01^a
Phosphorus	0.16±0.09^a	0.40±0.42^a	0.08±0.26^a	0.26±0.15^a	0.08±0.08^a	0.16±0.10^a

3.5 Oxidative stability of fish muscle

TBA values for the muscle at the 6th and 7th hour showed significant different compared with the other hours. No significant differences among others (Table 9). The TBA concentrations in the muscle (MDA/kg) for the control diet and diet 5 were significantly different with all the hours except the 2nd hour (Fig. 9A). Fig. 8B showed the TBA values of the diet 5 was the lowest within the hours compared with the others. The trend was observed at all post storage hours tested.

Table 9. TBA value[1] (mg malonaldehyde/kg sample) of tilapia muscle fed different sources from 1st hour till the 8th

	TBA ($mg.kg^{-1}$)					
Hour	C	1	2	3	4	5
1	0.051±0.00	0.047±0.012	0.051±0.00	0.044±0.00	0.035±0.01	0.030±0.01
2	0.047±0.00	0.045±0.02	0.040±0.01	0.043±0.00	0.036±0.01	0.038±0.00
3	0.052±0.00	0.042±0.01	0.042±0.00	0.043±0.00	0.040±0.00	0.036±0.00
4	0.053±0.00	0.047±0.01	0.047±0.00	0.044±0.01	0.041±0.01	0.041±0.00
5	0.052±0.01	0.044±0.01	0.040±0.01	0.047±0.00	0.045±0.01	0.040±0.00
6	0.052±0.01	0.046±0.011	0.045±0.01	0.051±0.01	0.050±0.00	0.037±0.01
7	0.065±0.00	0.044±0.011	0.047±0.01	0.045±0.01	0.045±0.00	0.040±0.01
8	0.051±0.00	0.049±0.00	0.048±0.00	0.043±0.00	0.042±0.00	0.037±0.00

[1] Values are the mean of triplicate groups of five fish

Hour[b]	TBA ($mg.kg^{-1}$)
1	ab
2	a
3	a
4	ab
5	ab
6	b
7	b
8	ab

Diet[b]	TBA ($mg.kg^{-1}$)
C	c
1	b
2	b
3	b
4	b
5	a

[b] Mean at the same column with the same alphabet is not significantly different (*P<0.05*).

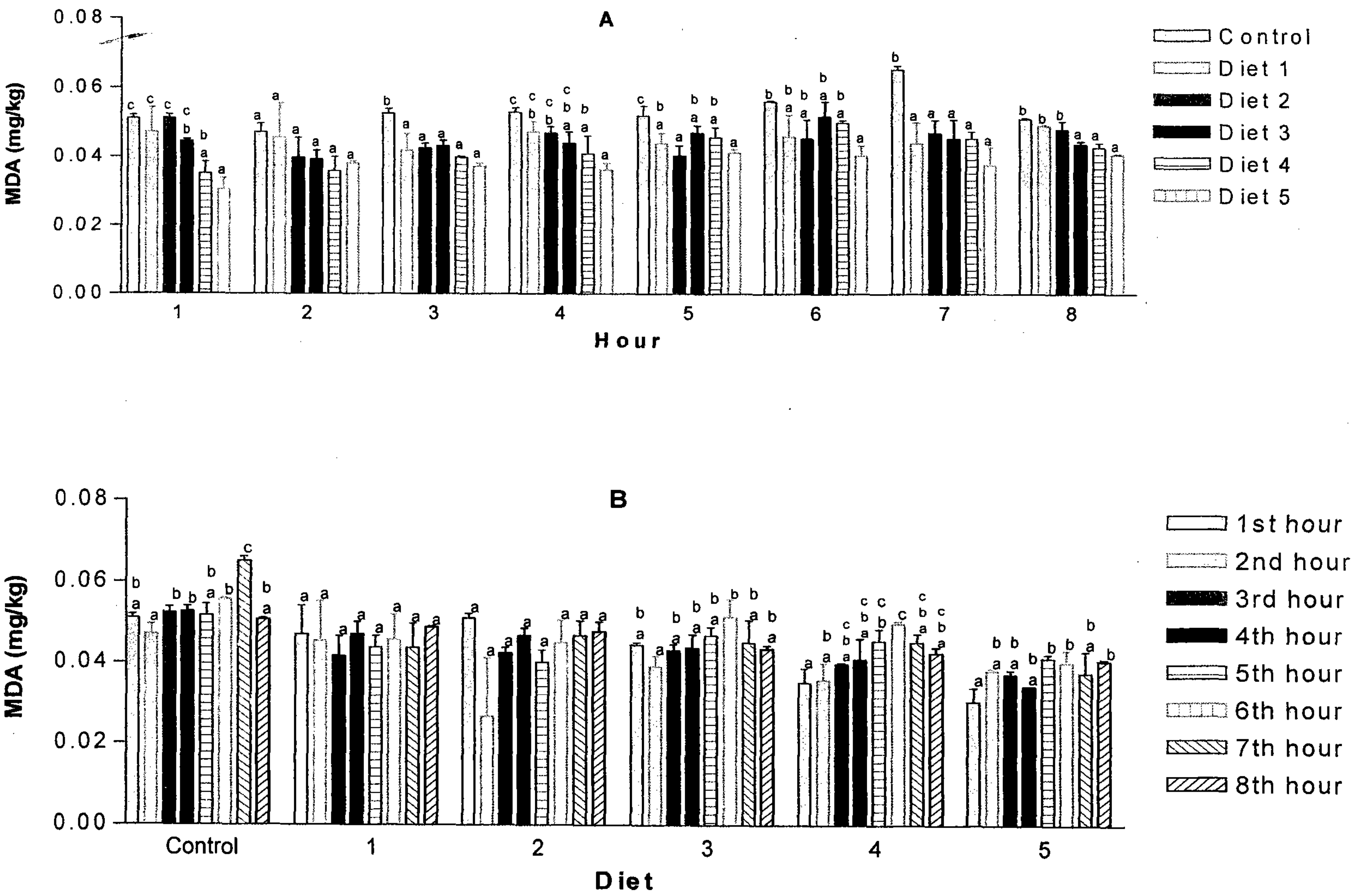

Fig. 9A, B. Effects of different dietary sources with TBA method. Values are the mean of triplicate groups of five fish. Mean values with the same alphabet are not significantly different (*P<0.05*)

3.6 Fatty acid analysis

Even though there were no significant different between SFA (total saturated fatty acid), MUFA (monounsaturated fatty acid) and PUFA (polyunsaturated fatty acid), but still there were some differences of the percentage fatty acid among the diet (Table 10). For example, palmitic acid (16:0) was the highest percentage content among the SFA. There were significantly different for myristic acid (C14.0) between diet 2 with diet 4 and diet 5 yet the percentage content of the fatty acid was increased with increasing dietary extract. No significant different between the concentrations of palmitic acid and stearic acid (C18:0) in the diet.

In the example of MUFA, no significant different were showed among the diet. Concentrations of oleic acid (C18:1n9) was increased from diet 1 till diet 5, while the concentrations for the control diet was between diet 2 and 3.

For PUFA, concentrations of DHA (C22:6n3, docosahezaenoic acid) was decreased from diet 1 until diet 5, while the concentrations for the control diet was between diet 1 and 2. For C20:5n3 (EPA, eicosapentaenoic acid), the control diet had the highest concentrations followed by diet 2, 3, 4, 1 and 5. For linoleic acid (C18:2n6), there were significantly different between diet 1 with diet 4 and 5. Diet 5 had the highest concentrations of linoleic acid followed by diet 4, 2, control, 3 and 1. Meanwhile the concentrations of α-linolenic acid (C18:3n3) was increased from the control diet until diet 5.

No significant different for the n-3 among the diet. Concentrations of it decreased from diet 1 until diet 5 and a concentration of the control diet was between diet 1 and 2. Meanwhile for the n-6, there were significant different between diet 1 and diet 5, and after all, concentrations of the n-6 were increased throughout the experiment. Ratios for the n-3/n-6 were decreased from diet 1 until diet 5 and the control diet was between diet 1 and 2.

Table 10. Fatty acid composition (% of total fatty acid) of experiment diets

Fatty Acid	**Lipid source**					
	Diet K	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
C14:0	1.61 ± 0.13^{ab}	1.00 ± 0.43^{a}	1.70 ± 0.26^{ab}	1.78 ± 0.35^{ab}	1.86 ± 0.69^{b}	2.03 ± 0.21^{b}
C16:0	15.43 ± 0.19^{a}	13.06 ± 6.12^{a}	16.16 ± 0.81^{a}	15.33 ± 0.24^{a}	14.23 ± 1.16^{a}	14.92 ± 0.80^{a}
C18:0	7.46 ± 0.55^{a}	5.68 ± 3.11^{a}	7.03 ± 0.28^{a}	6.05 ± 0.52^{a}	6.11 ± 2.26^{a}	6.34 ± 0.66^{a}
∑ saturated	24.49 ± 0.16^{a}	19.74 ± 5.53^{a}	24.89 ± 0.55^{a}	23.15 ± 0.08^{a}	22.20 ± 1.34^{a}	23.29 ± 0.30^{a}
C16:1n7	2.37 ± 0.3^{a}	1.72 ± 0.67^{a}	1.83 ± 0.30^{a}	3.01 ± 0.56^{a}	2.80 ± 1.28^{a}	2.88 ± 0.45^{a}
C18:1n9	15.96 ± 0.23^{a}	12.71 ± 5.55^{a}	14.97 ± 0.54^{a}	16.78 ± 1.73^{a}	17.56 ± 4.20^{a}	18.29 ± 1.56^{a}
C18:1n7	2.58 ± 2.17^{a}	2.89 ± 1.29^{a}	3.48 ± 0.36^{a}	3.41 ± 0.24^{a}	3.07 ± 1.79^{a}	4.25 ± 0.19^{a}
C20:1n9	3.68 ± 0.36^{a}	4.28 ± 2.32^{a}	2.34 ± 0.07^{a}	3.60 ± 0.28^{a}	3.42 ± 0.97^{a}	2.31 ± 0.08^{a}
C22:1n11	0.93 ± 0.74^{a}	2.20 ± 1.53^{a}	1.55 ± 0.12^{a}	2.47 ± 0.29^{a}	2.12 ± 0.84^{a}	2.23 ± 0.34^{a}
∑monosaturated	25.52 ± 1.14^{a}	23.80 ± 2.31^{a}	24.17 ± 0.40^{a}	29.26 ± 1.63^{a}	28.97 ± 4.31^{a}	29.96 ± 1.19^{a}
C16:4n3	0.31 ± 0.15^{a}	0.22 ± 0.05^{a}	0.19 ± 0.22^{a}	0.28 ± 0.23^{a}	0.47 ± 0.19^{a}	0.44 ± 0.33^{a}
C18:3n3	0.70 ± 0.13^{a}	0.92 ± 0.35^{a}	0.94 ± 0.26^{a}	0.98 ± 0.14^{a}	2.22 ± 2.35^{a}	2.30 ± 1.08^{a}
C20:4n3	0.54 ± 0.18^{a}	0.58 ± 0.44^{a}	0.35 ± 0.04^{a}	0.49 ± 0.01^{a}	0.51 ± 0.08^{a}	0.34 ± 0.08^{a}
C20:5n3	1.83 ± 0.14^{a}	1.55 ± 0.44^{a}	1.74 ± 0.10^{a}	1.68 ± 0.27^{a}	1.68 ± 0.29^{a}	1.34 ± 0.03^{a}
C22:5n3	5.33 ± 0.39^{a}	4.07 ± 0.98^{a}	4.78 ± 0.11^{a}	5.15 ± 0.30^{a}	4.70 ± 0.41^{a}	4.01 ± 0.19^{a}
C22:6n3	23.05 ± 0.73^{ab}	27.73 ± 9.17^{b}	22.86 ± 0.63^{ab}	20.67 ± 3.17^{ab}	19.08 ± 5.72^{ab}	16.80 ± 0.82^{a}
C18:4n3	0.20 ± 0.03^{a}	0.23 ± 0.11^{a}	0.20 ± 0.03^{a}	0.32 ± 0.18^{a}	0.21 ± 0.27^{a}	0.70 ± 0.80^{a}
Total n-3	31.95 ± 0.63^{a}	35.30 ± 5.22^{a}	31.06 ± 0.62^{a}	29.57 ± 2.02^{a}	28.87 ± 4.11^{a}	25.93 ± 1.42^{a}
C18:2n6	15.16 ± 1.21^{ab}	12.27 ± 5.28^{a}	16.36 ± 0.77^{ab}	15.10 ± 0.72^{ab}	17.38 ± 2.25^{b}	18.35 ± 0.26^{b}
C20:4n6	2.43 ± 0.04^{ab}	3.63 ± 0.91^{c}	3.01 ± 0.24^{bc}	2.38 ± 0.32^{ab}	2.11 0.61^{ab}	1.88 ± 0.30^{a}
Total n-6	17.58 ± 0.72^{ab}	15.90 ± 2.65^{a}	19.37 ± 0.56^{ab}	17.49 ± 0.31^{ab}	19.50 ± 0.96^{ab}	20.23 ± 0.28^{b}
C16:3n4	0.28 ± 0.09^{a}	0.24 ± 0.12^{a}	0.33 ± 0.09^{a}	0.30 ± 0.09^{a}	0.21 ± 0.18^{a}	0.34 ± 0.01^{a}
C16:2n4	0.13 ± 0.11^{b}	0.12 ± 0.07^{b}	0.18 ± 0.03^{bc}	0^{a}	0.25 ± 0.05^{c}	0.25 ± 0.04^{c}
C18:3n4	0.05 ± 0.09^{a}	0^{a}	0^{a}	0.22 ± 0.39^{a}	0^{a}	0^{a}
Total PUFA	49.99 ± 1.27^{a}	51.56 ± 7.39^{a}	50.94 ± 0.95^{a}	47.57 ± 1.59^{a}	48.83 ± 3.06^{a}	46.75 ± 1.36^{a}
n-3/n-6	1.82 ± 0.05^{a}	2.22 ± 0.90^{a}	1.61 ± 0.05^{a}	1.69 ± 0.14^{a}	1.51 ± 0.27^{a}	1.28 ± 0.08^{a}

^{a} – Values are the mean of triplicate. Mean values in rows with different superscripts are significantly different ($P<0.05$).

3.7 SDS-PAGE

The electrophoresis results in this study showed that different dietary lipid sources caused the changes in protein muscle of tilapia from the 1st hour till the 8th hour. The molecular weight and intensities of the proteins found in the experiment fish are presented in Table 11, where it was analyzed by Gel Doc (Versa Doc, Imaging System Model 4000) automatically.

In order to evaluate, the gels were divided into three parts (a, b, c) as it's shown in Fig. 10A (Fig. 10A-H). As we can see in Table 12, the numbers of the protein bands in each diet were increased as the length of storage increased. Table 13 showed the number of the protein bands compare among the diet within the hours. Even though there were not very much significant differences among the diet, but still the samples diet (diet 1-5) showed the numbers of the protein bands were lower or comparable with the control diet. For example, diet 5 showed the lower numbers of protein bands than the control diet at the 2nd, 4th, 5th, 7th and 8th hour.

Table 11. Molecular weight and peak intensity from 1st hour till the 8th hour.

GEL 1 (1st Hour)								
	Molecular weight KDa	Peak Intensity		Molecular weight KDa	Peak Intensity		Molecular weight KDa	Peak Intensity
Control diet (column 4)			Diet 2 (column 6)			Diet 4 (column 8)		
(a)	379.058	213.75	(a)	290.853	205.125	(a)	388.742	618.813
	191.826	1596.56		181.240	1558.688		177.845	1169.125
	132.547	83.75		97.164	229.125		140.038	138.25
	98.73	248.75		84.147	95.938		94.409	124.563
	91.731	66.313	(b)	67.938	44		90.277	76.75
	83.344	83.625		59.008	66.188	(b)	65.303	93.188
(b)	76.208	44.5		51.378	122.375		60.336	95.875
	71.736	59.063		42.723	1366.688		57.567	113.063
	67.436	96.813		36.942	204.563		49.441	131.438
	56.3	51.438		35.755	503		41.4	919.375
	51.378	147		31.132	433.25		35.811	197.563
	42.531	1530.56		29.163	264.375		34.66	278.063
	40.117	72	(c)	27.617	305.313	(c)	30.131	114
	37	131.5		25.71	361.938		17.463	304.75
	35.589	541.375		18.084	649.438		16.76	224.813
(c)	31.035	254.938		17.321	524.063		15.856	278.875
	17.789	283.313		15.124	1701.938	Diet 5 (column 9)		
	17.356	143.5	Diet 3 (column 7)			(a)	379.058	132.75
	15.124	1433.06	(a)	358.142	358.188		281.402	254.188
Diet 1 (column 5)				193.04	777.812		154.805	1921.688
(a)	371.954	61.438		97.787	127.75		105.652	214.375
	199.224	858.625		94.409	75.438		99.046	121.563
	98.73	146.563		79.696	92.375		88.562	374.625
	93.209	134.375	(b)	74.079	82.625		85.502	245.188
	88.846	93.625		67.436	93.625	(b)	80.722	71.063
(b)	70.679	67.25		63.081	92.563		61.846	73.813
	57.283	132.938		59.154	76.813		56.161	156.688
	51.378	120.188		50.999	86.688		53.452	170
	43.498	589.938		43.205	666.5		45.702	157.688
	42.056	86.75		36.77	91.438		38.268	1545.938
	36.828	179.438		35.755	167.438		34.178	445.375
(c)	35.811	277.25		34.072	52.563		33.131	563.188
	33.86	345.625	(c)	32.116	59.188		29.027	255.313
	23.644	591.375		30.891	104.438	(c)	24.031	84.188
				30.178	77.625		22.517	47.438
				17.608	202.25		20.758	91.875
				16.487	173.938		17.073	357.688
							15.954	410.438

GEL 2 (2nd Hour)

Control diet (column 4)

	Molecular weight KDa	Peak Intensity
(a)	353.574	70.25
	216.05	902.25
(b)	176.766	76.5
	137.156	59.5
	109.365	142.625
	70.339	71.813
	58.024	71.313
	54.839	51.438
	52.633	76.5
	49.176	71.563
	44.696	607.875
(c)	43.415	86.375
	38.409	139.688
	37	204.688
	34.737	222.188
	31.546	225.938
	29.225	98.125
	26.321	188.813
	25	160.188
	19.419	78.875
	17.774	60.5
	15.853	131.188

Diet 1 (column 5)

	Molecular weight KDa	Peak Intensity
(a)	401.738	297.313
	373.465	246.125
	197.214	1534.375
(b)	162.834	142.563
	106.523	187.875
	99.035	157.688
	93.136	126.063
	88.156	126
	81.576	167.438
	63.968	176.938
	54.419	432.313
	52.633	176.125
	44.419	578.188
(c)	38.091	240.875
	36.816	330.125
	34.679	425.25
	33.546	428.5
	31.494	469.063
	19.707	934.313
	16.879	1023.063

Diet 2 (column 6)

	Molecular weight KDa	Peak Intensity
(a)	366.713	252.313
	325.706	149.563
	180.021	1734.063
(b)	147.649	195.438
	127.408	97.75
	102.129	252.625
	96.819	134.625
	86.463	102.5
	51.432	114.688
	43.325	1336.75
(c)	41.134	132.125
	38.972	96.875
	37.776	230.125
	36.451	455.938
	31.285	277
	27.574	125.875
	16.632	286.563
	15.679	107.688
	13.985	118.875
	12.474	250.625

Diet 3 (column 7)

	Molecular weight KDa	Peak Intensity
	173.571	1582.313
(b)	134.297	314.563
	95.576	121.813
	51.696	99.438
	43.778	936.125
(c)	42.523	154.438
	38.012	259.813
	36.694	327.188
	36.03	132.625
	33.882	200.313
	31.494	275.563
	16.693	602.313
	13.985	602.25
	12.428	609.375

Diet 4 (column 8)

	Molecular weight KDa	Peak Intensity
(a)	302.784	270.5
	271.39	194.875
	147.649	2229.625
(b)	130.807	406.625
	115.887	169.063
	96.819	291.813
	92.536	280.375
	62.028	175.25
	60.147	84
	50.776	297.938
	42.878	1554.188
(c)	41.649	454.75
	37.386	423.188
	36.21	453.875
	34.737	424.75
	32.128	165.25
	31.285	189.438
	27.897	242.25
	16.632	516.688
	13.985	502
	12.428	547.688

Diet 5 (column 9)

	Molecular weight KDa	Peak Intensity
	149.212	1295.375
(b)	91.347	128.313
	71.431	155.563
	49.176	270.625
	47.866	323.875
	43.146	596.125
(c)	41.219	292.313
	36.939	485.438
	35.85	185.25
	33.323	225.688
	31.285	157.75
	29.469	166.313
	12.246	280.813

GEL 3 (3^{rd} Hour)

Control diet (column 3)

	Molecular weight KDa	Peak Intensity
(a)	336.322	169.625
	188.921	1231.563
(b)	103.049	192.5
	95.12	137.438
	72.303	151.688
	66.323	53.688
	49.501	203.125
	42.753	1190.875
(c)	41.402	283.813
	37.373	276.563
	36.184	250.875
	34.439	154.5
	31.249	209.25
	27.64	85.125
	25.975	93.25
	22.986	82.188
	18.309	270.313
	16.719	322.938
	15.461	328

Diet 1 (column 4)

	Molecular weight KDa	Peak Intensity
(a)	294.784	109
	164.23	1094
(b)	119.746	122.188
	98.141	166.438
	66.671	76
	59.422	214.125
	42.925	1200.313
(c)	37.524	208.75
	36.241	393.063
	32.936	48.688
	31.499	197.438
	25.892	100
	22.986	76.875
	18.402	251.875
	17.408	81.688
	15.539	270.563

Diet 2 (column 5)

	Molecular weight KDa	Peak Intensity
(a)	273.716	121.125
	153.754	1198.25
(b)	131.037	341.313
	116.203	107.438
	94.823	134
	90.478	87.625
	64.442	156.938
	49.302	195.125
	42.497	915.188
(c)	37.223	157.688
	36.068	313.938
	31.349	156.75
	26.224	265.688
	24.609	240.938
	18.495	240.5
	17.147	132.25
	15.539	144.875

Diet 3 (column 7)

	Molecular weight KDa	Peak Intensity
	147.026	1604.375
(b)	125.265	348.625
	94.232	135.125
	90.478	65.625
	86.332	41.563
	70.806	58.813
	49.105	156.75
	42.411	1175.25
(c)	37.373	216
	36.184	332.188
	34.549	84.125
	31.499	174.688
	26.183	74.5
	23.412	75.875
	18.263	181.25
	16.635	78.688
	15.5	237.5

Diet 4 (column 8)

	Molecular weight KDa	Peak Intensity
	145.562	1435.313
(b)	94.232	145.125
	89.914	149.625
	60.048	237.813
	49.401	293
	42.753	970.25
(c)	41.402	256.375
	37.298	271.688
	36.184	185.625
	31.954	151.813
	18.495	151.563
	16.262	187.875

Diet 5 (column 9)

	Molecular weight KDa	Peak Intensity
	144.835	1036.375
(b)	125.265	247.625
	93.06	104.063
	88.796	91.375
	64.611	56.438
	54.082	232.688
	48.517	142
	45.957	84
	42.157	807.313
(c)	40.824	168.063
	36.824	177.688
	35.782	167.813
	31.001	93.25
	27.684	59.25
	23.659	130.938
	18.08	136.375

GEL 4 (4th Hour)

	Molecular weight KDa	Peak Intensity
Control diet (column 4)		
(a)	365.626	874.375
	199.229	2134.875
(b)	135.984	689.438
	113.23	382.375
	101.317	804.375
	95.758	706.75
	92.421	818.5
	89.553	887.125
	78.632	233.375
	71.493	123.625
	67.5	269.25
	59.598	380.5
	55.733	150.438
	51.787	166.125
	47.63	590.313
	41.072	2265.188
(c)	39.22	2119.875
	37.361	1149.125
	35.028	1185.625
	33.388	1736.875
	29.974	1148.625
	26.498	225.688
	25.301	207.063
	23.124	322.188
	22.192	200.688
	19.631	436.688
	16.978	1645
	15.695	806.875
	14.241	514.813
	14.09	977.688
Diet 1 (column 5)		
(a)	406.884	522.5
	365.626	409.813
	152.502	2439.25
(b)	134.217	359.875
	120.883	180.5
	99.215	280.625
	95.758	399.938
	93.152	210.25
	87.116	160.313
	68.586	346.375
	59.788	96.813
	47.514	253.313
	46.827	176.813

	Molecular weight KDa	Peak Intensity
	41.878	1811.625
	35.268	450.438
	33.56	1200.063
	30.129	614.188
	23.225	215.25
	22.482	222.875
	19.762	423.063
	18.39	127.375
	17.183	891.625
	16.82	326
	15.926	643.375
	14.374	423.125
Diet 2 (column 6)		
	230.052	1663.625
(b)	148.051	429
	136.876	156.688
	100	275.25
	68.806	199.563
	48.8	266.563
	42.39	1621.688
(c)	40.478	978.813
	38.747	252.563
	35.755	306.375
	33.907	1432.5
	30.44	656.938
	23.631	275.188
	19.92	444.5
	17.183	1230.563
	16.843	517.875
	15.863	769.125
Diet 3 (column 7)		
	268.47	369.375
(b)	70.361	227.5
	43.962	459.375
(c)	42.596	380.25
	34.315	1316.875
	30.702	759.688
	17.228	738.188
	16.686	398.125
	15.905	680.125
	14.393	473.813

	Molecular weight KDa	Peak Intensity
Diet 4 (column 8)		
(a)	357.041	298.313
	221.997	1963.438
(b)	181.401	603.438
	148.051	274.75
	133.342	81.938
	117.762	281.063
	105.371	344.375
	98.049	290.625
	90.618	118.75
	69.468	118.125
	61.335	235.563
	57.541	191.375
	48.564	367.688
	43.116	1241.375
(c)	41.675	1091.625
	40.675	989.688
	39.03	339.938
	35.816	622.563
	34.257	544.75
	30.544	321.188
	28.572	334.5
	28.087	437.75
	27.002	526
	26.726	467.688
	23.024	262
	19.867	211.438
	17.183	799.438
	15.884	253
	14.393	396.375
Diet 5 (kolum 9)		
(a)	374.417	202.125
	211.695	1600.375
(b)	113.973	175.188
	91.695	206.25
	58.281	111.188
	47.055	224.875
	42.082	817.625
(c)	40.675	584.438
	39.893	524.875
	37.726	317.063
	35.028	333.375
	33.56	273.688
	32.99	165.75
	20.571	111.813
	16.91	280.688

GEL 5 (5th Hour)

	Molecular weight KDa	Peak Intensity
Control diet (column 4)		
	391.892	784.5
(a)	329.411	732.125
	171.308	2502
	118.877	409
	96.073	1124.688
	85.506	209.313
(b)	82.148	255.5
	80.081	170.5
	65.519	311.75
	48.65	543.375
	47.837	354.375
	40.763	2677.625
	36.042	920.875
	34.367	1934.063
	30.042	1285.938
	24.116	262.313
	20.683	396
(c)	19.13	245.563
	18.788	432.125
	17.502	1219.688
	16.975	368.75
	16.124	299.75
	15:21	741.688
Diet 1 (column 5)		
	387.909	1143.813
(a)	309.825	864
	244.944	754.563
	150	2355.688
	125.431	1261.438
	106.779	401.625
	91.631	1112.063
(b)	80.96	321.75
	63.52	255.875
	58.705	238.813
	49.685	228.938
	48.039	609.313
	40.082	2470.938
	35.513	922.5
	34.031	1798.375
	29.845	1039.063
	27.722	101.875
(c)	26.261	168.813
	23.886	498.938
	22.603	183.813
	21.287	184.438
	20.683	400.625
	18.892	307.938
	17.453	1135.5
	16.999	472.438
	16.282	168.875
	15.359	973.125
Diet 2 (column 6)		
	380.063	1188
(a)	303.559	690
	252.567	227.313
	148.222	2532.688
	106.779	489.188
	90.966	1091.188
	80.373	317
(b)	62.632	302.438
	51.864	229.938
	51.139	138.813
	47.536	326.438
	39.83	2676.063
	35.455	435.188
	33.808	1806.25
	29.602	1279.688
(c)	23.601	389.25
	20.437	489.5
	17.453	1387.5
	16.904	381.813
	15.359	943.625
Diet 3 (column 7)		
	350.236	825.75
(a)	291.403	494.563
	164.448	2616
	137.166	573
	118.17	262.125
	96.073	915.25
(b)	92.976	522.063
	83.354	194.5
	71.093	199.063
	48.14	340.75
	40.592	2485.375
	35.806	555.188
	34.367	1293.25
	29.944	665.375
	24.001	198.25
	20.783	254.313
(c)	18.709	494.688
	18.477	393.625
	17.673	832.188
	17.07	168.125
	16.487	244.25
	15.444	502.875
Diet 4 (column 8)		
	447.556	107.688
(a)	336.211	494.125
	297.419	287.375
	169.560	2480.25
	138.812	417.5
	119.588	263.188
	97.129	607.938
	93.315	559.313
	84.269	172.563
(b)	75.000	208.063
	64.967	130.625
	59.705	175.938
	57.559	137.938
	47.938	603.75
	46.939	260.875
	40.677	2355
	35.747	588.75
	34.367	969
	29.944	458.188
(c)	29.168	392.438
	17.551	544.625
	16.327	257.25
	15.337	325.813
	14.013	419.625
Diet 5 (column 9)		
	490.66	98.563
(a)	357.466	674.375
	313.007	330.875
	167.841	2556.313
	137.166	526.75
	117.468	347.813
	110.01	91.188
	95.724	772.625
	91.298	878.375
(b)	83.051	275.125
	77.781	297.688
	64.603	202.875
	59.036	396.063
	56.914	365.313
	52.897	136.563
	47.536	972.438
	40.251	2443.625
	35.397	1022.25
	34.086	1125.625
	29.748	455.438
(c)	23.886	250.313
	17.478	570.063
	16.147	419.688
	15.168	240
	14.013	796.188

GEL 6 (6th Hour)

	M. weight KDa	Peak Intensity
Control diet (column 4)		
(a)	407.313	551.75
	328.291	352.25
	179.375	2365.75
(b)	141.062	644.063
	122.474	248
	98.125	1089.938
	94.123	1058.75
	88.592	728.438
	74.788	214.5
	65.643	288.563
	60.29	261.938
	48.621	664.813
	41.024	2445.75
(c)	39.381	1003.438
	35.991	1035.25
	34.556	1642.75
	30.29	1234.625
	26.551	318
	25	422.688
	21.246	371.313
	19.262	159.938
	18.986	166.313
	17.713	1128.938
	17.01	729.563
	16.478	749.75
	16.241	495.688
	15.285	841.375
Diet 1 (column 5)		
(a)	407.313	836.313
	328.291	583.188
	164.26	2252.563
(b)	131.037	821.125
	113.77	483.75
	94.48	1074.563
	90.971	936.438
	82.757	350.563
	63.989	362.375
	58.937	400.875
	47.895	746.375
	44.136	353.813
	40.411	2502.875
(c)	36.462	202.125
	35.583	1093.188
	34.11	1842.688
	29.899	1139.75
	24.031	429.813
	20.806	454.813
	17.383	1109.75
	16.888	524.625
	16.312	529.5
	15.174	913.25
Diet 2 (column 6)		
(a)	488.437	439.688
	407.313	850.5
(a)	380.495	620.625
	328.291	444.875
	[illegible]	2314.75
	135.125	949.313
(b)	118.77	549.25
	96.651	1233.875
	93.413	1012.75
	84.979	447.438
	64.902	559.25
	48.517	523.75
	40.848	2541.875
(c)	35.932	846.688
	34.277	1983.313
	29.996	1369.625
	24.143	501.438
	20.952	594.75
	17.585	1533.5
	16.961	440.688
	16.43	259.75
	15.285	1149.375
Diet 3 (column 7)		
(a)	411.964	695.25
	343.542	574.125
	[illegible]	2470.063
	155.196	485.438
(b)	128.644	226.188
	100.616	683.813
	96.285	537.438
	87.261	234.25
	71.268	197
	66.203	218.563
	61.324	180.938
	49.253	520.688
	41.736	2456.188
(c)	40.151	676.438
	36.403	703.125
	34.895	1272.688
	33.669	232.438
	30.439	804.625
	25.286	155.75
	24.539	209.813
	21.197	279.125
	17.713	1015.313
	16.43	759.063
	16.124	989.938
	15.307	533.875
Diet 4 (column 8)		
(a)	426.235	1087.375
	347.464	827.313
	[illegible]	1818
(b)	140.198	669.313
	121.724	585.938
	113.77	702.75
	100	1146.25
	94.839	1404.313
(b)	89.265	639.25
	82.133	484.313
	74.576	210.313
	68.107	540.688
	62.376	1065.438
	60.29	662.563
	56.323	381
	49.678	1367.938
	45.977	588.563
	41.826	2358.563
(c)	39.551	1796.875
	36.285	1847.313
	34.895	2007.938
	33.723	744.063
	30.737	1472.438
	26.899	759.563
	25.369	625.875
	24.942	571.875
	23.208	351.625
	21.395	585.938
	19.123	539.313
	18.767	291.313
	17.687	1463.625
	17.084	453.125
	16.454	1376.25
	16.148	735
	15.285	1156.938
	14.137	549.813
Diet 5 (column 9)		
(a)	355.442	500
	190.38	2461.813
(b)	146.359	627.75
	126.295	359.688
	99.622	970.188
	95.922	853.375
	87.592	305.313
	81.205	125.688
	66.769	281.688
	63.989	213.75
	61.673	473.625
	49.678	620.125
	41.826	2461
(c)	40.237	878.563
	36.581	925.188
	35.066	1676.938
	30.587	985.25
	26.768	331.75
	24.711	533.313
	22.993	174.688
	21.295	358.563
	17.687	1157.875
	16.986	385.438
	16.454	576.813
	16.055	393.25
	15.329	723.438

GEL 7 (7th Hour)

	M. weight KDa	Peak Intensity
Control diet (column 4)		
(a)	360.58	494
	312.043	448.938
	175.011	1472.375
	135.54	819.375
(b)	123.13	976.188
	118.616	540.25
	96.317	931.313
	88.243	512.375
	81.352	347.25
	67.382	463.938
	62.261	326.438
	60.539	172.875
	49.307	815.5
	41.537	2045.313
(c)	35.941	1185.625
	32.847	1732.625
	26.113	1311.5
	22.339	378.25
	20.359	691.75
	16.696	1008.625
	15.657	1174.125
	12.946	867.438
	12.656	2063.875
	11.987	888.188
	11.508	504
	10.463	1276.25
Diet 1 (column 5)		
(a)	424.776	693.5
	353.696	1005.313
	224.852	1717.75
	176.706	1611.375
	126.458	776.188
	119.251	706.563
	99.688	491.438
	91.904	244.813
	50.256	254.813
	43.399	1707.25
(c)	39.913	123.688
	37.371	596.813
	34.71	997.813
	27.514	612.313
	20.847	452.5
	17.914	431
	14.078	371.938
	12.976	986.438
	10.727	817
Diet 2 (column 6)		
(a)	408.712	527.25
	327.449	405.688
	252.421	1291.375
	160.460	1904
(b)	135.54	962.563
	114.879	380.563
(b)	65.019	485.688
	61.003	426.063
	58.714	208.75
	54.113	124.813
	48.527	687.063
	46.816	110.375
	41.619	1767.625
(c)	39.675	1023
	35.837	1041.313
	33.134	1043.875
	30.903	142.313
	26.189	602.625
	24.754	201.563
	22.075	131.125
	20.48	250.313
	17.005	349.563
	14.532	201.563
	12.917	653
	12.041	159.938
	10.703	529.188
Diet 3 (column 7)		
(a)	424.776	292.813
	340.319	190.938
	288.867	408.5
	[illegible]	1619.688
(b)	146.052	864.563
	122.474	318.625
	107.755	161.938
	693.5	643.688
	94.823	539.5
	88.243	121.625
	79.591	253.625
	65.854	146.125
	61.315	230.5
	59.467	194.563
	56.511	121.25
	55.228	101.063
	49.307	309.313
	42.037	1815.813
	40.232	483.875
(c)	36.045	481.813
	33.134	738.5
	25.962	624.25
	21.859	110.938
	20.642	221.563
	20.159	222.563
	16.798	140.063
	14.932	563.688
	527.25	603.438
	14.015	225.813
	12.772	519.313
	11.852	295.313
	11.508	315.375
	10.559	375.563
	173.332	1821.813
(b)	141.45	1005.188
	120.53	270.313
	98.141	659.438
	94.527	625.813
	87.146	379
	79.591	205.063
	73.861	513.75
	65.352	328.063
	60.539	246
	58.714	174
	48.141	563.688
	40.879	1929.375
(c)	38.969	789.125
	34.811	761.25
	31.999	1128.5
	30.106	194.125
	25.073	774.438
	20.079	957.375
	18.134	290.938
	15.898	262.375
	13.92	168.188
	12.234	973.5
	11.56	586.938
	11.276	442
	10.416	521.75
Diet 5 (column 9)		
(a)	412.67	482.063
	333.822	249.938
	252.421	1138.313
	173.332	1771.625
(b)	139.205	991.063
	117.985	335.188
	95.12	859.313
	91.331	675.125
	83.674	407.625
	77.868	108.625
	64.195	350.063
	59.467	237.313
	57.09	180.813
	47.379	520.938
	40.152	2148.125
(c)	38.352	856
	34.21	807.063
	31.174	1436.438
	29.672	187.563
	25.887	487.625
	24.754	1273
	21.263	219.313
	19.696	256.25
	19.044	441.25
	17.427	166.813
	15.514	546.438
	13.67	153.125

	94.527	806.313	Diet 4 (column 8)				12.068	963.188
(b)	91.046	723.875		428.89	494.563		11.301	672.563
	83.674	243.75	(a)	346.943	294.188	(c)	10.973	477.125
	78.602	189.813		267.449	1195.25		10.114	572.5

GEL 8 (8^{th} Hour)

	M. weight KDa	Peak Intensity		M. weight KDa	Peak Intensity		M. weight KDa	Peak Intensity
Control diet (column 4)			Diet 2 (column 6)			Diet 4 (column 8)		
	381.644	540		317.637	435.063		375.6	698
(a)	360.906	316.5	(a)	293.27	243.125	(a)	317.637	386.25
	305.211	207.313		252.003	284.313		190.582	2332
	184.594	1969.875		[illegible]	2196.75		148.639	729.25
	148.639	129.125		136.314	692.875		132.639	380.125
	124.443	187.75		121.64	270.75		110.542	796
	114.124	226.063		100.915	959.25		104.661	701.063
	101.376	357.625		96.153	1045.625		94.594	321.5
	96.784	359.625		88.607	317.375		91.852	244.875
	86.038	304.25		82.728	206.875	(b)	88.897	105.563
(b)	68.745	331.313	(b)	79.546	154.625		79.027	160.188
	62.5	307		76.987	133.375		71.415	397.938
	59.512	154.75		67.816	252.563		66.176	328.375
	55.447	199.938		62.33	393.375		57.915	199.625
	51.379	160.125		60.164	293.188		54.401	339.938
	49.389	504.188		56.054	163.063		51.66	369.813
	46.351	322.563		49.187	598.188		47.504	175.25
	45.04	325.75		41.839	1781.75		43.41	1736.688
	42.443	2002.313		39.831	861.563		41.243	446.5
	40.324	473.438		36.588	877.563		38.547	478.25
	36.646	612.125		34.985	1122.813		37.688	663.75
	35.097	830.75		33.829	207.563		35.777	1104.125
	33.721	264.875		31.579	282.938		33.721	323.625
	33.026	175.813		30.83	853.875		32.037	297.313
	30.683	502.938		26.91	363.188		31.328	710.5
	29.621	98.375	(c)	26.02	422.063	(c)	29.479	212.375
(c)	28.46	105.25		25.403	673.5		28.143	168.313
	25.813	206.813		23.676	120.688		27.652	226.438
	24.932	215.25		21.642	228.688		22.915	504.813
	22.605	271.75		19.19	187.563		22.119	540.188
	18.505	486.313		17.378	939.188		17.609	884.25
	16.924	429.75		16.702	728.063		16.757	609.188
	16.185	325.563		15.504	610.125		16.347	245.375
	14.877	248.625		14.827	552.313		15.504	625.125
Diet 1 (column 5)			Diet 3 (column 7)			Diet 5 (column 9)		
	366.713	402.875		360.906	795.375		366.713	930.563
(a)	295.62	734	(a)	300.377	412.938	(a)	322.749	576.563
	170.43[illegible]	2546.563		180.226	2207.875		199.931	2204.938
	126.732	294.688		129.062	408		152.414	883.125
	121.64	418.188		106.586	740.625		141.374	384.875
	99.348	1033.5		100.915	720.125		135.078	407.688
	95.215	1122.25		92.152	338.375		111.554	1038.063
	87.742	317.063	(b)	76.736	264.375		105.619	906.188
(b)	81.387	156.188		69.12	258.313	(b)	96.468	401.188
	67.082	296		64.399	260.313		89.188	172.688
	61.823	391.813		61.992	225		71.415	376.063
	59.351	287.875		50.962	434.438		66.176	261.188
	55.296	136.438		43.232	1891		63.184	263.25

Gel 1 (1^{st} Hour)

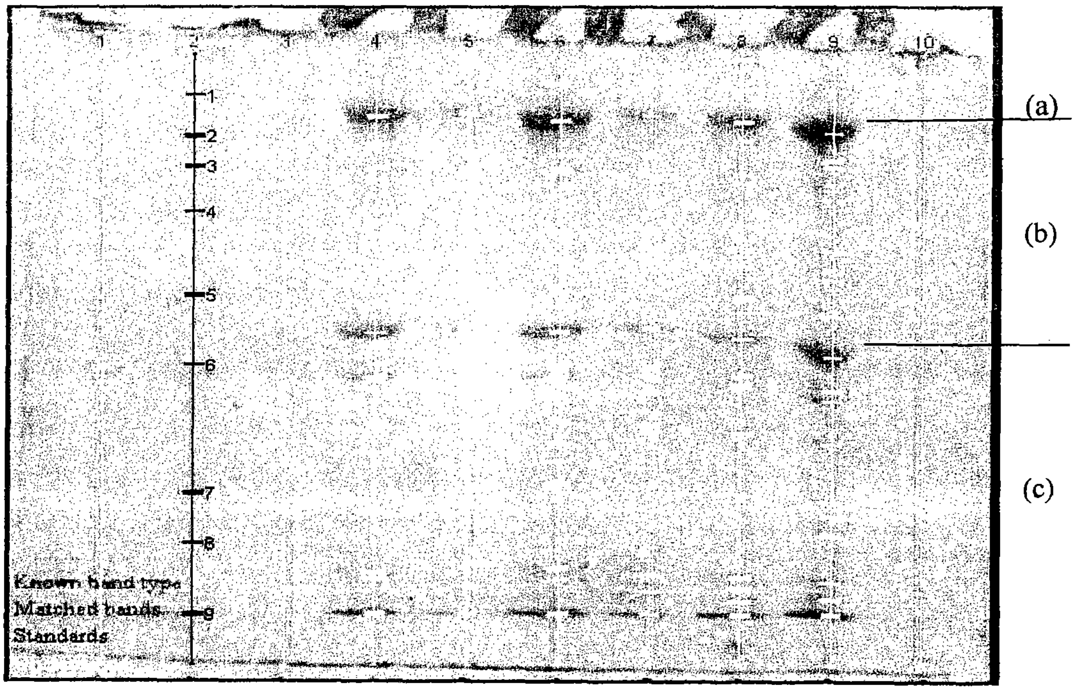

Fig. 9A SDS-PAGE patterns of tilapia muscle fed different dietary at 1^{st} hour room temperature storage. Blue lane: protein marker. Yellow lane: protein samples

Gel 2 (2^{nd} Hour)

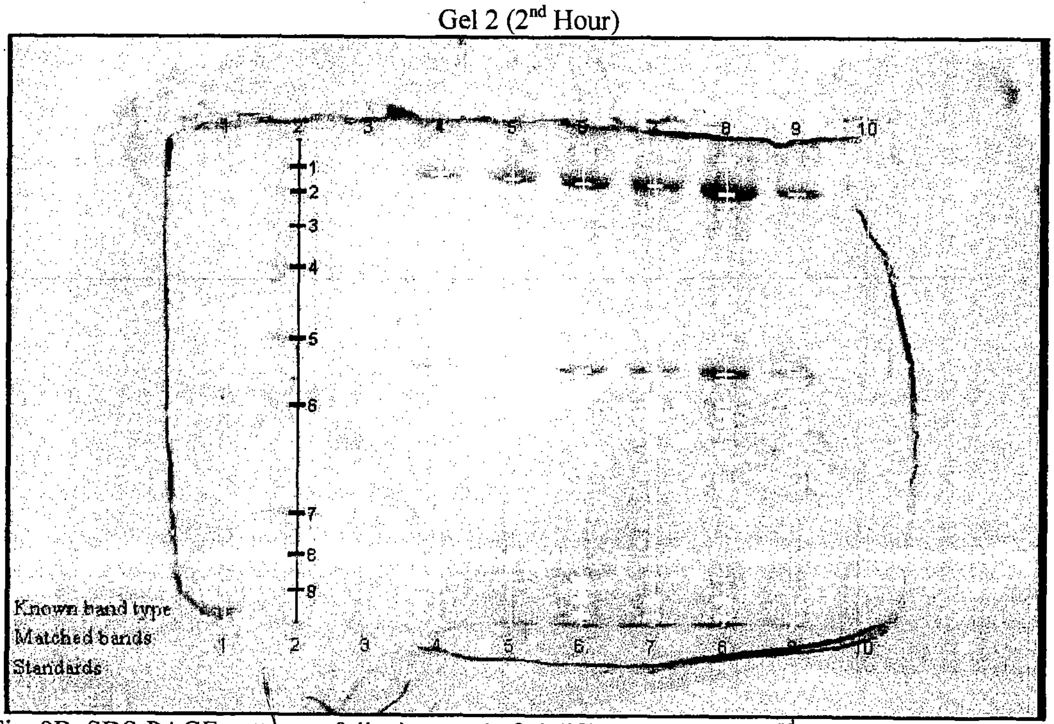

Fig. 9B SDS-PAGE patterns of tilapia muscle fed different dietary at 2^{nd} hour room temperature storage. Blue lane: protein marker. Yellow lane: protein samples

Gel 3 (3rd Hour)

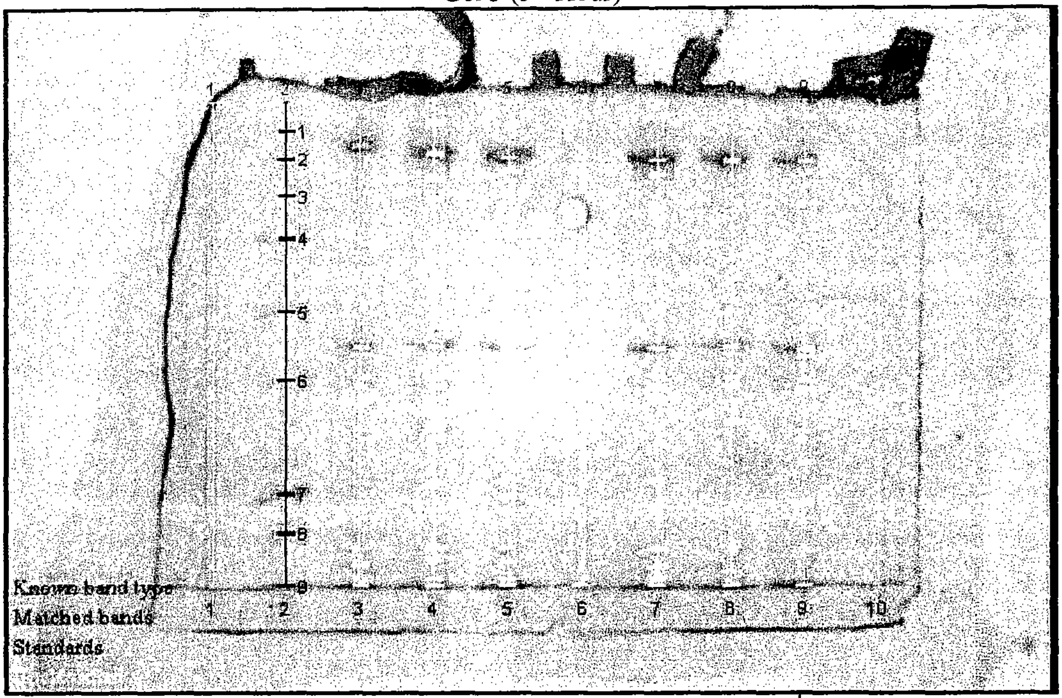

Fig. 9C SDS-PAGE patterns of tilapia muscle fed different dietary at 3rd hour room temperature storage. Blue lane: protein marker. Yellow lane: protein samples

Gel 4 (4th Hour)

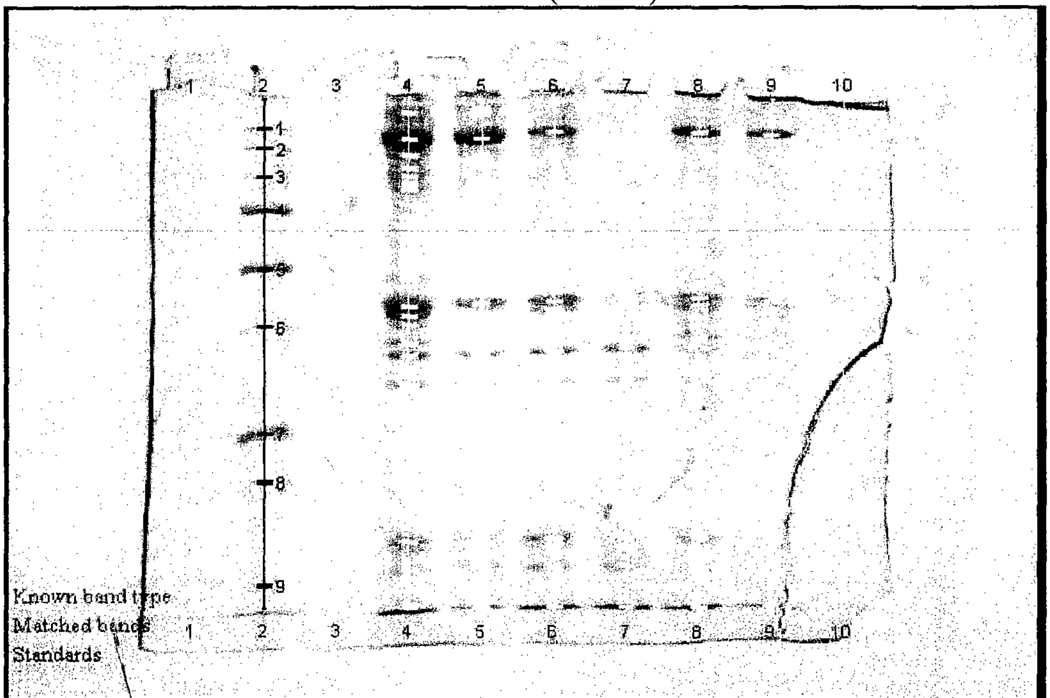

Fig. 9D SDS-PAGE patterns of tilapia muscle fed different dietary at 4yh hour room temperature storage. Blue lane: protein marker. Yellow lane: protein samples

Gel 5 (5th Hour)

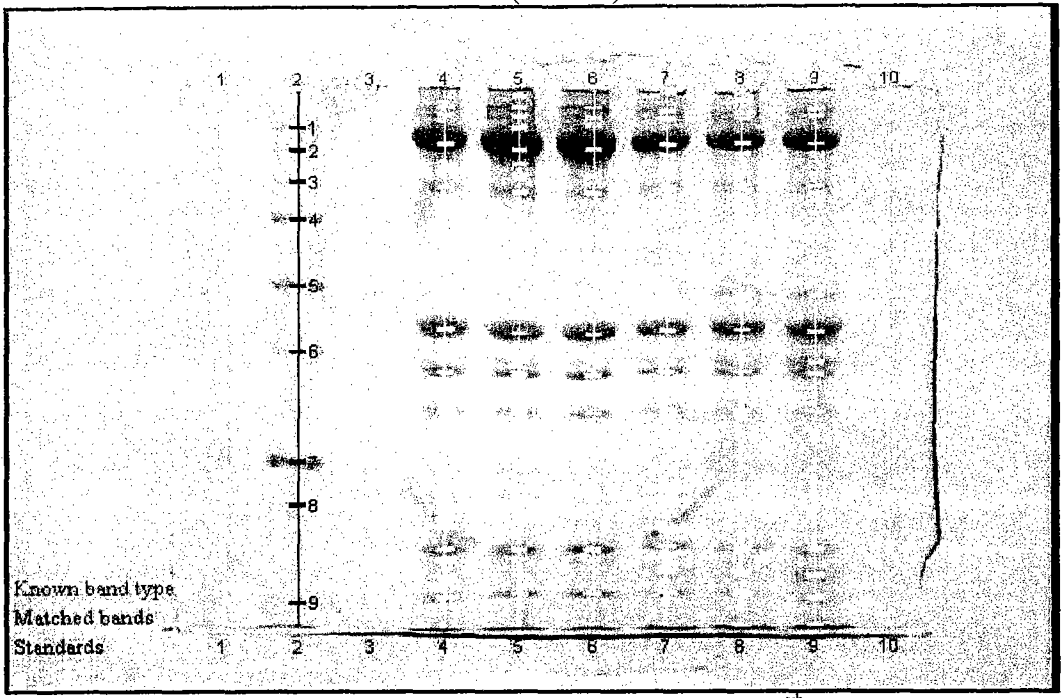

Fig. 9E SDS-PAGE patterns of tilapia muscle fed different dietary at 5yh hour room temperature storage. Blue lane: protein marker. Yellow lane: protein samples

Gel 6 (6th hour)

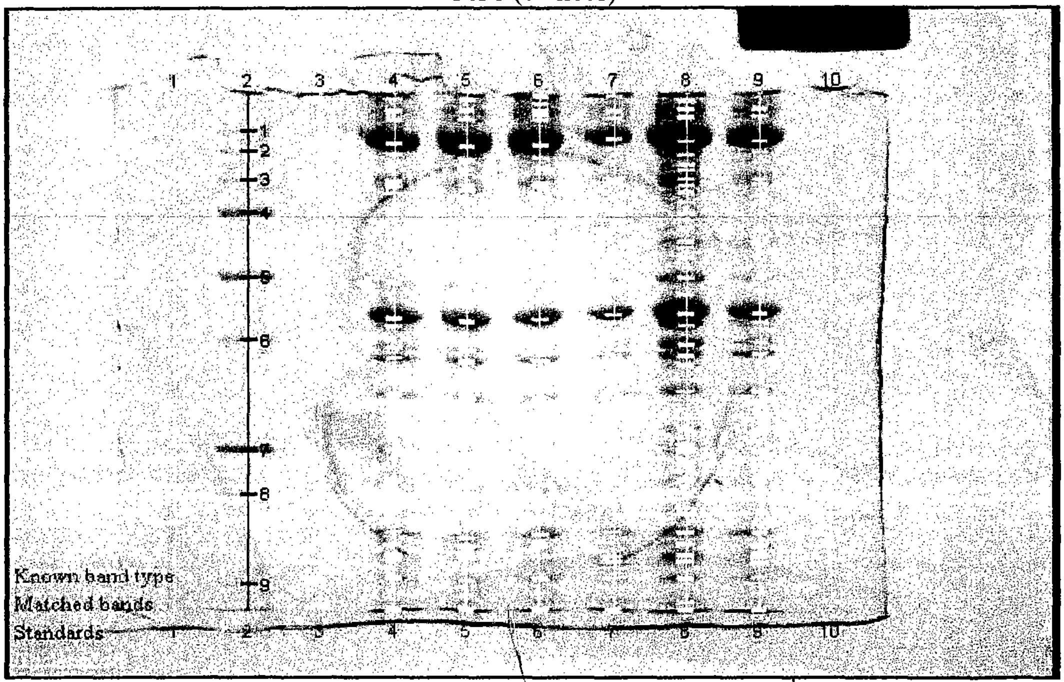

Fig. 9F SDS-PAGE patterns of tilapia muscle fed different dietary at 6yh hour room temperature storage. Blue lane: protein marker. Yellow lane: protein samples

Gel 7 (7th Hour)

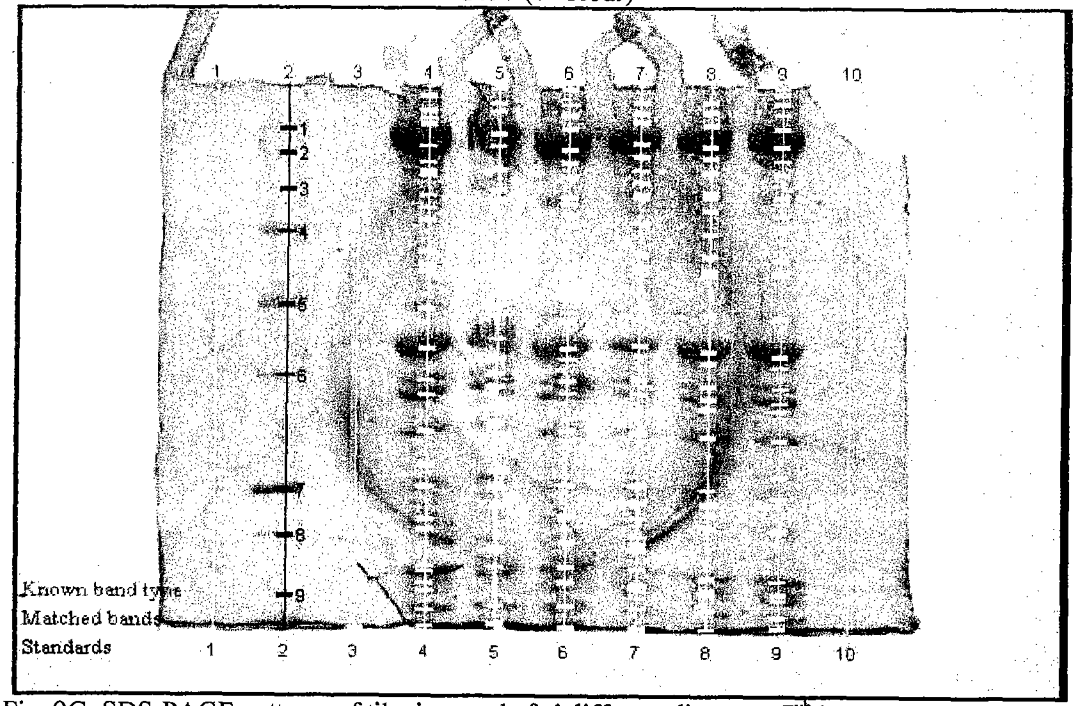

Fig. 9G SDS-PAGE patterns of tilapia muscle fed different dietary at 7^{yh} hour room temperature storage. Blue lane: protein marker. Yellow lane: protein samples

Gel 8 (8th Hour)

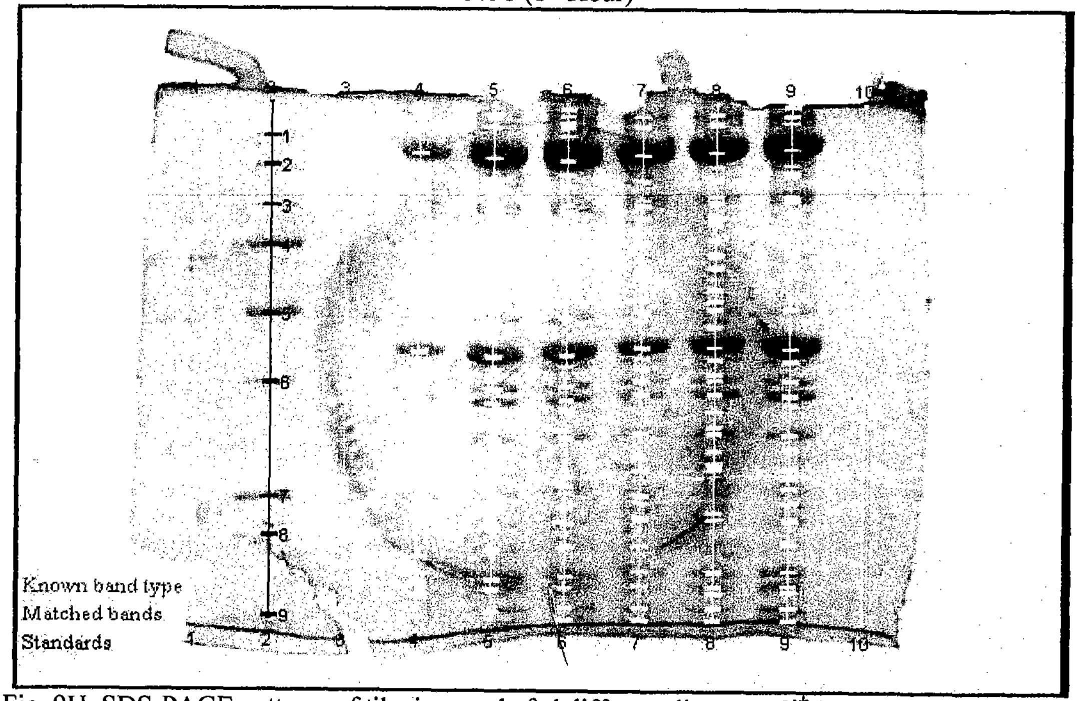

Fig. 9H SDS-PAGE patterns of tilapia muscle fed different dietary at 8^{yh} hour room temperature storage. Blue lane: protein marker. Yellow lane: protein samples

Table 12. The total numbers of the protein bands among each diet within the hours

Control diet									
Molecular weight (kDa)		**Hour**							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
(a)	300-410	1	1	1	1	2	2	2	3
	100-200	1	3	1	3	1	2	3	4
	90-99	2	–	1	2	1	2	1	1
	80-89	1	–	–	1	3	1	2	1
(b)	70-79	2	1	1	2	–	1	–	–
	60-69	1	–	1	1	1	2	3	2
	50-59	2	3	–	3	–	–	–	3
	40-49	–	1	1	12	1	1	1	3
Total band 40-200		9	9	5	13	8	9	10	14
	[illegible]	1	1	1	–	–	–	–	1
(c)	[illegible]	3	4	4	4	3	4	2	5
	[illegible]	–	3	3	5	2	3	3	5
	10-19	2	3	3	5	6	7	7	4
Total band 10-50		6	11	11	14	11	14	13	15

Diet 1									
Molecular weight (kDa)		**Hour**							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
(a)	300-410	1	2	1	2	3	2	2	3
	100-200	–	2	1	2	2	2	3	2
	90-99	2	2	1	3	1	2	2	2
	80-89	1	2	–	1	1	1	–	2
(b)	70-79	1	–	–	–	–	–	–	–
	60-69		1	1	1	1	1	–	2
	50-59	2	2	1	1	1	1	1	3
	40-49	–	–	–	2	2	2	–	1
Total band 40-200		6	9	4	10	8	9	6	12
	[illegible]	1	–	–	–	–	–	–	–
(c)	[illegible]	3	5	4	3	2	3	3	5
	[illegible]	1	–	2	2	7	3	2	5
	10-19	–	2	3	6	5	4	4	4
Total band 10-50		5	7	9	11	14	10	9	14

Diet 2

Molecular weight (kDa)		Hour							
		1	2	3	4	5	6	7	8
(a)	300-410	1	2	1	—	3	4	3	3
	100-200	—	3	2	3	1	2	2	3
	90-99	1	1	2	—	1	2	2	1
	80-89	1	1	—	—	1	1	1	2
(b)	70-79	—	—	—	—	—	—	—	2
	60-69	1	—	1	1	1	1	2	3
	50-59	2	1	—	—	2	—	2	1
	40-49	—	—	1	1	1	1	2	1
Total band 40-200		5	6	6	5	7	7	12	13
[redacted]			1	—	1	—	—	—	—
[redacted]		3	4	3	4	2	2	4	6
[redacted]		3	1	2	1	3	3	4	5
[redacted]		2	4	3	4	3	4	5	5
Total band 10-50		8	10	8	10	8	9	13	16

Diet 3

Molecular weight (kDa)		Hour							
		1	2	3	4	5	6	7	8
(a)	300-410	1	—	—	—	2	2	3	2
	100-200	—	1	1	—	2	3	3	3
	90-99	2	1	2	—	2	1	2	1
	80-89	—	—	1	—	1	1	1	—
(b)	70-79	2	—	1	1	1	1	1	1
	60-69	2	—	—	—	—	2	2	3
	50-59	2	1	—	—	—	—	3	1
	40-49	—	—	1	—	1	1	1	—
Total band 40-200		8	3	6	1	7	9	13	9
[redacted]		—	1	—	1	—	1	1	1
[redacted]		6	5	4	2	2	4	2	4
[redacted]		—	—	2	—	3	3	4	5
[redacted]		2	3	3	4	6	4	8	5
Total band 10-50		8	9	9	7	11	12	15	15

Diet 4

Molecular weight (kDa)		Hour							
		1	2	3	4	5	6	7	8
(a)	300-410	1	2	—	1	3	2	3	2
	100-200	1	2	—	5	2	4	2	4
	90-99	2	2	1	2	2	1	2	2
	80-89	—	—	1	—	1	2	1	1
(b)	70-79	—	—	—	—	1	—	2	2
	60-69	2	2	1	2	1	4	2	1
	50-59	1	1	—	1	2	1	1	3
	40-49	1	—	1	1	2	2	1	1
Total band 40-200		7	7	4	11	11	14	11	14
[illegible]	[illegible]	—	1	1	2	—	—	—	1
[illegible]	[illegible]	3	5	3	4	2	5	4	6
	[illegible]	—	1	—	5	2	5	2	5
	[illegible]	3	3	2	4	4	8	7	4
Total band 10-50		6	10	6	15	8	18	13	16

Diet 5

Molecular weight (kDa)		Hour							
		1	2	3	4	5	6	7	8
(a)	300-410	2	—	—	1	3	1	3	2
	100-200	1	—	1	1	3	2	2	5
	90-99	1	1	1	1	2	2	2	1
	80-89	3	1	1	—	1	2	1	1
(b)	70-79	—	—	—	—	1	—	1	1
	60-69	1	—	1	—	1	3	1	2
	50-59	2	—	1	1	3	—	2	1
	40-49	1	2	2	1	1	1	1	—
Total band 40-200		9	4	7	4	12	10	10	11
[illegible]	[illegible]	—	1	1	1	—	1	—	1
[illegible]	[illegible]	2	4	3	5	2	3	3	4
	[illegible]	4	1	2	1	2	4	4	6
	[illegible]	2	1	1	1	4	5	9	6
Total band 10-50		8	7	7	8	8	13	16	17

Table 13. The total numbers of the protein bands among the hours for different dietary fed

1st Hour							
Molecular weight (kDa)		**Diet-diet**					
		Control diet	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
(a)	300-410	1	1	1	1	1	2
	100-200	1	—	—	—	1	1
	90-99	2	2	1	2	2	1
	80-89	1	1	1	—	—	3
(b)	70-79	2	1	—	2	—	—
	60-69	1	—	1	2	2	1
	50-59	2	2	2	2	1	2
	40-49	—	—	—	—	1	1
Total band 40-200		9	6	5	8	7	9
	40-50	1	1	—	—	—	—
(c)	30-39	3	3	3	6	3	2
	20-29	—	1	3	—	—	4
	10-19	2	—	2	2	3	2
Total band 10-50		6	5	8	8	6	8

2nd Hour							
Molecular weight (kDa)		**Diet-diet**					
		Control diet	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
(a)	300-410	1	2	2	—	2	—
	100-200	3	2	3	1	2	—
	90-99	—	2	1	1	2	1
	80-89	—	2	1	—	—	1
(b)	70-79	1	—	—	—	—	—
	60-69	—	1	—	—	2	—
	50-59	3	2	1	1	1	—
	40-49	1	—	—	—	—	2
Total band 40-200		9	9	6	3	7	4
	40-50	1	—	1	1	1	1
(c)	30-39	4	5	4	5	5	4
	20-29	3	—	1	—	1	1
	10-19	3	2	4	3	3	1
Total band 10-50		11	7	10	9	10	7

3rd Hour							
Molecular weight (kDa)		**Diet-diet**					
		Control diet	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
(a)	300-410	1	1	1	—	—	—
(b)	100-200	1	1	2	1	—	1
	90-99	1	1	2	2	1	1
	80-89	—	—	—	1	1	1
	70-79	1	—	—	1	—	—
	60-69	1	1	1	—	1	1
	50-59	—	1	—	—	—	1
	40-49	1	—	1	1	1	2
Total band	40-200	5	4	6	6	4	7
(c)	40-50	1	—	—	—	1	1
	30-39	4	4	3	4	3	3
	20-29	3	2	2	2	—	2
	10-19	3	3	3	3	2	1
Total band	10-50	11	9	8	9	6	7

4th Hour							
Molecular weight (kDa)		**Diet-diet**					
		Control diet	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
(a)	300-410	1	2	—	—	1	1
(b)	100-200	3	2	3	—	5	1
	90-99	2	3	—	—	2	1
	80-89	1	1	—	—	—	—
	70-79	2	—	—	1	—	—
	60-69	1	1	1	—	2	—
	50-59	3	1	—	—	1	1
	40-49	12	2	1	—	1	1
Total band	40-200	13	10	5	1	11	4
(c)	40-50	—	—	1	1	2	1
	30-39	4	3	4	2	4	5
	20-29	5	2	1	—	5	1
	10-19	5	6	4	4	4	1
Total band	10-50	14	11	10	7	15	8

5th Hour							
Molecular weight (kDa)		Diet-diet					
		Control diet	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
(a)	300-410	2	3	3	2	3	3
	100-200	1	2	1	2	2	3
	90-99	1	1	1	2	2	2
	80-89	3	1	1	1	1	1
(b)	70-79	—	—	—	1	1	1
	60-69	1	1	1	—	1	1
	50-59	—	1	2	—	2	3
	40-49	1	2	1	1	2	1
Total band	40-200	8	8	7	7	11	12
	40-50	—	—	—	—	—	—
(c)	30-39	3	2	2	2	2	2
	20-29	2	7	3	3	2	2
	10-19	6	5	3	6	4	4
Total band	10-50	11	14	8	11	8	8

6th Hour							
Molecular weight (kDa)		Diet-diet					
		Control weight	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
(a)	300-410	2	2	4	2	2	1
	100-200	2	2	2	3	4	2
	90-99	2	2	2	1	1	2
	80-89	1	1	1	1	2	2
(b)	70-79	1	—	—	1	—	—
	60-69	2	1	1	2	4	3
	50-59	—	1	—	—	1	—
	40-49	1	2	1	1	2	1
Total band	40-200	9	9	7	9	14	10
	40-50	—	—	—	1	—	1
(c)	30-39	4	3	2	4	5	3
	20-29	3	3	3	3	5	4
	10-19	7	4	4	4	8	5
Total band	10-50	14	10	9	12	18	13

7th Hour						
Molecular weight (kDa)	Diet-diet					
	Control diet	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
(a) 300-410	2	2	3	3	3	3
100-200	3	3	2	3	2	2
90-99	1	2	2	2	2	2
80-89	2	—	1	1	1	1
(b) 70-79	—	—	—	1	2	1
60-69	3	—	2	2	2	1
50-59	—	1	2	3	1	2
40-49	1	—	2	1	1	1
Total band 40-200	10	6	12	13	11	10
40-50	—	—	—	1	—	—
(c) 30-39	2	3	4	2	4	3
20-29	3	2	4	4	2	4
10-19	7	4	5	8	7	9
Total band 10-50	13	9	13	15	13	16

8th Hour						
Molecular weight (kDa)	Diet-diet					
	Control diet	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
(a) 300-410	3	3	3	2	2	2
100-200	4	2	3	3	4	5
90-99	1	2	1	1	2	1
80-89	1	2	2	—	1	1
(b) 70-79	—	—	2	1	2	1
60-69	2	2	3	3	1	2
50-59	3	3	1	1	3	1
40-49	3	1	1	—	1	—
Total band 40-200	14	12	13	9	14	11
40-50	1	—	—	1	1	1
(c) 30-39	5	5	6	4	6	4
20-29	5	5	5	5	5	6
10-19	4	4	5	5	4	6
Total band 10-50	15	14	16	15	16	17

4.0 DISCUSSION

In the present study, crude extracted from *M. pigra* was added as a supplementation into fish diet to improve the growth and feed utilization along with inhibiting lipid oxidation in tilapia. After feeding the diets to tilapia for 8 weeks, RGR, SGR, FCR and PER were not significantly different. Similarly, Wang *et al.* (2005) reported that tilapia fed fish meal diets supplemented with 30 to 240 mg total vitamin E derived from a tocotrienol-rich fraction (TRF) extracted from crude palm oil/kg diet for 9 weeks did not show differences in growth. Similar report was made by Huang *et al.* (2003) when feeding hybrid tilapia for 14 weeks with diets supplemented with 0, 100, 200, 450 and 700 mg α-tocopheryl acetate/ kg diet. The lack of differences in growth among tilapia in the present study could be due to the temperature stress, particularly cold temperature where there were rainfalls for those days (Table 6). It would be believed that those tilapia were having temperature shock, physiological stress induces by sudden or rapid changes in temperature, defined by some as any change greater than 3 degrees per hour (Parker, 1995), as we can see that the temperature dropped from ±30 °C to ±26 °C (Table 6). The tilapia expressed or handled their stress by peeling off their scales and a break in their skin (Plate 3). Any break in the skin or removed scale creates an opening invasion by pathogenic organisms where it can completely halt the activity of antibodies of the immune system, eliminating an important first defense (Parker, 1995). Similarly, Imsland *et al.* (1995) also suggested that freshwater fish species are more sensitive to photoperiod than marine and diadromous species.

MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA concentrations is an indicator of lipid peroxidation (Emanuel & Lyaskovskaya, 1967). There were significantly different between control diet and diet 5 (Table 9). Similar report was made by Huang *et al.* (2003) where TBARS values for muscle decreased with increasing dietary vitamin E up to 63 mg/kg and also Wang *et al.* (2005) reported that lipid peroxidation in muscle o tilapia fed low dietary vitamin E diets (E0 and E30) was significantly higher than those of fish fed high dietary vitamin E diets (E60 to E240). Those reports indicated that the higher antioxidant consumption by the fish, the greater antioxidant concentrations in fish tissue, thus resulted in lower lipid peroxidation.

As expected in our primary hypothesis, the supplementations of crude extract *M. pigra* can induce an increase of linoleic acid (C18: 2n-6) and linolenic acid (C18:3n-3), and following a decrease of highly unsaturated fatty acids such C20: 5n-3 (EPA, eicosapentaenoic acid) and C22: 6n-3 (DHA, docosahezaenoic acid). This due to linoleic acid and linolenic acid are commonly found in plants or vegetables such as flaxseed, soybean, sunflower seed and so on (Tocher *et al.*, 2002; Tocher *et al.*, 2000). The high values of *n*-3 (omega-3) play a crucial role in the prevention of atherosclerosis, heart attack, depression and cancer (Simopoulos Artemis, 1991). From the present study, the results showed that crude extracts *M. pigra* contain less omega-3. Freshwater fish normally consist of more *n*-6 polyunsaturated fatty acid such as linoleic acid (Parker, 1995) whereas the marine fish are rich in *n*-3, especially DHA and EPA (Wang *et al.*, 1990). Therefore, there were some reports suggested that in certain cases, it might be needed to manipulate the nutritional quality of a fish in terms of its *n*-3 content by feeding it a higher fish oil-based diet to restore its DHA and EPA before harvest and selling to the market (Ng, 2002; Ng, 2004).

In the present study, the level of PUFA decreased with increasing dietary crude extract. Well, it has been reported that high levels of PUFA or HUFA lead to increased oxidative stress, resulting in feed spoilage (Parker, 1995) for the fish and result in pathological conditions (Ng, 2004). The low level of PUFA in the dietary crude extracts can be supported by the TBA results (Table 9) where concentrations of MDA decreased with increasing dietary crude extracts within the 8 hours.

There are a few reports describing the relationship between lipid oxidation and protein denaturation. Losada, *et al.* (2005) reported that when storage of horse mackerel specimens in slurry ice conditions compared with flake ice conditions, it allowed an inhibitory effect on chemical changes related to quality loss. Thus, development of different fish damage pathways, such as nucleotide autolytic degradation, lipid hydrolysis and oxidation, non-enzymatic browing and protein profile modifications, showed a partial inhibition. This can be seen at the results of the report that showed comparison between the two icing conditions for the TBA – i method, flake ice treatment showed higher values yet the appearance of two extra polypeptides could be observed (denatured) in the SDS-Excel Gel.

Results obtained by electrophoresis in the present study showed that the number of the protein bands were almost similar among all samples. Maybe this is due to the storage time that not long enough to let the crude extracts to show its effectiveness on inhibiting protein denatured. As we can see for most of the previous reports, they tested their storage behaviors for at least 1 day or more (Losada *et al.*, 2005; Verrez-Bagnis, *et al.*, 2001; Benjakul *et al.*, 1997).

It is difficult to compare the results obtained in this study with those obtained in studies carried out on other tilapia species due to differences in experimental conditions and methodology. Many factors are known to modify feed intake in fish such as water temperature, water quality, fish size, frequency of feeding, photoperiod, stocking density and feed quality (NRC, 1987). Much more research data to be collected before reliable feeding guides under various management and environmental conditions can be developed from *M. pigra.*

At last, considering the lower price and high availability of *M. pigra* in the tropics, it has the potential as an alternative dietary natural antioxidant for fish compare by using synthetic antioxidant (BHT, BHA). By using the natural products, these will have a positive impact on the aquaculture industry and also the crops industry.

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Plate 1 *Mimosa pigra* tree and it flowers

Plate 2 Red tilapia (*Oreochromis spp.*)

Plate 3 Scales and skin damaged on tilapia