Original Research Article

Effect of Temperature and Long Storage Of Fatty Acid Profile, Value Peroxide and Value TBA Fillet of Snapper (*Lutjanus sp*)

Rahim Husain[1], Rieny Sulistijowati[2]

[1] Faculty of Fisheries and Marien Science, Gorontalo State University. Jend.Sudirman, No.6, Gorontalo City
[2] Faculty of Fisheries and Marien Science, Gorontalo State University. Jend.Sudirman, No.6, Gorontalo City
Coressponding author: imrahim76@yahoo.co.id

ABSTRACT

This research was conducted at the Fisheries Technology Laboratorium Department of Fisheries and Marine Sciences, Gorontalo State University, at the Mey 2018 for three months in a month. .Fish has a high nutritional value and is a major food source in many countries. Lipid fish has a high content of polyunsaturated acids (PUFA), especially eicosapentaenoic acid (EPA;20:5n-3) and docosahexenoate acid (DHA; 22: 6n-3). This study aims to the impact effect of temperature and storage time of the fatty acid snapper fillet *(Lutjanus sp*) and damage caused by the storage process. The sample selection stage is Snapper (Lutjanus sp) fresh weeds, and the meat is released by gills, bones, then washed and rinsed with ice water to remove blood and other debris. Contineus does the cleavage from head to tail without causing his back to be disturbed. Filleting is done by slicing the meat ribs longitudinally to produce boneless meat. Stored at temperatures of 0, 10, 20, 30 and 40^{o}C for 45 days. The results of fatty acid analysis snapper fillet (Lutjanus sp) has a saturated fatty acid lauric acid, acid tridecanoic, myristate acid, pentadecanoic acid, palmitic acid, stearic acid, heneicosanoic acid, behenic acid, lignoceric acid, acid and acid heptadecanoate arachidic. Monounsaturated fatty acids oleic acid, nervonic acid, palmitoleic acid, acid and acid erucat eicosanoic, while polyunsaturated fatty acids eicosatetraenoate acid, dokosadinoat acid, eicosapentaenoic acid, eikosatrioanat acid, arachidonic acid and linolenic acid are susceptible to oxidative damage. Increased primary peroxide snapper fillet (*Lutjanus sp)* the higher the storage temperature increases from 0°C to 40°C. TBA value increases from 0° to 40°C with increased storage time of one day to 45 days storage time. from the results of this study found a way for Snapper fish fillets *(Lutjanus sp)* to have a longer expiration in storage, from 1 day to 45 days without reducing the Omega and DHA content.

Keywords: Fillet Snapper (Lutjanus sp), fatty acids, peroxide value and TBA value

INTRODUCTION

In general, the fish has no nutritional value and is a major food source in many countries. Lipid fish has a high content of polyunsaturated acids (PUFA), especially eicosapentaenoic acid {EPA; 20: 5n-3} and docosahexenoate acid (DHA; 22: 6n-3). {Pazos et al., 2005; Bayir et al., 2006}[17]. Processing by freezing the fish has been used for thousands of years because of the quality and high product {Persson and Londahl., 20013}[18]. The concept of storage by freezing depends on the product temperature decrease to slow decay so that when the fish melted freshness can be maintained (Kolbe et al., 2004)[12].

However, fish and fishery products may undergo undesirable changes during storage and can damage the storage time limit. Unwanted changes resulting from the oxidation of proteins {Fujiwara et al., 2008; Benjakul et al., 2005}[10] and lipid oxidation {Sarma et al., 2000; Richards and Hultin., 201)[20]. Fish protein experienced a number of changes (cause can not form aggregates) that modify the structural and

UNDER PEER REVIEW

functional properties of fish muscle{Badii and Howell., 2012}[4].

Degradation of polyunsaturated fatty acids (PUFA) by lipid oxidation during storage led to the formation of volatiles associated with rancidity (Pazos et al., 2005)[17]. The high level of unsaturated fats makes the fish tissue is very susceptible to peroxidation and easily damaged. Oxidative changes primarily related to the taste and texture of the fish. In later stages of the process of lipid peroxidation, changes in color and nutritional value will be observed or secondary products of lipid (Dragoev et al., 2008)[9]. This study aims to determine the effect of temperature and storage time of the fatty acid snapper fillet (*Lutjanus sp*) and damages resulting from the storage process.

MATERIAL AND METHODS

Raw material

Raw materials snapper (*Lutjanus sp*) obtained from the fish auction place (TPI) kobong, Village Kaligawe, Semarang, Central Java.

Sample Preparation Fish fillets

The sample selection stage is Snapper (Lutjanus sp) fresh weeds, and the meat is released by gills, bones, then washed and rinsed with ice water to remove blood and other debris. Contineus does the cleavage from head to tail without causing his back to be disturbed. Filleting is done by slicing the meat ribs longitudinally to produce boneless meat. Stored at temperatures of 0, 10, 20, 30 and 40^{o}C for 45 days.

Proximate analysis

Chemical analysis: Proximate moisture content, ash content, protein and fat by AOAC (2006).

Fatty Acid Analysis Procedure

a. Hydrolysis

After selecting the sample, then the sample is cleaned with water, then mashed and weighed ± 10 g of the sample. Hydrolysis with 10 ml HCl. then the water is heated at a temperature of 70 ° C, boiling. Cold. Extract with 15 ml of diethyl ether and 15 ml of oil, take the top layer. Extract again with 15 ml of diethyl ether and 15 ml of oil, take the top layer, make one with the previous results. Steamed in a water bath with the help of N2.

b. Analysis of saturated fatty acids and unsaturated

Taken 0.5 ml sample, add 1.5 ml of sodium methanolic solution, cover and heat at 70°C for 5-10 minutes while being shaken. Chill. Add 2 ml Boron trifluoride ethanoic, heat at 70°C for 5-10 minutes. Chill. Extract with 1 ml of heptane and 1 ml saturated NaCl. Take the top layer and put in Eppendorf. Injected into the GC. Injected as much 1µ samples on GC - 2010 SHIMADZU.

Analysis Value Peroxide

Samples (0.5 g) put in a test tube, then added 0.1 ml of a solution of ammonium thiocyanate and 0.1 ml of solution feroklorida. The test tube is shaken for 5 seconds and heated at 50^{0}C for 2 minutes, then cooled to a temperature of 25^{0}C. Use absorbance using a spectrophotometer at a wavelength of 510 nm. A blank solution prepared using all solvent without the sample (Hills and Thiel, 1946 modified Adnan, 1980).

Analysis Value TBA (expand)

Analysis of figures TBA performed according to the method Tokur and Korkmaz (2007)[24]. Oil added 0.5 g in 50 ml of distilled water, then add another 2.5 mL N HCl then distilled. Absorb distilled to 50 ml, 5 ml of distilled download then added with 5 ml TBA. Thereafter, heated for 30 minutes and cooled. Absorbance at a wavelength of 528 nm. Value TBA = mg malonaldehyde / kg of oil.

RESULTS AND DISCUSSION

Proximate Fish Snapper (*Lutjanus sp*)

Based on Table 1. fat content snapper (*Lutjanus sp*) in the study was 1.96% compared to that reported by de Castro et al., (2010) [8] on tilapia (*Oreochromis niloticus*), i.e 0.79% and tambagui (*Colossoma macropomum*) i.e 1.30%. The fat content rough on snapper (1.96%) is still higher, it indicates that the

UNDER PEER REVIEW

snapper (*Lutjanus sp*) belong to the fish that contains high fat enough.

Table 1. Proximate Fish Snapper (*Lutjanus sp*) and Fe content

No	Chemical composition	amount (%)
1.	Moisture	78,39
2.	Ash	1,58
3.	Protein	18,77
4.	Fat	1,96
5.	Carbohydrate by difference	0,30
6.	Fe (ppm)	121,7

Description: Data is derived from repeat 3x

Differences in fish fat content are strongly influenced by the type of fish, fish size, fishing season and the environment in which the fish live. According to (Shaviklo 2006)[23] demersal fish higher in fat than pelagic fish that lives in surface waters. Demersal fish usually live in the bottom waters and rarely engage in the activity. In Table 1 also shows the snapper (*Lutjanus sp*) containing Fe total was 121.47 ppm. Okada report (2010)[15] describes the color of the flesh of fish due to the Fe content in the meat is very high because it is rich hemoprotein (80%) mainly myoglobin and hemoglobin. Based Okada (2010), the content of white meat hemoprotein red snapper low so the meat is white.

Composition Fatty Acid

Table. 2. Composition fatty acids of snapper fillet (*Lutjanus sp*) during storage

No	Profil asam lemak (%)	Storage temperature (°C)				
		0°C	10°C	20°C	30°C	40°C
1	Lauric acid	0,703	-	-	0,99	-
2	Tridecanoac acid	0,001	-	-	0,86	-
3	Myristic acid	4,687	0,74	4,44	2,69	0,51
4	Pentadecanoac acid	0,001	0,90	1,03	2,46	0,38
5	Palmetic acid	2,283	1,67	4,39	3,58	12,94
6	Stearic acid	0,708	-	9,19	15,46	12,14
7	Heneicosanoac acid	13,462	-	0,78	2,03	-
8	Behenac acid	-	1,04	0,65	0,68	1,54
9	Lignoserat acid	0,762	-	0,74	-	0,55
10	Heptadecanoac acid	17,811	-	1,96	-	-
11	Arachidac acid	2,945	-	-	-	-
I	Saturated Fatty Acid (SFA)	25,552	4,35	32,73	31,75	28,06
1	Oleic acid	8,807	12,63	16,37	22,70	10,82
2	Nervonac acid	1,64	1,34	1,03	1,27	-
3	Palmitoleic acid	13,239	2,59	-	-	-

UNDER PEER REVIEW

4	Erucat acid	-	5,43	5,26	5,43	3,58
5	Eicosanoac acid	0,001	8,34	-	-	-
II	Mono Unsaturated Fatty Acid (MUFA)	23.687	23,72	22,66	29,4	14,4
1	Eicosatetraenoic acid	-		-	5,60	1,05
2	Docosadinoac acid	0,827	-	0,43	-	0,70
3	Eicosapentanoac acid	6,055	3,52	2,29	0,43	-
4	Eicosatrienoac acid	1,557	2,6,23	9,82	-	-
5	Arachidonac acid	0,692	-	-	-	-
6	Docosaheksanoac acid	5,31	-	0,17	0,25	-
7	Linoleict acid	10, 612	1,52	3,27	2,56	3,57
III	Poly Unsaturated Fatty Acid (PUFA)	25,055	14,99	15,98	8,84	10,32
Total		74,294	103.07	101.37	99,99	82,78

Description: Data is derived from repeat 2x

Based on the analysis of fatty acids snapper fillet (*Lutjanus sp*) are listed in Table 4.2, snapper fillet (*Lutjanus sp*) has saturated fatty acids (Saturated Fatty Acid, SFAs), which consists of lauric acid, acid tridecanoic, acid myristate, acid pentadecanoic , palmitic acid, stearic acid, heneicosanoic acid, behenic acid, lignoceric acid, acid and acid heptadecanoate arachidic. Acids monounsaturated fats (MUFA) consisting of: oleic acid, acid nervonat, palmitoleic acid, acid erucat and sour eicosanoic, while the unsaturated fatty acid compound (Poly Unsaturated Fatty Acids, PUFAs) consisting of acid eicosatetraenoate, dokosadinoat acid, eicosapentaenoic acid, eikosatrioanat acid, arachidonic acid and linolenic acid are susceptible to oxidative damage.

According Nazemroaya et al., (2011); Karami et al., (2013)[14] that when the fish are kept in a long time then, the amount of saturated fatty acids (Saturated Fatty Acid, SFA) will increase as well as fatty acids monounsaturated (Mono Unsaturated Fatty Acid, MUFA) will be slightly experience improved although not too significant. While polyunsaturated fatty acids (Poly Unsaturated Fatty Acids, PUFAs) experienced a reduction process. This is similar to the research conducted at the snapper fillet (*Lutjanus sp*) during storage.

Aranda et al., (2005)[3] states that the oxidation of PUFAs begins with the production of hydrogen peroxide with three different ways: (1) autooksidasi, (2) enzymatic oxidation, and (3) photooxidation, which can also occur simultaneously. Product peroxide decomposes by different mechanisms, forming a secondary oxidation products; both the primary and secondary oxidation products can react with amino groups of proteins, producing compounds that modify the interaction of taste, smell and functional properties of proteins.

Furthermore, the fraction of volatile compounds is indicated as the cause of rancidity in fatty fish (Aranda et al., 2005)[3]. Serbecic and Beutelspacher, (2005)[21] states that the oxidation is the process of fat breakdown and lead to the formation of off-flavor compounds and condition is called rancid (rancid). Processed food products were rancid, may experience discoloration and loss of nutritional value for the oxidation of unsaturated fatty acids (PUFA) that impact on the quality. The compound oxide such as peroxides, aldehydes, and ketones harmful to human health. While McClement and Decker (2000)[13] states the factors that affect the speed of oxidation include the number and type of oxygen, the chemical structure of lipids, antioxidant and prooxidant form: ferrous metals, sensitiser, storage temperature and properties of packaging materials.

Effect of Temperature and Time Against Value Peroxide

Value peroxide as primary products of oxidation snapper fillet (*Lutjanus sp*) during storage at different temperatures and times can be seen in Table 3.

Table 3. Date analysis peroxide value snapper fillet (*Lutjanus sp*)

Temperature 0°C

UNDER PEER REVIEW

N0	Storage temperature (day)	Peroxide Value (meq/kg)
1	0	1.7411
2	5	2.9437
3	10	4.06055
4	15	5.296
5	20	6.7797
6	25	6.29125
7	30	10.905
8	35	11.4923
9	40	12.98225
10	45	13.21835
	Temperature 10°C	
N0	Storage temperature (day)	Peroxide Value (meq/kg)
1	0	1.7411
2	3	3.26525
3	6	5.63635
4	9	6.90375
5	12	6.3911
6	15	9.60175
7	18	10.78685
8	21	10.4857
9	24	14.5174
10	27	15.9977
	Temperature 20°C	
N0	Storage temperature (day)	Peroxide Value (meq/kg)
1	0	1.7411
2	1	4.21665
3	2	6.96335
4	3	8.3699
5	4	8.5134
6	5	11.22005
7	6	11.963
8	7	15.59645
9	8	15.9638
10	9	18.0701
	Temperature 30°C	
N0	Storage temperature (day)	Peroxide Value (meq/kg)
1	0	1.7411
2	0.5	4.5663

UNDER PEER REVIEW

3	1	7.06935
4	1.5	9.2808
5	2	10.422
6	2.5	12.1345
7	3	11.83165
8	3.5	16.974
9	4	18.703
10	4.5	20.4086

Temperature 40°C		
N0	Storage temperature (day)	Peroxide Value (meq/kg)
1	0	1.7411
2	0.25	4.67575
3	0.5	7.7808
4	0.75	10.7164
5	1	12.06535
6	1.25	12.6637
7	1.5	18.4146
8	1.75	18.42355
9	2	22.9266
10	2.25	24.62795

Description: Data is derived from repeat 3x

In the treatment of frozen storage temperature (0°C) shows the formation of peroxides to 45 days of storage. Speed peroxide formation increased 9.6 times at a temperature of 0°C with a storage temperature increases, while at a temperature of 10, 20, 30, and 40°C peroxide value increased respectively 14.33, 16.12, 19.19, and 23.05 times meq/kg sample with increased storage temperature. The peroxide values increased from 0° to 40°C with increased storage time of one day to 45 days storage time. Increased primary peroxide snapper fillet (*Lutjanus sp*) the higher the storage temperature increases from 0°C to 40°C.

According to Pak et al, (2005) [16], peroxide value is an indicator of the stability of the oil against oxidation, the oxidation products of primary lipid parameters, namely hydroperoxide. Oxidation of lipids/oils naturally easily occurs, because tuna fish oil rich in PUFAs (6 double bonds), while oil containing many double bonds susceptible to lipid oxidation reactions. Thus, the oxygen molecules that are attached to the double bond susceptible to oxidation.

Effect of Temperature To Value TBA

Value TBA is used to measure the secondary products of lipid oxidation, especially coming from PUFA (hide no, 2012) and indicates the level of rancidity, especially in the high PUFA-containing oil (Cheng et al., 2014). TBA formation as a secondary oxidation products snapper fillet (*Lutjanus sp*) during storage can be seen in Table 4.

Table 4. Data analysis value TBA snapper fillet (*Lutjanus sp*)

Temperature 0°C		
No	Storage temperature (day)	Value TBA (mg MDA/kg)
1	0	2.307378

UNDER PEER REVIEW

2	5	3.504316
3	10	5.277034
4	15	6.573477
5	20	6.315832
6	25	9.565049
7	30	11.91097
8	35	11.75207
9	40	13.71696
10	45	14.62961

Temperature 10°C		
No	Storage temperature (day)	Value TBA (mg MDA/kg)
1	0	2.307378
2	3	4.299369
3	6	6.748229
4	9	6.105547
5	12	10.76352
6	15	13.41159
7	18	13.2912
8	21	15.61061
9	24	16.80039
10	27	17.21704

Temperature 20°C		
No	Storage temperature (day)	Value TBA (mg MDA/kg)
1	0	2.307378
2	1	4.424119
3	2	6.195105
4	3	6.932098
5	4	10.79865
6	5	13.33775
7	6	13.45672
8	7	17.74074
9	8	18.62311
10	9	20.31495

Temperature 30°C		
No	Storage temperature (day)	Value TBA (mg MDA/kg)
1	0	2.307378
2	0.5	5.887911
3	1	7.589422

UNDER PEER REVIEW

4	1.5	8.931236
5	2	8.773049
6	2.5	13.85214
7	3	14.5131
8	3.5	14.5922
9	4	20.7325
10	4.5	22.03099

Temperature 40°C

No	Storage temperature (day)	Value TBA (mg MDA/kg)
1	0	2.307378
2	0.25	5.975746
3	0.5	7.946435
4	0.75	8.185896
5	1	8.54802
6	1.25	10.79128
7	1.5	15.43709
8	1.75	15.59018
9	2	20.52694
10	2.25	23.57116

Description: Data is derived from repeat 3x

Value TBA increased 7.21 times at a temperature of 0°C with an increase in storage temperature, whereas at a temperature of 10, 20, 30, and 40°C peroxide value increased respectively to 9.19, 9.89, 10.63, and 12.59 times the MDA mg/kg of the sample with increased storage temperature. Value TBA increases from 0° to 40° with increased storage time of one day to 45 days storage time. In the treatment of storage temperature of 0°C-40°C showed the formation of value TBA during storage.

CONCLUSION

Differences in fat content of the fish are strongly influenced by the freshness of the fish is used as a critical factor in resulted isolates, methods of isolation/extraction is used, homogenization of meat processing, the ratio of fish and solvents (viscosity) used, length of extraction, time and temperature processing and dissolution protein. Snapper fillet (*Lutjanus sp*) has a saturated fatty acid comprising: lauric acid, acid tridecanoic, myristate acid, pentadecanoic acid, palmitic acid, stearic acid, heneicosanoic acid, behenic acid, lignoceric acid, acid and acid heptadecanoate arachidic. Fatty acids are monounsaturated consisting of: oleic acid, acid nervonat, palmitoleic acid, acid erucat and sour eikosanoat, while the unsaturated fatty acid compound consisting of: acid eicosatetraenoate, acid dokosadinoat acid, eicosapentaenoic acid eikosatrioanat, arachidonic acid and linolenic acid susceptible to oxidative damage.

Speed formation of peroxide value increased 9.6 times at a temperature of 0°C with a storage temperature increases, while at a temperature of 10, 20, 30, and 40°C peroxide value increased respectively 14.33, 16.12, 19.19 and 23.05 times meq/kg sample with increased storage temperature. Value TBA increased 7.21 times at a temperature of 0°C with an increase in storage temperature, whereas at a temperature of 10, 20, 30, and [10]40°C peroxide value increased respectively to 9.19, 9.89, 10.63, and 12.59 times the MDA mg/kg of the sample with increased storage temperature.

REFERENCES

UNDER PEER REVIEW

[1] Adnan, M. (1980). *Lipid Properties and Stability of Partially Defatted Peanuts*. Doctor Thesis, Department of Food Science, University of Illinois, Urbana-Champaign.

[2] AOAC., 2006, "Official Methods of Analysis of the Association of Official Analytical Chemistry", *Association of Official Analytical Chemistry, Washington, DC*.

[3] Aranda, M., Mendoza, N., & Villegas, R. (2005). Lipid damage during frozen storage of whole jack mackerel (Trachurus symmetric murphy). *Journal of Food Lipids*, 13, 155–166.

[4] Badii, F., and Howell, N. K., 2012. Changes in the texture and structure of cod and haddock fillet during frozen storage. *Food Hydrocolloids*, 16(4), 313-319.

[5] Bayir, A. and Sirkecioglu, A. N., 2006. Fatty acid composition in some selected marine fish species living in Turkish water. *Journal of Science and Food Agriculture*, 86(1), 163-168.

[6] Benjakul, S., Viessanguan, W., Thongkaew, C. and Tanaka, M., 2005. Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. *Food hydrocolloids*, 19(2), 197-207.

[7] Boran G, Karach, Boran M. (2006). Changes In The Quality Of Fish Oils Due To Storage Temperature And Time. *Food Chemistry***. 98**: 693–698.

[8] De Castro, F.A.F., Sant'Ana a, P.M.H., Campos,M.F., Costa, B.M.N., Silva, C.T.M., Salaro, L.A., Franceschini, C.D.S., (2007). Fatty acid composition of three freshwater fishes under different storage and cooking processes. Food Chemistry. 103: 1080–1090.

[9] Dragoev, S.G., Kiosev, D.D., Danchev, S.A., Ioncheva, N.I. and Genov, N.S., 2008. Study on the oxidative processes in frozen fish. *Journal of Agriculture and Science*, 4(1), 55-65.

[10] Fijuwara, K., Oosawa, T. and Saeki, H., 2008**.** Improved thermal stability and emulsifying proper-Ties of carp myofibrillar proteins by conjunction with dextran. *Journal of Agricultural and Food Chemistry*, 46(4), 1257-1261.

[11] Karami, B., Moradi, Y., Motallebi, A. A., Hosseini, E., Soltani, M. (2015). Effects of frozen storage on fatty acids profile, chemical quality indices and sensory properties of red tilapia (*Oreochromis niloticus* × *Tilapia mosambicus*) fillets. *Iranian Journal of Fisheries Sciences* 12(2) 378-388.

[12] Kolbe, E., Craven, C., Sylvia, G. and Morrissey, M., 2004. Chilling and freezing guidelines to Maintain Onboard Quality and Safety of Albacore Tuna Agricultural Experiment Station. Astoria, Oregon, USA: Oregon State University.

[13] McClements, D.J.dan Decker, E.A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of moleculer environment on a chemical reaction in heterogenous food system. *Journal of Food Science***. 65**: 1270-1282.

[14] Nazemroaya, S., Sahari, A.M., and Rezaei, M., (2009). Effect of frozen storage on fatty acid composition and changes in lipid content of Scomberomorus immersion and Carcharhinus dussumieri. *Journal Application Ichthyology*. 25: 91–95

[15] Okada, M. (2010). Fish and raw material. In the science of processing marine food product. Vol. **I**. editor. T. Motohiro, H. Kadota. K. Hashimoto. M. Katayama and T. Tokunaga. *Japan International Cooperation Agency. Hyoga International Centre Japan*.

[16] Pak, C, S., (2005). Stability and Quality Of Fish Oil During Typical Domestic Application, Fisheries Training Programme, *The United Nations University*, Iceland.

[17] Pazos, M., Gallardo, J. M., Torres, J. L. and Medina, I., (2005)**.** The activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chemistry*, 92, 547-557.

[18] Persson, P. O. and Londahl, G., (2013). Freezing technology. In C. P. mallet (Ed.), Frozen food technology. Glasgow, UK: Blackie Academic & Professional.

[19] Richards, M. P. and Hultin, H. O., 2012. Contributions of blood and blood components to lipid oxidation in fish Muscle, Journal Agriculture Food Chemistry, 50(3), 555-564.

[20] Sarma, J., Reddy, G. V. S. and Srikar, L. N., 2000. Effect of frozen storage on lipids and functional properties of proteins of dressed Indian oil sardine (*Sardinella longiceps*). *Food Research International*, 33(10), 815-820.

[21] Secbecic, N dan Beutelspecher, S.C. 2005. Anti-oxidative vitamins prevent lipid-peroxidation

UNDER PEER REVIEW

and apoptosis in corneal endothelial cell. Cell tissue Res **320**: 465-475.

[22] Semb, T, N., (2012). Analytical Methods for determination of the oxidative status in oils. *Department of Biotechnology*, Norwegian University of Science and Technology.

[23] Shaviklo, G.R., (2006). Quality assessment of ash protein isolates using surimi standard methods.
Reykjavik, Ice/and: The United Nations University.

[24] Tokur, B., Korkmaz, K., (2007). The effects of an iron-catalyzed oxidation system on lipids and proteins of dark muscle fish. *Food Chemistry*, **104**: 754-760.

UNDER PEER REVIEW