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Proximate and Nutritional Content of Rainbow Trout (*Oncorhynchus mykiss*) Flesh Cultured in a Tropical Highland Area

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HIGHLIGHTS

- Body composition and carcass length were significantly higher in fish aged 24 months
- Higher pH, moisture, shear force, TBARS, and collagen were found in 24-month fish
- Lipid content and n-6/n-3 ratios were higher in 12-month fish and in ventral fillets
- PUFA: SFA ratio was higher in 24-month fish and in dorsal fillets

Abstract: The present study was performed to assess the proximate and nutrient content of rainbow trout flesh, cultured in the Doi Inthanon Fisheries Research Unit, Chiang Mai Inland Fisheries Research and Development Center, Thailand. 240 fish were randomly distributed across 12 cages with 20 fish cage-1. Sixteen individual fish from each cage were randomly collected at different ages of 10, 12 and 24 months. Body composition, pH, water-holding capacity, shear force, collagen content analysis, sensory, lipid oxidation, and fatty acids profile were evaluated. The results indicated that body composition and carcass length were significantly higher in fish aged 24 months, except for carcass and viscero-somatic index percentages ($P \leq 0.05$). Fish at 24 months showed significantly higher pH, moisture, fat, shear force, thiobarbituric acid reactive substances, and total collagen content values than fish at 10 and 12 months ($P \leq 0.05$). However, protein percentage, sensory measurement and water-holding capacity were significantly higher in younger fish at 10 and 12 months. The average lipid content and n-6/n-3 ratios were significantly greater in fish at 12 months and in ventral fillets ($P \leq 0.05$). However, polyunsaturated fatty acids: saturated fatty acid ratio was higher in fish at 24 months and in dorsal fillets. In conclusion, rainbow trout cultured in sub-tropical, montane conditions can be valuable sources of protein, unsaturated fatty acids, eicosapentaenoic acids, and docosahexaenoic acids.

Keywords: rainbow trout; flesh quality; age; muscle type; fatty acids.

INTRODUCTION

Rainbow trout, *Oncorhynchus mykiss* is a fish native to the temperate climate of North America that can adapt very well to cool water conditions in mountainous areas of tropical or sub-tropical regions [1]. This is one of the most commonly farmed fish species of the Salmonidae family, enjoying high demand from global markets [1]. It was first introduced to Thailand in 1973 by His Majesty King Bhumibol Adulyadej to be cultured in the Northern highlands as an alternative form of livelihood opportunity and protein source for ethnic Karen people. Because of its tolerance to relatively high-water temperatures and its fast growth rate, excellent flesh, rainbow trout has become preferred popular salmonid species for aquaculture worldwide, including in several highland areas of tropical countries [1]. However, the nutrient content in cultured fish flesh depends on several factors, such as species, seasonality, nutrition, area, and age [2]. It has been reported that salmonids are heterothermal animals and their body temperature can vary from 6 °C in winter to 20–22 °C in summer [3]. This usually implies a pronounced effect on both the general level of lipid metabolism and the lipid composition of poikilotherms. Cold temperatures are normally associated with an increased unsaturation degree in body fat, in particular with a conversion of saturated fatty acids of the biological membrane phospholipids typical of the warm season into the corresponding mono- and dienic fatty acids typical of the cold season [4]. In addition, fatty acids of fish flesh play an important role in human health, varies with season, age, and diet [5].

The variation in the fatty acid profile of fish may have effects on the nutritional value, texture and organoleptic properties [5]. Fish flesh is a well-known source of proteins with high biological value, polyunsaturated n-3 fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), minerals and vitamins [6]. These fatty acids, particularly EPA and DHA, have been found to have anti-inflammatory and immunomodulatory properties. They also have been proved to be beneficial to cardiac, musculoskeletal, gastrointestinal, and immune systems in humans [7-9]. Evidence from epidemiological and preclinical studies indicates that n-3 fatty acids, especially EPA and DHA, have anti-cancer properties.

The identification of optimal rainbow trout fillet quality from cultured fish is complicated since quality infers a broad range of traits and variables [10]. It's also complicated because the manufacture and sale of products involve many levels with varying perceptions of quality [10]. Therefore, a combination study between growth performance and fillet quality of rainbow trout in a tropical area could provide valuable information for fish farmers and food industry representatives in the determination of best harvest endpoints. Moreover, it would be particularly valuable for trout farmers using or planning to use innovative fish production technologies that recirculate water and optimize environmental variables. Therefore, the objective of this study was to investigate the proximate and nutritional contents in the flesh of rainbow trout, *Oncorhynchus mykiss* culture under Thai climate, and hydrological conditions.

MATERIAL AND METHODS

Experimental diets and design

Fingerlings rainbow trout were obtained from Doi Inthanon Fisheries Research Unit, Chiang Mai Inland Fisheries Research and Development Center, Thailand. The farming of rainbow trout (*Oncorhynchus mykiss*) is located at the height of about 1300 m above sea level on a small tributary stream of the River Klang near the base of Siriphum Waterfall on Doi Inthanon National Park. These raceways are supplied with water from the waterfall at the rate of 250 L/min in the summer season and 500 L/min in the rainy season. Two hundred and forty individual fish were allocated into 12 cages with 20 fish cage^{-1} (2x2x1.5 m) in the same water body. The diet was hand-fed to the fish twice a day at 8:00 a.m. and 5:00 p.m. Water temperature was at 20 – 25 ^{o}C all year round. Fish were fed with a diet containing dry matter, crude protein, ether extract, crude fibre, ash percentage, and water content of 93.36, 42.63, 12.15, 0.62, 12.65%, 10%, respectively. The amount of feed was adjusted based on temperature and fish biomass according to the method described by Pornsopin [11]. The feeding trial was last for 24 months.

Sampling method

Sixteen fish from each treatment were randomly selected at three different ages 10, 12 and 24 months. They were anesthetized, followed by slaughter, and packed on tissues in polystyrene iced boxes and transported to the laboratory within 4 hours. Upon arrival, the fish were weighed, measured, and the Viscera-somatic index (VSI) and Hepatosomatic index (HSI) were calculated. Fish were then filleted along the insertion line of the ribs to obtain a dorsal (DF) and ventral fillet (VF). These samples were stored at -25 ^{o}C

and then analysed in duplicate for pH, water-holding capacity, moisture, crude protein, sensory, shear force, collagen content, total lipids, and fatty acids, as described in the next section.

Determination of fillet pH

The pH of dorsal and ventral fins fillets was determined at 5,45 min, and 24 hours (h) post-mortem, respectively, by using pH meter (pH meter model 191, Knick, Berlin, Germany). The electrode was inserted into the longissimus muscle at the anterior cut surface of the 10th rib location.

Colour measurements

After the measurement of pH, the samples were kept in polyethylene bags, chilled at 4 °C for 48 h. They were then stored at 4 °C outside the bag for 1h ('blooming') before conducting colour measurements with the use of Chroma Meter (Minolta, CR-300, Osaka, Japan). The colour parameters included L* = Lightness; white=100, black=0, a* =redness; green=-80, red=100, b* =yellowness; blue=-50, yellow=70.

Water-holding capacity

Water-holding capacity was determined according to the method described by Honikel [12].

Shear force measurement

Shear force measurement was detected following the method of Roth and coauthors [13]. For boiled samples, shear force was measured using TA-XTplus Texture Analyzer from Stable Micro Systems equipped with a Warner-Bratzler test cell. For muscles, they were sliced at a constant speed of 2.0 mm/s, 45° angle inverted knife. The shear force was determined by the maximum force (N) and the total amount of work (J) after slicing through the sample.

Sensory analysis

For sensory measurement, 9 panelist testers were assigned to each group (total 3 groups), panelists being selected from students and faculty members who have taken sensory measurement training according to the methods of [14].

Chemical composition

Samples of the dorsal fillet (DF) and ventral fillet (VF) were minced and analysed in duplicate for moisture, fat and protein contents (Kjeldahl; 6.25 x N) according to Cunniff and Association of Official Analytical [15].

Lipid oxidation

Susceptibility of the lipids to oxidation was assessed by the 2-Thiobarbituric acid (TBARS, Thiobarbituric acid reactive substances) as the method described by Rossell [16].

Fatty acids profile

Fatty acids in the feed and fillet were extracted by a mixture of chloroform/methanol according to Folch and coauthors [17]. Fatty acid methyl esters were prepared, according to the method of Morrison and Smith [18].

Statistical analysis

All statistical analyses were performed using SAS version 6.12 19. Descriptive statistics of analysis results were calculated for each treatment. The results of meat quality were determined by two-way analysis of variance (ANOVA) considering slaughter ages and muscle types as fixed effects. When significant difference was found, the means were compared with Duncan's New Multiple Range Test.

RESULTS

Body composition and biometric data of rainbow trout at different ages

Data on body composition and biometric data of rainbow trout at different ages are presented in Table 1. The results showed that body composition and carcass length of rainbow trout at 24 months were significantly higher than those at 10 and 12 months. However, carcass percentage was significantly higher in fish at 10 and 12 months compared to 24 months. In contrast, the dorsal and ventral fillets were significantly higher in fish at 24 months compared to fish at 10 and 12 months. Regarding the viscero-somatic index, the present study revealed that fish at 10 months had significantly higher VSI than those of 12 and 24 months. However, no significant difference in hepato-somatic was observed in fish at different ages.

Table 1. Body composition and biometric data of rainbow trout at different age

Criteria	Age (months)			SEM	P-Value
	10	12	24		
Body composition					
Whole body weight (g)	339.04^{c}	500.74^{b}	1133.64^{a}	11.584	<0.001
Carcass weight[1/] (g)	287.99^{c}	432.44^{b}	930.98^{a}	10.307	<0.001
Visceral weight (g)	35.93^{b}	34.43^{b}	64.50^{a}	1.438	<0.001
Liver weight (g)	4.52^{c}	6.49^{b}	15.15^{a}	0.311	<0.001
Carcass (%)	84.94^{a}	86.48^{a}	81.90^{b}	0.286	<0.001
Dorsal fillet weight (g)	77.63^{c}	142.98^{b}	261.40^{a}	3.758	<0.001
Ventral fillet weight (g)	67.30^{c}	129.53^{b}	208.25^{a}	3.557	<0.001
VSI[2] (%)	11.23^{a}	7.45^{b}	7.02^{b}	0.002	<0.001
HSI[3] (%)	1.34	1.31	1.30	0.000	0.858
Carcass length					
Total length (mm)	290.22^{c}	334.68^{b}	439.80^{a}	2.006	<0.001
Standard length (mm)	263.15^{c}	305.08^{b}	387.45^{a}	2.264	<0.001
Snout length (mm)	56.71^{c}	66.55^{b}	93.98^{a}	0.628	<0.001
Depth (mm)	71.34^{c}	80.83^{b}	104.86^{a}	0.596	<0.001
Thickness (mm)	36.53^{c}	43.68^{b}	60.20^{a}	0.400	<0.001

[1/] Carcass without visceral. [2/] VSI (Viscero-somatic index) = 100[(weight of all visceral including gonads and heart)/body weight]. [3/] HSI **(**Hepato**-**somatic index**) =** 100**[(**weight of liver**)/**body weight**].**

Flesh quality of different trout ages and muscle types

Meat quality of different trout ages and muscle types are shown in Table 2. pH at different slaughter ages and muscle types decreased 24 hours post-mortem, from 6.61 to 6.25 after 24 hours. Fish at 24 months of age had higher meat pH value than other groups, and ventral fillet showed significantly greater pH than ventral fillet. The chemical analysis showed that moisture and protein percentages were significantly higher in rainbow trout meat at 10 months compared with 12 and 24 months. However, rainbow trout at 10 and 12 months had a lower fat percentage than that of 24 months. For muscle types, dorsal fillets had higher moisture and protein than ventral fillets; however, fat content was lower in dorsal fillets compared to ventral fillets. For water-holding capacity, drip loss was higher in fish at 10 and 12 months, whereas thawing loss was higher in fish at an older age (24 months). Boiling loss was higher in fish at 10 months compared to fish at 12 and 24 months. No significant difference in water-holding capacity between dorsal and ventral fillets was observed. For fillet colour, it was found that the mean values of a^* and b^* was higher in fish fillets at 24 months compared with fish at 10 and 12 months of age. However, L^* was highest in fish at 12 months following by 10 and 24 months of age. For fillet colour, L^*, a^*, and b^* of the ventral fillet (VF) were significantly higher than dorsal fillet (DF) muscle. Results with regards to sensory measurement indicated that firmness and overall acceptability of rainbow trout at 10 months was significantly higher than other groups. Surprisingly, ventral fillets indicated more tenderness than dorsal fillets. Regarding the shear force, our results showed that maximum force and energy value increased as rainbow trout age increased. Additionally, boiled dorsal fillets presented a higher maximum shear force than ventral fillets. For lipid oxidation, a significant increase in TBARS was observed in rainbow trout flesh at all ages after 9 days of storage. Fish at older ages (24 and 12 months) showed higher TBARS compared to the younger age (10 months). In addition, TBARS in dorsal fillets was significantly higher than that of ventral fillets.

T**able 2.** Meat quality characteristics of rainbow trout at different age and muscle

Criteria	Age (months)			Muscle		SEM	P-Value		
	10	12	24	DF[1]	VF[2]		Age	Muscle	Inter[3]
pH value									
pH_i (5 min pm[4].)	6.53[b]	6.48[b]	6.61[a]	6.50[y]	6.55[x]	0.001	<0.001	0.267	<0.001
pH_{ii} (45 min pm.)	6.37[b]	6.38[b]	6.45[a]	6.38	6.40	0.001	0.018	0.596	0.060
pH_u (24 h pm.)	6.34[a]	6.25[b]	6.37[a]	6.37	6.32	0.000	<0.001	0.828	0.779
Chemical composition, %									
Moisture	72.49[a]	72.79[a]	71.30[b]	72.22[x]	71.83[y]	0.139	<0.001	<0.001	0.683
Protein	21.83[a]	20.03[c]	20.79[b]	20.38[x]	19.02[y]	0.074	<0.001	<0.001	<0.001
Fat	5.46[c]	6.48[b]	7.71[a]	7.07[y]	8.70[x]	0.148	<0.001	<0.001	0.064
Fillet color									
L*[5]	48.51[b]	49.86[a]	46.90[c]	48.07[y]	48.86[x]	0.009	<0.001	0.054	0.002
a*	2.48[b]	2.10[c]	4.54[a]	2.31[y]	3.73[x]	0.004	<0.001	<0.001	0.365
b*	11.32[c]	13.07[b]	15.05[a]	12.27[y]	13.98[x]	0.009	<0.001	<0.001	0.828
Sensory evaluation[6]									
Firmness	5.94[a]	5.24[b]	5.51[b]	5.48	5.70	0.003	<0.001	0.089	0.244
Odour	5.29	5.33	5.37	5.41	5.24	0.004	0.887	0.287	0.248
Juiciness	5.68	5.73	6.03	5.85	5.76	0.004	0.097	0.515	0.798
Tenderness	5.89	5.96	6.01	5.80[y]	6.10[x]	0.003	0.742	0.021	0.894
Acceptability	5.94[a]	5.54[b]	5.55[b]	5.63	5.76	0.003	0.006	0.232	0.541
Shear force									
Max. Force *(N)*									
Raw fillet	5.04[b]	5.89[b]	11.52[a]	7.35	7.04	0.068	<0.001	0.718	0.947
Boiled muscle	9.73[c]	14.20[b]	19.01[a]	16.30[x]	12.82[y]	0.139	<0.001	0.040	0.840
Work *(J)*									
Raw fillet	0.11[b]	0.13[b]	0.25[a]	0.16	0.16	0.002	<0.001	0.951	0.957
Cook fillet	0.35[b]	0.52[b]	0.72[a]	0.58	0.45	0.006	<0.001	0.183	0.860
Water holding capacity, %									
Drip loss	9.75[a]	11.26[a]	6.38[b]	8.65	9.60	0.057	0.002	0.385	0.277
Thawing loss	8.13[b]	6.08[c]	9.53[a]	7.02	8.86	0.056	0.024	0.076	0.544
Grilling loss	14.75	13.06	12.18	13.94	13.13	0.150	0.681	0.659	0.100
Boiling loss	8.07[a]	4.65[b]	3.26[b]	3.94	6.72	0.053	<0.001	<0.001	0.077
TBARS, mg malondaldehyde/ kg fillet									
Day 0	1.71[b]	5.00[a]	5.49[a]	3.5[y]	4.64[x]	0.124	<0.001	<0.001	0.178
Day 3	7.35[c]	8.44[b]	9.91[a]	7.95[y]	9.19[x]	0.103	<0.001	<0.001	<0.001
Day 6	10.12[b]	8.68[c]	10.72[a]	9.64[y]	10.05[x]	0.098	<0.001	0.039	<0.001
Day 9	9.47[b]	9.49[b]	12.53[a]	9.26[y]	11.7[x]	0.101	<0.001	<0.001	<0.001

[a,b,c] Mean within the same row with different superscripts differ significantly (*P<0.001*) by age effect.
[x,y] Mean within the same row with different superscripts differ significantly (*P<0.001*) by muscle effect.
[1] Dorsal fillet
[2] Ventral fillet
[3] Interaction between age and muscle
[4] post mortem,
[5] L* = Lightness; white=100, black=0, a* =redness; green=-80, red=100, b* =yellowness; blue=-50, yellow=70 and
[6] 1= low intensity 10= high intensity

Lipid and fatty acid composition

Results of lipid and fatty acid composition are presented in Table 3. The results indicated that the highest value was SFAs (67.079, 66.546, and 61.952%), followed by MUFAs (18.333, 18.881, and 20.103%), and PUFAs (14.592, 14.577, and 17.951%) at 10, 12, and 24 months, respectively. Furthermore, SFAs were higher in the ventral compared to the dorsal fillets, whereas PUFAs were significantly greater in the dorsal compared to ventral fillets. However, no significant difference in MUFAs was observed between dorsal and

ventral fillets. The concentration of individual fatty acids showed that the highest amount was the C18:0, followed by C16:0 and C22:6 (n-3) when fish weights increased. In the case of muscle types, C18:0 was higher in VF than DF muscles, whereas C22:6 (n-3) was higher in DF than VF muscles. However, no significant difference in C16:0 was observed between DF and VF muscles. Regarding the PUFA: SFA ratio, the present study revealed that differences in the PUFA: SFA ratio occurred among different slaughter ages and two muscle parts of the rainbow trout were observed. Fish at age 24 months had significantly higher PUFA/SFA ratio than those at 10 and 12 months, and the DF was significantly higher than that for the VF. For n-6/n-3 PUFA, the highest value was observed in rainbow trout at 12 months (0.144). This value was significantly higher than in fish at 10 and 24 months. However, no significant difference in n-6/n-3 ratio was found between 10 and 24 months. This value in VF was higher than that in the DF muscle.

Table 3. Fatty acids profile and total fatty acids (TFA, mg/100g fillet) of rainbow trout at different age and muscle

Criteria	Age (months)			Muscle		SEM	P-Value		
	10	12	24	DF	VF		Age	Muscle	Inter[3]
C 14:0	2.634^{a}	2.609^{a}	2.119^{b}	2.405^{y}	2.503^{x}	0.001	<0.001	<0.001	0.248
C 14:1	0.491^{a}	0.488^{a}	0.431^{b}	0.466^{y}	0.474^{x}	0.000	<0.001	0.024	0.661
C 15:0	9.314^{a}	7.785^{b}	6.612^{c}	7.682	8.125	0.015	<0.001	0.265	0.995
C 15:1	0.343^{b}	0.220^{c}	0.403^{a}	0.326	0.319	0.001	<0.001	0.657	0.614
C 16:0	23.270^{a}	23.820^{a}	20.152^{b}	22.437	22.391	0.015	<0.001	0.911	0.451
C 16:1	0.580^{b}	0.614^{a}	0.603^{a}	0.593	0.604	0.000	<0.001	0.127	0.326
C 17:0	0.563^{a}	0.520^{b}	0.484^{c}	0.513^{y}	0.532^{x}	0.000	<0.001	0.009	0.017
C 17:1	7.156^{a}	6.474^{b}	6.163^{b}	6.700	6.496	0.009	0.002	0.372	0.378
C 18:0	29.609^{c}	30.063^{b}	30.628^{a}	29.480^{y}	30.720^{x}	0.007	<0.001	<0.001	<0.001
C 18:1	9.475^{c}	10.925^{b}	12.304^{a}	10.800	11.003	0.006	<0.001	0.187	0.085
C 18:2n6c	0.209^{b}	0.226^{a}	0.220^{ab}	0.216	0.220	0.000	0.011	0.326	0.425
C 18:2n6t	0.343^{b}	0.914^{a}	0.277^{b}	0.475^{y}	0.547^{x}	0.001	<0.001	0.023	<0.001
C 18:3n6	0.772^{b}	0.281^{c}	0.922^{a}	0.677	0.639	0.001	<0.001	0.217	<0.001
C 18:3n3[1]	0.723^{a}	0.443^{b}	0.496^{b}	0.494^{y}	0.613^{x}	0.001	<0.001	<0.001	<0.001
C 20:0	0.164^{c}	0.191^{b}	0.206^{a}	0.180^{y}	0.194^{x}	0.000	<0.001	<0.001	<0.001
C 20:1	0.232^{a}	0.215^{b}	0.227^{a}	0.220^{y}	0.230^{x}	0.000	<0.001	0.005	<0.001
C 20:3n3	1.270^{b}	1.293^{b}	1.535^{a}	1.431^{x}	1.301^{y}	0.001	<0.001	<0.001	0.777
C 20:3n6	0.158^{c}	0.204^{b}	0.327^{a}	0.216^{y}	0.243^{x}	0.000	<0.001	0.027	0.002
C 20:4n6	0.126^{ab}	0.118^{b}	0.128^{a}	0.123	0.125	0.000	0.037	0.631	0.677
C 20:5n3[2]	0.748^{b}	0.633^{c}	0.870^{a}	0.788^{x}	0.713^{y}	0.001	<0.001	<0.001	0.026
C 21:0	1.694^{c}	1.892^{b}	2.020^{a}	1.776^{y}	1.961^{x}	0.001	<0.001	<0.001	0.041
C 22:1	0.114^{a}	0.085^{b}	0.103^{a}	0.106^{x}	0.096^{y}	0.000	<0.001	0.034	<0.001
C 22:2	0.268^{c}	0.658^{a}	0.386^{b}	0.470^{x}	0.404^{y}	0.001	<0.001	<0.001	<0.001
C 22:6n3[3]	9.991^{b}	9.825^{b}	12.809^{a}	11.838^{x}	9.911^{y}	0.008	<0.001	<0.001	0.015
C 24:0	0.279^{a}	0.182^{c}	0.251^{b}	0.239	0.236	0.000	<0.001	0.571	<0.001
C 24:1	0.061^{a}	0.048^{b}	0.035^{c}	0.045^{y}	0.050^{x}	0.000	<0.001	<0.001	<0.001
SFA[4]	67.079^{a}	66.546^{a}	61.952^{b}	64.211^{y}	66.174^{x}	0.012	<0.001	<0.001	0.214
MUFA	18.333^{b}	18.881^{b}	20.103^{a}	19.081	19.130	0.009	<0.001	0.844	0.076
PUFA	14.592^{b}	14.577^{b}	17.951^{a}	16.713^{x}	14.700^{y}	0.009	<0.001	<0.001	0.182
PUFA:SFA	0.218^{b}	0.219^{b}	0.292^{a}	0.263^{x}	0.224^{y}	0.000	<0.001	<0.001	0.088
n-6 PUFA	1.599^{c}	1.731^{b}	1.861^{a}	1.697^{y}	1.764^{x}	0.001	<0.001	0.013	0.442
n-3 PUFA	12.725^{b}	12.189^{b}	15.705^{a}	14.547^{x}	12.532^{y}	0.009	<0.001	<0.001	0.233
n-6:*n*-3	0.128^{b}	0.144^{a}	0.122^{b}	0.119^{y}	0.144^{x}	0.000	<0.001	<0.001	0.422
TFA	5242.2^{b}	6516.5^{a}	4054.6^{c}	4050.4^{y}	6491.7^{x}	6.852	<0.001	<0.001	<0.001

$^{a, b, c}$ Mean within the same row with different superscripts differ significantly (*P<0.001*) by age effect. ^{x,y} Mean within the same row with different superscripts differ significantly (*P<0.001*) by muscle effect. [1]ALA=alphalinolenic acid, [2] DHA= docosahexaenoic acid, [3] EPA= eicosapentaenoic acid. [4]SFA=saturated fatty acids, MUFA=monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

DISCUSSION

Carcass quality of different trout age and muscle type

The present study indicated that both body composition and the carcass length of rainbow trout at 24 months were significantly higher than those at 10 and 12 months. It is natural that older fish accumulate more protein and lipid compared to young ones. However, carcass percentage was significantly higher in fish at 10 and 12 months compared to 24 months. The result agreed with previous results in rainbow trout, *O. mykiss* [1]. The reason for this may be attributable to the evolution of gonadal development since gonad size increases with the growth of the animal during its reproductive stage [20]. Moreover, carcass yield also depends on the animal's sex [1]. For carcass yield, our result indicated that fish at 10 months had significantly higher VSI than those of 12 and 24 months. Similarly, rainbow trout at 14 months had higher VSI than those of 24 and 26 months, according to Davidson and coauthors [10]. By contrast, rainbow trout at weights ranging from 371-440 g showed significantly higher VSI than fish at weights between 300-370 g^{-1}. The differences in these findings may be due to the different culture conditions and diets.

Flesh quality of different trout age and muscle type

The present study revealed that pH at different slaughter ages and muscle types rapidly decreased after 24 hours post-mortem. Similar results were observed in cod, *Gadus morhua* [21]. In contrast, no significant decrease of pH after post mortem was observed in the same species, *O. mykiss* [22]. It has been well-documented that the rapid decrease in pH more or less depends on the fish stage, slaughter, and storage conditions [23]. This could be a reason for significant differences in pH at different slaughter ages found in the present study.

For chemical analysis, present results revealed that younger trout had significantly higher moisture and protein percentages than older fish, whereas lipid content was higher in older fish compared to younger fish. The results were in line with previous results in rainbow trout, *O. mykiss* [1]. However, no significant differences in moisture and fat percentages have been reported in rainbow trout at different weights [6]; and in yellow croaker, *Pseudosciaena crocea* at ages of 1 and 2 years [24]. The decrease in protein content of older fish may be attributable to the conversion of protein into fat or protein used for energy [25]. Nargis [26] reported that protein content in moderate-sized Koi carp, *Cyprinus carpio* was higher in older fish. This decrease in muscle protein may be due to the use of energy for growth [27]. Significant differences in moisture, protein, and fat percentages between different types of fillet were also observed in the present study. Similarity, significant differences in chemical composition of different muscle types and portions were observed in Pacific bluefin tuna, *Thunnus orientalis* [28] and Asian catfish, *Pangasius bocourti* [5]. The fluctuations in fish chemical composition are linked to intake-rations since protein rates in muscle tissue slightly increased in feeding time and consequently increased fat rates [29].

In terms of water holding capacity (WHC), our study indicated that the mean value WHC of 10 months rainbow trout was higher than those at 12 and 24 months. Several factors have been reported to affect the WHC of fish flesh. Suárez and coauthors [30] have demonstrated that the WHC directly related to muscle structure, which was strongly influenced by the structural changes in the proteins comprising the muscle, fibre contraction and by water distribution both intra- and extra-cellular. The free water is maintained in the interior of the tissue by capillary action and surface tension, while loss results from changes in myofibrils volume. Ang and Haard [31] indicated that the rate of pH decline in the muscle post-mortem was important, as a rapid pH decline may cause soft texture and poor water holding capacity of the meat. Nonetheless, a negative correlation between flesh pH and WHC has been revealed by Toldrá [32]. Recently, Roth and coauthors [33] have proven that stressed fish with softer flesh texture and drip loss almost 3-fold higher than rested fish, presumably as a result of physical stress of muscle fibrils or connective tissue combined with protease-mediated muscle tissue degradation. These could be the reason for a significant difference in WHC amongst different aged fish in the present study.

Our study showed that the mean values of a^* and b^* were higher in fish fillets at 24 months compared with fish at 10 and 12 months of age. However, L^* was highest in fish at 12 months, followed by 10 and 24 months of age. Our results were in agreement with previous results reported by Werner and coauthors [34]. For muscle colour, L^*, a^*, and b^* of VF were significantly higher than the DF muscle. Significant differences in colour parameters of different body parts of fish have been demonstrated in previous studies. Suárez and coauthors [35] indicated that higher values of L^* in dorsal and ventral muscles of rainbow trout compared with other lots were observed. In addition, b^* values were higher in the skin and flesh of fish. Many factors have been demonstrated to be responsible for colour changes in fish flesh. It has been well-documented that

higher muscle fat contents resulted in higher L^* and b^* values [36]. Stress also affected the flesh colour [36] by an isolubilization of muscle proteins concerning intense muscle activity before death [37]. Different culture conditions also affected lightness and skin colour distribution of gilthead seabream [38].

Regarding the sensory measurement, in this trial, firmness and overall acceptability of 10 months trout were significantly higher than the other groups. Surprisingly, ventral fillets were tenderer than dorsal fillets. The reason for this could be attributable to the fat content in fish flesh. Valente and coauthors [38] indicated that significant differences between lipid content and both fatty flavour and the perception of fatty texture was observed in gilthead seabream from different production systems. It has been reported that ventral fillets of turbot showed a more pronounced odour than dorsal ones. The reason was probably attributable to the relatively higher fat content of the ventral fillet [39]. Fillet lipid content shows a correlation with flesh texture and affects texture attributes [40]. Moreover, fillet fatty acid composition may be connected to fattiness and a so-called "juiciness experience" [41]. Izquierdo and coauthors [42] revealed that slightly lower hardness was found in the fillets of gilthead seabream fed with vegetable oils due to slightly higher lipid content and a significantly lower percentage of saturated fatty acids found in their flesh. Nonetheless, no significant differences between the firmness degree during chewing and resistance to force applied in the mouth, despite the differences in lipid content and fatty acid profile were observed [43]. For blackspot seabream, *Pagellus bogavaveo* "firmness" was similar in wild and farmed fish [44]. Fat-rich tissues normally tasted smooth and juicy, whereas dryness was found in the tissue with low fat. Lipid content, water content, and fibre characteristics are thought to contribute to the juiciness of the fish in organoleptic tests [45].

For shear force, present results were similar to those observed in triploid brown trout, *Salmo trutta* [46]. The shear force was affected by several factors, such as firmness, collagen content, and others. It has been reported that flesh firmness was positively related to the collagen content of muscle in Atlantic salmon [47]. Roth and coauthors [13] studied shear-force of salmon flesh according to different pre-slaughter techniques and found that carbon oxides technique showed minimum force (N) and lowest energy (J). An increase in shear force due to handling stress has been indicated in the raw muscle of farmed cod, although no significant difference was observed [48]. Similarly, cod chased to exhaustion in reduced water level tended to have a softer texture than dip-netted cod [49].

In the present study, a significant increase in TBARS was observed in rainbow trout flesh at all ages after 9 days of storage. In addition, TBARS in dorsal fillets was significantly higher than that of ventral fillets. These findings were in agreement with previous results reported by Daniel and coauthors [50] and Secci and coauthors [51]. The significant increase of TBARS after storage may be due to the stress pre-slaughter. It has been reported that stressful killing methods influence oxidative stress during frozen storage, both reducing the length of the induction phase and increasing the rate of lipid oxidation [51]. There is well-documented literature concerning the interaction between peroxides and lipid oxidation. Therefore, it could be assumed that the higher level of hydroperoxide might have negatively affected the flesh oxidative stability.

Lipid and fatty acid composition

Fish, especially sea fish, have considerable amounts of n-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [28]. The present study indicated that the level of SFAs (average value, 67.079%) was highest, followed by MUFAs (18.333%), and PUFAs (14.592%). Similar trends were also observed in previous studies in blackspot seabream, *Pagellus bogaraveo* [44] and yellow croaker, *Pseudosciaena crocea* [24]. However, the results disagreed with findings on *O. mykiss* [6,51], and Pacific Bluefin tuna, *Thunnus orientalis* [28], and Chinook salmon, *O. tshawytscha* [52]. Interestingly, our result indicated that fish at 10 and 12 months of age had significantly higher SFAs compared to 24 months and VF muscle was greater than DF muscle. These were in line with previous findings in *O. tshawytscha* [28]. In contrast, yellow croaker at 2-year old had significantly higher SFAs than the one-year-old fish [24]. In terms of MUFAs, the older fish (24 months) contained more than younger fish (10 and 12 months). The result agreed with findings in chinook salmon [52] but disagreed with results in yellow croaker [24]. For PUFAs, our result agreed with findings in yellow croaker [24] but disagreed with results in chinook salmon [52]. We also found that DF muscle had significantly higher PUFAs than VF muscle. However, no significant difference in PUFAs between DF and VF was observed in Pacific bluefin tuna [28]. The difference in these findings may be attributable to the differences in culture condition, diet, species, and sex.

Regarding the concentrations of individual fatty acids in the lipid fraction, the C18:0 content was dominant compared to the other ones. A similar order in the level of these fatty acids were observed in *O. mykiss* [51] and Asian catfish, *Pangasius bocourti* [5]. In contrast, a different predominance order in fatty acids were observed in yellow croaker, *P. crocea* [24] and blackspot seabream, *P. bogaraveo* [44], where the

predominant fatty acid orders were C16:0, C18 and C22:6 n-3 or C22:6 n-3, C16:0 and C18, respectively. This could be due to the use of different lipid sources in the diet because the fatty acid composition of the muscular tissue in fish reflects that of the diet [53]. Significant differences in individual predominance fatty acid were observed in rainbow trout at different ages. The results were similar to those reported by Kiessling and coauthors [52]. Finding a different order, Tang and coauthors [24] indicated that yellow croaker at 2-year old had significantly greater C16:0 than that of fish at 1 year old. In terms of C18, fish at 24 months of age showed significantly higher levels than those at 10 and 12 months. This was similar to the result reported by Kiessling and coauthors [52], but disagreed with the result of Tang and coauthors [24]. Another predominant fatty acid was C22: 6 (n-3), with our result indicating that older fish accumulated more fatty acid than younger fish. This agreed with the previous result in yellow croaker [24], but it did not agree with results obtained in Chinook salmon [52]. Different parts of the fish body also had an effect on the fatty acids profile. Rebolé and coauthors [6] have reported that lipid content and the saturated fatty acids/polyunsaturated fatty acids and n-6/n-3 ratios were higher in the skin than in the muscle; whereas, the proportion of docosahexaenoic acid (C22:6 n-3) was higher in the muscle. The present results also indicated that C18:0 was higher in VF than DF muscles, whereas C22:6 n-3 was higher in DF than VF muscles. However, no significant differences between these fatty acids were observed between dorsal ordinary muscles and ventral ordinary muscles in Pacific Bluefin tuna [28] or between different body parts of Asian catfish [5].

It indicated that differences in the PUFA/SFA ratio occurred among the three different slaughter ages and two muscle parts of the rainbow trout fillet were observed. The results agreed with a previous study in rainbow trout [6], where significant differences in PUFA/SFA and n-6/n-3 of fish at different ages and muscle types were detected. However, the PUFA/SFA and n-6/n-3 ratios of present study were lower than those reported by Rebolé and coauthors [6] and Secci and coauthors [51]. It was well-documented that fatty acid compositions of fish vary due to several factors such as the geographical location, season, food availability, water temperature, age, and size of the fish and the maturation status [54]. In addition, diets containing only *n*-3 PUFA-poor vegetable oils such as soybean and palm oil as lipid sources could lead to a decrease of EPA and DHA in farmed fish with an increase of SFA and *n*-6 PUFA [55]. All in all, the n-6/n-3 PUFA ratios of different age and muscle types are in the recommended range for a healthy diet.

CONCLUSION

The three age stages and two flesh body parts tested for rainbow trout showed different nutrient compositions. The younger fish contained significantly higher carcass percentage, protein, moisture content and acceptability; however, lower long-chain PUFA and MUFA were lower than older fish. Based on body composition, meat quality, and the n-6/n-3 PUFA ratio, the nutritional quality of younger fish is better than the older ones tested. It may be concluded that rainbow trout cultured under highland water source conditions in a tropical or sub-tropical region may be considered a valuable food source for human consumption.

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REFERENCES

1. Souza MLRd, Macedo-Viegas EM, Zuanon JAS, Carvalho MRBd and Goes ESdR, Processing yield and chemical composition of rainbow trout (*Oncorhynchus mykiss*) with regard to body weight. Acta Sci., Anim. Sci. 2015 Jun;37**(**2**)**:103-08.
2. Drazen JC, Depth related trends in proximate composition of demersal fishes in the eastern North Pacific. Deep Sea Res. Part I*:* Oceanogr. Res. Pap. 2007 Feb;54**(**2**)**:203-19.
3. Calabretti A, Cateni F, Procida G and Favretto LG, Influence of environmental temperature on composition of lipids in edible flesh of rainbow trout (*Oncorhynchus mykiss*). J. Sci. Food Agric. 2003 Oct;83:1493-98.
4. Hazel JR and Prosser CL, Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev. 1974 Jul;54(3):620-77.
5. Thammapat P, Raviyan P and Siriamornpun S, Proximate and fatty acids composition of the muscles and viscera of Asian catfish (*Pangasius bocourti*). Food Chem. 2010 Sep;122*(*1*):*223-7.
6. Rebolé A, Velasco S, Rodríguez ML, Treviño J, Alzueta C, Tejedor JL and Ortiz LT, Nutrient content in the muscle and skin of fillets from farmed rainbow trout (*Oncorhynchus mykiss*). Food Chem. 2015 May;174*(*1*):*614-20.
7. Yang P, Jiang Y and Fischer SM, Prostaglandin E 3 metabolism and cancer. Cancer lett. 2014 Jun;348*(*0*):*1-11.

8. Aliko V, Qirjo M, Sula E, Morina V and Faggio C, Antioxidant defense system, immune response and erythron profile modulation in gold fish, *Carassius auratus*, after acute manganese treatment. Fish Shellfish Immunol. 2018 May;76:101-9.
9. Carbone D and Faggio C, Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of *Sparus aurata* and *Dicentrarchus labrax*. Fish Shellfish Immunol. 2016 Jul;54:172-8.
10. Davidson JW, Kenney PB, Manor M, Good CM, Weber GM, Aussanasuwannakul A, Turk PJ, Welsh C and Summerfelt ST, Growth Performance, Fillet Quality, and Reproductive Maturity of Rainbow Trout (*Oncorhynchus mykiss*) Cultured to 5 Kilograms within Freshwater Recirculating Systems. J. Aquac. Res. Development 2014;5(4):1-9.
11. Pornsopin P, Performance Comparison of Rainbow Trout *(Oncorhynchus Mykiss)* Under the Specific Environmental Condition in the Highland of Northern Thailand. Cuvillier 2004 Sep;0:152.
12. Honikel KO, How to Measure the Water-Holding Capacity of Meat? Recommendation of Standardized Methods, in Evaluation and Control of Meat Quality in Pigs, ed. by Tarrant PV, Eikelenboom G and Monin G. Springer Netherlands; 1987. p 129-42.
13. Roth B, Moeller D, Veland JO, Imsland A and Slinde E, The Effect of Stunning Methods on Rigor Mortis and Texture Properties of Atlantic Salmon (*Salmo Salar*). J. Food Sci. 2006 Jul;67(4):1462-6.
14. ISO, Sensory analysis, in General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors, Ed. International Organisation for Standardisation (2012).
15. Cunniff P and Association of Official Analytical C, Official methods of analysis of AOAC international. Association of Official Analytical Chemists, Washington, DC (1995).
16. Rossell JB, Measurement of rancidity, in *Racidity in Foods*, ed. by Allen JC and Hamilton RJ. Chapman & Hall, London, England; 1994. p. 22-53.
17. Folch J, Lees M and Sloane Stanley GH, A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 1957 May;226(1):497-509.
18. Morrison WR and Smith LM, Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride - methanol. J. Lipid Res. 1964 Oct;5:600-8.
19. SAS, SAS/STAT Sofware: Changes and Enhancements, Relaese 8.2, Ed by Inc. I, Cary, NC. USA (1996).
20. Britto ACPd, Rocha CB, Tavares RA, Fernandes JM, Piedras SRN and Pouey JLOF, Rendimento corporal e composição química do filé da viola (*Loricariichthys anus*). Ciênc. Anim. Bras. 2014 Jan;15(1):38-44.
21. Hultmann L, Phu TM, Tobiassen T, Aas-Hansen Ø and Rustad T, Effects of pre-slaughter stress on proteolytic enzyme activities and muscle quality of farmed Atlantic cod (*Gadus morhua*). Food Chem. 2012 Oct;134(3):1399-408.
22. Lefèvre F, Bugeon J, Aupérin B and Aubin J, Rearing oxygen level and slaughter stress effects on rainbow trout flesh quality. Aquaculture 2008 Nov;284(1-4):81-9.
23. Roth B, Slinde E and Robb DHF, Field evaluation of live chilling with CO2 on stunning Atlantic salmon (*Salmo salar*) and the subsequent effect on quality. Aquac. Res. 2006 May;37(8):799-804.
24. Tang H-g, Chen L-h, Xiao C-g and Wu T-x, Fatty acid profiles of muscle from large yellow croaker (*Pseudosciaena crocea* R.) of different age. J. Zhejiang Univ. Sci. B. 2009 Fed;10(2):154-8.
25. Vieira VAR, Hilsdorf AW and Moreira RG, The fatty acid profiles and energetic substrates of two Nile tilapia (*Oreochromis niloticus*, Linnaeus) strains, Red-Stirling and Chitralada, and their hybrid. Aquac. Res. 2012 Apr;43(4):565-76.
26. Nargis A, Seasonal variation in the chemical composition of Body flesh of Koi Fish *Anabas testudineus* (Bloch)(Anabantidae: Perciformes). Bangladesh J. Sci. Ind. Res. 2006;41(3):219-26.
27. Alemu L, Melese A and Gulelat D, Effect of endogenous factors on proximate composition of nile tilapia (*Oreochromis niloticus* L.) fillet from lake zeway. Am. J. Res. Commun. 2013;1(11):405-10.
28. Nakamura Y-N, Ando M, Seoka M, Kawasaki K-i and Tsukamasa Y, Changes of proximate and fatty acid compositions of the dorsal and ventral ordinary muscles of the full-cycle cultured Pacific bluefin tuna *Thunnus orientalis* with the growth. Food Chem. 2007;103(1):234-41.
29. Boran G and Karaçam H, Seasonal Changes in Proximate Composition of Some Fish Species from the Black Sea. Turk. J. Fish. Aquat. Sci. 2011 Dec;11(01-05).
30. Suárez MD, Abad M, Ruiz-Cara T, Estrada J and García-Gallego M, Changes in muscle collagen content during post mortem storage of farmed sea bream (*Sparus aurata*): influence on textural properties. Aquacult. Int. 2005 Jul;13:315-25.
31. Ang JF and Haard NF, Chemical composition and postmortem changes in soft textured muscle from intensely feeding Atlantic cod (*Gadius morhua,* L). J. Food Biochem. 1985 Mar;9(1):49-64.
32. Toldrá F, Muscle Foods: Water, Structure and Functionality. Food Sci. Technol. Int. 2003 Jun;9:173-7.
33. Roth B, Slinde E and Arildsen J, Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on rigor mortis and the physical properties of flesh. Aquaculture 2006 June;257(1-4):504-10.
34. Werner C, Poontawee K, Mueller-Belecke A, Hoerstgen-Schwark G and Wicke M, Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. Arch. Anim. Breed. 2008;51:71-83.
35. Suárez MD, García-Gallego M, Trenzado CE, Guil-Guerrero JL, Furné M, Domezain A, Alba I and Sanz A, Influence of dietary lipids and culture density on rainbow trout (*Oncorhynchus mykiss*) flesh composition and quality parameter. Aquac. Eng. 2014 Dec;63:16-24.

36. Erikson U and Misimi E, Atlantic salmon skin and fillet color changes effected by perimortem handling stress, rigor mortis, and ice storage. J Food Sci. 2008 Mar;73*(2):*C50-59.
37. Robb DHF, Kestin SC and Warriss PD, Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. Aquaculture 2000 Feb;182*(*3-4*):*261-9.
38. Valente LMP, Cornet J, Donnay-Moreno C, Gouygou JP, Bergé JP, Bacelar M, Escórcio C, Rocha E, Malhão F and Cardinal M, Quality differences of gilthead sea bream from distinct production systems in Southern Europe: Intensive, integrated, semi-intensive or extensive systems. Food Control 2011 May;22*(*5*):*708-17.
39. Regost C, Arzel J, Robin J, Rosenlund G and Kaushik SJ, Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*): 1. Growth performance, flesh fatty acid profile, and lipid metabolism. Aquaculture 2003 Mar;217(1-4):465-82.
40. Johansson L, Kiessling A, Kiessling KH and Berglund L, Effects of altered ration levels on sensory characteristics, lipid content and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). Food Qual. Prefer. 2000 May; 11*(*3*):*247-54.
41. Waagbø R, Sandnes K, Torrissen OJ, Sandvin A and Lie Ø, Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of N-3 polyunsaturated fatty acids at two levels of vitamin E. Food Chem. 1993;46*(4):*361-66.
42. Izquierdo MS, Montero D, Robaina L, Caballero MJ, Rosenlund G and Ginés R, Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture 2005 Nov;250*(*1-2*):*431-44.
43. Turchini GM, Mentasti T, Frøyland L, Orban E, Caprino F, Moretti VM and Valfré F, Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). Aquaculture 2003 Jul;225*(*1-4*):*251-67.
44. Rincón L, Castro PL, Álvarez B, Hernández MD, Álvarez A, Claret A, Guerrero L and Ginés R, Differences in proximal and fatty acid profiles, sensory characteristics, texture, colour and muscle cellularity between wild and farmed blackspot seabream (*Pagellus bogaraveo*). Aquaculture 2016 Jan;451*:*195-204.
45. Johnston IA, Muscle development and growth: Potential implications for flesh quality in fish. Aquaculture 1999 Jul;177*(*1-4*):*99-115.
46. Regost C, Arzel J, Cardinal M, Laroche M and Kaushik SJ, Fat deposition and flesh quality in seawater reared, triploid brown trout (*Salmo trutta*) as affected by dietary fat levels and starvation. Aquaculture 2001 Feb;193*(*3-4*):*325-45.
47. Sriket C, Proteases in fish and shellfish: Role on muscle softening and prevention. Int. Food Res. J. 2014;21***(1):***433-45.
48. Bjørnevik M and Solbakken V, Preslaughter stress and subsequent effect on flesh quality in farmed cod. Aquac. Res. 2010 Apr;41***(***10***):***e467-74.
49. Digre H, Erikson U, Misimi E, Lambooij B and Van De Vis H, Electrical stunning of farmed Atlantic cod *Gadus morhua* L.: a comparison of an industrial and experimental method. Aquac. Res. 2010 Jul;41*(*8*):*1190-202.
50. Daniel AP, Ferreira LF, Klein B, Ruviaro AR, Quatrin A, Parodi TV, Zeppenfeld CC, Heinzmann BM, Baldisserotto B and Emanuelli T, Oxidative stability during frozen storage of fillets from silver catfish (Rhamdia quelen) sedated with the essential oil of *Aloysia triphylla* during transport. Ciênc. Rural 2016 May;46*(*3*):*560-66.
51. Secci G, Parisi G, Dasilva G and Medina I, Stress during slaughter increases lipid metabolites and decreases oxidative stability of farmed rainbow trout (*Oncorhynchus mykiss*) during frozen storage. Food Chem. 2016 Jan; 190:5-11.
52. Kiessling A, Pickova J, Eales JG, Dosanjh B and Higgs D, Age, ration level, and exercise affect the fatty acid profile of chinook salmon (*Oncorhynchus tshawytscha*) muscle differently. Aquaculture 2005 Jan;243*(*1-4*):*345-56.
53. Simmons CA, Turk P, Beamer S, Jaczynski J, Semmens K and Matak KE, The effect of a flaxseed oil-enhanced diet on the product quality of farmed brook trout (*Salvelinus fontinalis*) fillets. J. Food Sci. 2011 Apr;76***(****3****):***S192-7.
54. Ould Ahmed Louly AW, Gaydou EM and Ould El Kebir MV, Muscle lipids and fatty acid profiles of three edible fish from the Mauritanian coast: *Epinephelus aeneus*, *Cephalopholis taeniops* and Serranus scriba. Food Chem. 2011 Jan;124***(1)***:24-8.
55. Strobel C, Jahreis G and Kuhnt K, Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products. Lipids Health Dis. 2012 Oct;11:144.