INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 4, p. 1-7, 2015

RESEARCH PAPER OPEN ACCESS

Fatty acid profile of fresh betok (*Anabas testudineus* Bloch) fish and traditional salted fermented fresh betok fish with and without palm (*Arenga pinnata*) sugar and lime (*Citrus aurantifolia*) juice

Petrus

Departement of Fisheries, Faculty of Agriculture, Palangkaraya University, Palangkaraya, Central Kalimantan, Indonesia

Key words: Fatty acid profile, Fresh Betok fish, Fermentation, Palm sugar, Lime juice.

http://dx.doi.org/10.12692/ijb/6.4.1-7 Article published on February 21, 2015

Abstract

Fatty acid profile of fresh betok (*Anabas testudineus* Bloch) fish, traditional salted fermented fresh betok fish with and without palm (*Arenga pinnata*) sugar and lime (*Citrus aurantifolia*) juice had been studied. The changes of fatty acid profile of those samples were observed and it was found that addition of palm sugar and lime juice during preparation of salted fermented fresh betok (*Anabas testudineus* Bloch) fish reduced the amount of lauric acid, myristic acid, palmitic acid, stearic acid, EPA, DHA, SFA and PUFA. However, the addition of palm sugar and lime juice increased especially palmitic acid, oleic acid and MUFA. Therefore modified traditional salted fermentation by adding palm sugar and lime juice could improve a new healthy and extended shelf life of fermented fresh water fish product.

* **Corresponding Author:** Petrus ✉ petrussenas@yahoo.co.id

Introduction

Betok (*Anabas testudineus* Bloch) is a fresh water fish which abundantly found in water area of Kalimantan and people also know this fish as papuyu fish. This species is very popular and consumed widely in South and Central Kalimantan for decades and traditionally fermented product of this fish is known as wadi betok (Petrus,2009). According to Petrus *et al.* (2013[a]) the addition of 15% (w/w) salt and 7 days of fermentation resulted the best traditionally fermented betok fish (wadi betok) in Banjar regency, South Kalimantan Province. While Petrus *et al.* (2013[b]) reported that addition of 15% (w/w) palm (*Arenga pinnata*) and 6% (w/w) lime (*Citrus aurantifolia*) juice during fermentation process obtained the best modified traditional salted fermented betok fish from the point of view of physicochemical, microbial and organoleptic quality.

Cengiz *et al.* (2010),Hajirostamloo and Hajirostamloo (2010) and Mohamed and Al-Sabahi (2011) stated that fish is not only a good source of protein , but also rich in polyunsaturated fatty acids especially omega 3 and 6 which are beneficial for human health. Dincer *et al.* (2010) found changes in either amino acids and fatty acids composition of fermented sardine fish sauce, furthermore this product was a good source of EPA and DHA. Majumdar and Basu (2010) in their study on lona ilish (a traditional fermented fresh hilsa fish chunk) contained 9.51% fat and 18.3% fatty acid(oleic acid). While Ezeama and Udoh (2012[a]) reported that moisture, protein, fat , nitrogen free extracted of fermented and salted tilapia decreased, while free fatty acid, peroxide value and total volatile base of samples were increased.

As there are very limited information on fatty acid profile of fermented fresh water fish in Indonesia, therefore the aim of this study was to investigate the changes of fatty acid profile of fresh betok fish due to traditional salted fermentation process and addition of palm (*Arenga pinnata*) sugar and lime (*Citrus aurantifolia*) during fermentation process.

Materials and method

Samples for laboratory analysis

Fresh fish (*Anabas testudineus* Bloch), cooking salt, palm (*Arenga pinnata*) sugar and lime (*Citrus aurantifolia*) fruits used in this study were purchased from local market in Banjar Baru. Fresh fish with relatively same size approx.10.29 cm (weight : 13.48 g) after evisceration, descaled , washed in running tap water, drained and stored in polyethylene pouches then kept in freezer before laboratory analysis. Some of those fish were layered in fermentation bowl (size: 18.7 cm length and 20.0 cm diameter) and each layer was covered with coarse salt (15% w/w) only for traditional fermentation treatment and some were added with palm sugar (15% w/w) combined with lime juice (and 6% w/w) respectively beside 15% salt (w/w) as treatment in the modified traditional fermentation experiment, then sealed tightly before fermented at ambient temperature for 7 days. Each fermentation bowl containing approximately 15 - 20 fish.

Samples preparation for fatty acid profile analysis

Samples for fatty acid profile analysis were obtained by extracting the oil of either fresh fish, traditional salted fermented and modified traditional salted fermented fish and then these oil were converted to their FAMEs constituents according to the method described in AOAC (2000). 0.3 ml of oil sample were methylated using 1.5 ml of Na-metanolic and heated at 65^{o}C for 15 minutes in waterbath. Furthermore 1.5 ml of BF_3-methanol were added to the mixture, then heated at the same condition and the solution was allowed to cool down up to ambient temperature. Then 0.5 ml of N-heptane and 1 ml of saturated NaCl were added for solution extraction and the top-layer of solution (1 µl) was injected to Gas Chromatography (at the same condition with standard) as described in AOAC (2000).

The GC-MS (Gas Chromatography-Mass Spectrometry) used in this analysis was GCMS-QP2010S SHIMADZU - Japan) equipped with Agilent DB-1 - column (30m), Internal Diameter (ID): 0.25 mm and Helium (He) as gas carier and ionosation of E170 Ev . The column oven temperature was 80^{o}C

and split injector temperature was 310°C with pressure flow control: 16.5 kPa, total flow: 20.0 ml/min and column flow: 0.50 ml/min. While the linear velocity was 26.1 cm/sec , purge flow 3.0 ml/min and split ratio: 33.0. The GC program ion source temperature was 250°C , inteface temperature was 305°C, solvent cut time: 4.80 min, while the MS table start time 5 minutes and end time 40 minutes with ACQ mode: scan, event time: 0.50 sec, scan speed 1250,start m/z:28.00 and end m/z: 600.00.

Results and discussion

Fatty acid profile of the analyzed fresh betok fish, traditional salted fermented betok fish and modified traditional salted fermented betok fish samples are presented in Figure 1 and Table 1 . There were only eight quite high concentration fatty acids identified i.e. lauric acid, miristic acid, palmitoleic acid, palmitic acid, oleic acid, stearic acid, Eicosapentanoic acid (EPA) and Decohexanoic acid (DHA). Figure 1 showed the chromatogram of fatty acid profile of fresh betok fish, traditional salted fermented and modified traditional salted fermented betok fish.

While the relative percentage of those fatty acids are presented in Table 1 as follow:

Table 1. Fatty acid profile of fresh betok fish, traditional fermented and modified traditional fermented betok fish.

Fatty acids	Fresh betok fish. (%)	Traditional fermented betok fish. (%)	Modified traditional fermented betok fish. (%)
Lauric acid ($C_{12}H_{24}O_2$)	5.05	*nd*	*nd*
Miristic acid ($C_{14}H_{28}O_2$)	4.37	1.09	0.85
Palmitoleic acid ($C_{16}H_{30}O_2$)	3.56	4.29	3.66
Palmitic acid ($C_{16}H_{32}O_2$)	23.76	27.52	27.70
Oleic acid ($C_{18}H_{34}O_2$)	43.24	45.60	53.65
Stearic acid ($C_{18}H_{36}O_2$)	7.78	9.92	9.60
EPA ($C_{20}H_{25}O_2$)	2.02	3.00	0.92
DHA ($C_{22}H_{32}O_2$)	1.12	1.50	0.47
SFA	17.20	11.01	10.45
MUFA	48.82	52.89	58.23
PUFA	1.12	1.50	0.47

nd : not detected

SFA : *Saturated Fatty Acid*; MUFA: *Mono Unsaturated Fatty Acid*; PUFA: *Poly Unsaturated Fatty Acid*.

The results in Table 1 showed that lauric acid was not detected in either traditional or modified traditional fermented betok fish samples. After fermentation process only palmitic acid (23.76%), oleic acid (43.24%) and MUFA (48.82%) contents of fresh betok fish were significantly increased either in samples of traditional salted fermented (27.52%,45.60% and 52.89%) or modified traditional salted fermented betok fish samples (27.70%, 53.65% and 58.23%). While palmitoleic acid (3.56%), stearic acid (7.78%), EPA (2.02%) , DHA (1.12%) and PUFA (1.12%) content slightly increased in traditional salted fermented betok fish samples (4.29%, 9.92%, 3.00%, 1.50% and 1,50% respectively), but after the addition of palm sugar and lime juice these fatty acids content decreasing to 3.66%, 9.60%, 0.92%, 0.47% and 0.47% respectively). However the SFA (17.20%) content of fresh betok fish decreasing after traditional salted fermentation (11.01%) and modified traditional salted fermentation process (10.45%).

The fermentation process resulted not detected lauric acid either in salted fermented betok fish and in samples with and without palm sugar and lime juice. While the difference in EPA and DPA contents were believed due to the effect of salt and microorganisms

producing lipolytic enzyme which could produce EPA and DHA. Further decreasing EPA and DHA contents in salted fermented betok fish with palm sugar and lime juice added could caused combination preservative action of salt (15%), palm sugar (15%) and lime juice (6%) and hence reduced the number of microorganisms producing lipolytic enzyme which play an important role in the synthetic of EPA and DHA.

The fatty acid profile changed after fermentation process are possibly due to the reaction occured either by the presence of microorganisms involved in the process especially Lactic Acid Bacteria metabolism or metabolite produced. Ezeama and Udoh (2012[a]) reported that addition of salt 10% during fermentation slightly increased free fatty acid content(15.46%) compared to non salted unfermented tilapia *(Chromidotilapia guentheri)* free fatty acid content (14.36%), while addition of 15% salt slightly decreased free fatty acid content (13.44%). However addition of 1% garlic powder and 1% red chilli either in fermentation tilapia with 10% salt or 15% salt slightly decreased free fatty acid content to 13.64% and 13.44% compared to nonsalted unfermented tilapia free fatty acid content (14.36%). A similar effect of salt and spices added during fermentation of *Sardinella sp* on free fatty acid had also been reported by Achinewhu and Oboh (2002).

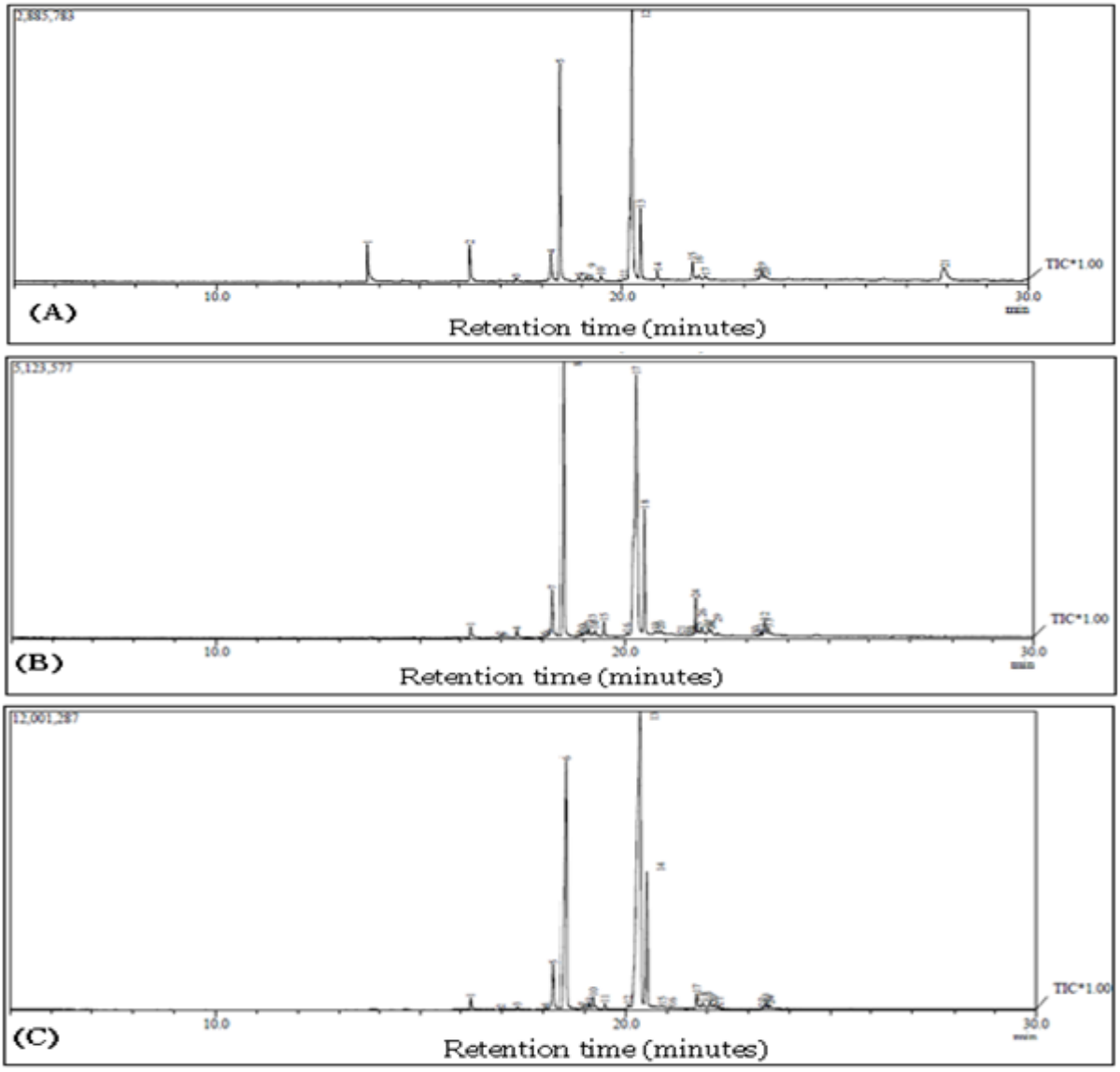

Fig. 1. Chromatogram of fatty acid profile of fresh betok fish (A), traditional salted fermented betok fish (B) and modified traditional, salted fermented betok fish (C).

Enzeama and Udoh (2012[b]) found a different pattern in their study using catfish (*Clarias buthupogon*), as the free fatty acid content of non salted unfermented catfish was 9.76% increased to 11.29% in 10% salt added during fermentation but in samples with 15% salt added during fermentation the free fatty acid content decreased slightly (9.45%). However if either in samples with 10% or 15% salt added with 1% garlic and 1% red pepper during fermentation the free fatty acid content slightly increased to 10.68% and 10.48% respectively. The differences of two research reports mentioned above could be due to different fish species used in their study, Endinkeau and Tan (1993); Nowsad *et al.*,(2012) , De Oliveira *et al.*, (2013) noted that climate, temperature, rainfall and water current, species, sex, weight, size, reproductive and especially the feeding system affected the fish food and diet and hence affected the fish components include the fatty acid profile.

The MUFA content of fresh betok fish was (48.82%) , SFA content (17.20%) and PUFA content only 1.12%. However, salted fermentaton process with and without palm (*Aringa pinnata*) sugar and Lime (*Citrus aurantifolia*) increased the MUFA content to 52.80% and 58.23% respectively. The SFA and PUFA contents only increased in salted fermented namely 11.01% and 1.50% respectively and by addition of palm sugar and lime juice slightly decreasing SFA content to 10.15% but significantly decreasing PUFA content to 0.47%.

According to Muhamad and Mohamad (2012) fresh water fish tend contained higher MUFA content compared to saturated fatty acids and PUFA and it is believed due to fresh water fish feeding are mostly vegetation or plant materials rather than zooplanktons wich contained high concentration of PUFA and feed by most marine fish.

In some western European countries traditional salted fermented were using a brine solution of a mixture of 6 – 18% salt and 0.3 – 2.0% acetic acid (vinegar) and in some region lime juice mixed with spices known as ceviche used for marinating marine fish before fermentation or lemon juice mixed with onion and tomato for marinating salmon to produce lomi- lomi. These organic acid and spices added could enhance the taste and flavour of end product although in fact organic acid, spices and salt added during fermentation act as preservative by reducing pH value of fish flesh and also as selective agent for microorganisms growth during fermentation process (Köse, 2010). While Huda *et al.* (2012) reported that salt, tamarind, salt, roasted rice powder and brown sugar were added in the production of Pekasam a fermented fresh tilapia (*Oreochromis mossambica*), spotted gourami (*Trichogaster trichopterus*), catfish (*Clarias batracus*), java barb (*Puntius javanicus*), or snake head (*Channa striatus*) fish product.

Petrus *et al.* (2013[b]) observed that addition of palm sugar (15%) and lime juice (6%) during salted fermentation process could prevent spoilage microrganisms growth hence promote the Lactic Acid Bacteria growth. This condition affected the fermentation process and the end product components include fatty acid profile.

Conclusion

The addition of palm (*Arenga pinnata*) sugar (15%) and lime (*Citrus auriaflora*) juice (6%) during salted fermentation of fresh betok (*Anabas testudineus* Bloch) could improve the quality of end product as it lowered the SFA content and increased the MUFA content. Therefore the modified salted fermented fresh betok fish could be considered as an improved method of new healthy product with exteded shelf life.

References

AOAC. 2000. Official methods of analysis of the association of official analysis.18th Edn. Washington D.C. Association of Official Analytical Chemists.

Awaludin MY, Rusky IP, Safri I. 2011. Fatty Acid Composition of Fish Tuna , Layur , and mackerel from Pameungpeuk , Garut . Faculty of Fisheries and Marine Sciences. Padjadjaran University. Bandung, Indonesia.

Bell JG, Tocher DR, Farndale BM, Cox DI, McKinney RW, Sargent JR. 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing Parr-smolt transformation. Lipid. **32,** 515-525.
http://dx.doi.org/10.1023/B:FISH.0000045722.44186.ee

Cengiz EI, Unlu E, Başhan M. 2010 Fatty acid composition of total lipids in muscle tissues of nine freshwater fish from the River Tigris (Turkey). Turkey Journal of Biology **34,** 433-438.
http://dx.doi.org/10.3906/biy-0903-19

De Oliveira ER, Agostinho AA, Matsushita M. 2013. Effect of biological variables and capture period on the proximate composition and fatty acids composition of the dorsal muscle tissue of hypophytalmus edentatus. Brazilian Archives of Biology and Technology an International Journals **46 (1),** 105- 114.

Dincer T, Cakli S, Kilinc B, Tolasa S. 2010. Amino acids and fatty acid composition of fish sauce. Journal of Animal and Veterinary advances **9(2),** 311 – 315.

Endinkeau K, Tan KK. 1993. Profile fatty acid contents in Malaysian freshwater fish. Journal Tropical Agriculture Science **16(3),** 213- 221.

Ezeama CF, Udoh EJ. 2012[a]. Effects of fermentation and salting on the bacterial, chemical and sensory characteristics of Tilapia (*Chromidototilapia guentheri*) based marinate in Nigeria. American Journal of Food and Nutrition **2(1),** 7-13.
http://dx.doi.org/10.5251/ajfn.2012.2.1.7.13

Ezeama CF, Udoh EJ. 2012[b]. The influence of fermentation and salting on the bacterial, chemical and sensory characteristics of catfish *(Clarias buthupogon)* based marinate in Nigeria. African Journal of Food Science **6(14),** 381-385.
http://dx.doi.org/10.5897/AJFS12.039

Hajirostamloo B, Hajirostamloo M. 2010.The omega 3 fatty acids content of some Iranian fresh water fish species. World Journal of Fish and Marine Sciences **2(4),** 285 – 290.

Huda N. 2012. Malaysian Fermented Fish Products. In : Hui YH and Evranuz E Ó (Eds). Handbook of Animal - Based Fermented Food and Beverage Technology, 2 nd edn. P. 709 – 715. London : CRC Press.

Köse S. 2010. Evaluation of seafood safety health hazards for traditional fish products: Preventives measures and monitoring issues.Turkish Journal of Fisheries and Aquatic Sciences **10,** 139 – 160.
http://dx.doi.org/10.4194/trjfas.2010.0120

Majumdar RK, Basu S. 2010. Characterization of the traditional fermented fish product Lona ilish of Northeast India. Indian Journal of Traditional Knowledge **9(3),** 453 – 459.

Mohamed EHA, Al-Sabahi GN. 2011. Fatty acids content and profile of common commercial Nile fishes in Sudan. International Journal of Fisheries and Aquaculture **3(6),** 99-104.
http://dx.doi.org/10.1155/2014/712134

Muhamad NA, Mohamad J. 2012. Fatty Acids Composition of Selected Malaysian Fishes. Sains Malaysiana **41(1),** 81–94.

Nowsad AKM, Mchanthy BP, Enamul Hq M, Shakuntala H. 2012. Nutritional value, consumption and utilization of Hilsa (*Tenuola ilisha*). Procedding Regional on Hilsa : Potensial of Aquaculture Dhaka,Bangladesh.

Petrus. 2009 Improvement of Wadi Ikan Betok (*Anabas testudineus* Bloch) by Incorporating Palm Sugar(*Arenga pinnata*) as Food Additive and Acetic Acid (CH_2COOH) (Perbaikan Mutu Fermentasi Wadi Ikan Betok (*Anabas testudineus* Bloch) dengan

Penambahan Food Additive Gula Merah Aren (*Arenga pinnata*) dan Asam Cuka (CH_2COOH), Banjar, Indonesia : University of Lambung Mangkurat. MSc thesis.

Petrus, Purnomo H, Suprayitno E, Hardoko. 2013[a] Physicochemical characteristics, sensory acceptability and microbial quality of *Wadi Betok* a traditional fermented fish from South Kalimantan, Indonesia. International Food Research Journal **20(2),** 933 – 939.

Petrus, Purnomo H, Suprayitno E, Hardoko. 2013[b]. Quality of fermented fresh water fish (Wadi Betok) added with palm (*Arenga pinnata*) sugar and Lime (*Citrus aurantifolia*) juice. International Food Research Journal **20(5),** 2849-2855.