International Food Research Journal 20(2): 933-939 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Physicochemical characteristics, sensory acceptability and microbial quality of *Wadi Betok* a traditional fermented fish from South Kalimantan, Indonesia

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Article history

Received: 28 June 2012
Received in revised form: 3 September 2012
Accepted: 17 September 2012

Keywords

Fermented fish
chemical characteristics
organoleptic characteristic
microbial quality

Abstract

The aims of this study were to find out the physicochemical characteristics, sensory acceptability and microbial quality of traditional fermented fish (*Wadi Betok*) which is popular in South Kalimantan society. *Wadi Betok* samples were collected from 20 processors at three production area and grouped base on the level of salt addition during sample preparation (5, 10, 15, 25, 50, 75 and 100% w/w). The physicochemical characteristics of these samples were pH : 6.74 - 7.87; a_w : 0.75 - 0.93; salt content 2.39 - 13.68% and protein content : 11.50 - 16.52%. The sensory evaluation showed that sample added with 15% salt had the highest score for texture: 5.90 ; aroma: 5.89 ; taste : 5.93 and colour: 5.64. (1: extremely undesirable and 7 : extremely desirable). While the highest microbial count was found in sample added with 5% salt : 2.44 x 10^6 cfu/g and the highest Lactic Acid Bacteria count also in sample added 5% salt: 2.13 x 10^6 cfu/g. It was found that physicochemical characteristics and sensory acceptability as well as microbial quality of traditional fermented fish samples from cottage level producers were in a very wide range, and this showed that there is still no standard operating procedures for such traditional fermenting fish processing steps. It can be concluded that physicochemical characteristics, sensory acceptability and microbial quality of *Wadi Betok* are influenced by the level of salt addition.

Introduction

Traditional fermentation in developing countries is one of the oldest processing method which are used for preserving fish ,where it is not only extend the shelf life but also enhanced a unique taste, flavor and nutritional quality of end products. (Anihouvi *et al*.,2006; Nayeem *et al*.,2010 and Dincer *et al*.,2010). There are a number of fish fermentation products such as fish sauce which are mainly prepared for flavor enhancers and condiments, however there are also fermented fish prepared as a staple food for years in almost every country in Europe, Africa, Midle East, Asia and South East Asia. In Indonesia Peda is a wet fermented mackerel (*Rastrilliger* sp), where fresh mackerel put in layers in basket and topping with 30% (w/w) salt and fermented at room temperature for 3 months or more. This traditional fermented fish product without drying process could be grouped as intermediate moisture food (Putro, 1993; Desniar *et al*., 2009). According to Zakaria (1998) and Desniar *et al*. (2009), this process can be classified as spontaneous fermentation where only natural microorganisms involved in this process and therefore wide variation of final products quality are found.

One of other popular traditional fermented fish product in South Kalimantan, Indonesia is fermented fresh water fish or locally known as *Wadi Betok*, which are widely consumed in South and Central Kalimantan for decades (Petrus, 2009).This fresh water fish is Climbing Perch (*Anabas testudineus* Bloch) or "walking fish" with local name ikan Betok or ikan Papuyu with protein content in the range of 10% - 15% are used as raw material in *Wadi Betok* production (Basrindu, 1987; Rahayu *et al*., 1992 and Arianti, 2004) and found abundant during dry season around May - July and difficult to find this fish during August - February ; therefore fermentation process have been chosen by fish processors to preserve it (Petrus, 2009). Furthermore, Petrus (2009) reported that whole fresh water fish after added with 30 -

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100% (w/w) salt were layered in plastic jars and left for spontaneous fermentation for 7 days to 4 months at room temperature.

Study on physical, chemical and microbial characteristics as well as consumers preference on traditional fermented fish similar to *Wadi Betok* had been carried out by some researchers such as effect of salt concentration on the amino acids profile and consumers acceptance of some salted and ripening of pellagic fish species in some European countries such as herring (*Clupea harengus*) in Rusia, Norway, Holland and England or salt ripened anchovies (*Engraulis encraschicolus*) in Germany, Spain and France (Stefánsson and Gudmundsdóttir, 1995); Fessiekh a traditional fermented fish in Sudan made from *Alestes* sp or *Hydrocynus* sp and prepared by layered this fish with salt in a tank and fermented for 7 days, then transferred in larger tank and salt added for further fermentation process up to 10 or 15 days. (Essuman, 1992).

Lanhouin is another traditional fermented fish in Republic Benin, Africa which prepared by salting fresh whole cassava fish (*Pseudotolithus* sp) with 20 - 30% salt (w/w) then followed by ripening process for 15 hours (Anihouvi *et al*., 2006; Anihouvi *et al*., 2011); Lona illish a fermented salted fish (Hilsa/ Temualosa ilisha Hambuch 1822) which was first salted dried in the dark for 48 hours, then packed in tin containers filled with saturated brine and left for 4 - 6 months for spontaneous fermentation and Ngari is a sun dried salted whole fish (*Puntius sophore*) for 3 - 4 days, then stacked in earthen pot for 4 - 6 months for spontaneous fermentation is a traditional fermented fish in North East India (Thapa *et al*., 2004 and Majumdar and Basu, 2010).

Shidal (a semi dried fermented *Puntius* sp) is also another traditional fermented whole fish in North East India as reported by Muzaddadi and Basu (2012). Chepa sutkhi a semi dried traditional fermented fish(*Puntius stigma*) in Bangladesh and fermented under anaerobic condition in earthen vats for 3 - 4 months (Nayeem *et al*., 2010), whilst Irianto and Irianto (1998) and Huda (2012) reported the chemical and microbial quality of Peda a traditional fermented fish in Indonesia which is similar to Wadi Betok in its preparation.

Although there are quite intensive studies on physical, chemical and organoleptic characteristics and microbial quality of traditional fermentation fish products in some countries as reported by Nayeem *et al*. (2010) and Dincer *et al*. (2010), however informations on traditional fermentation of Betok (*Anabas testudineus* Bloch) in South Kalimantan are very limited. Therefore the aims of this study were to find out the physicochemical characteristics and sensory acceptability as well as microbial quality of this product.

Materials and Methods

Samples for laboratory analysis

Samples were purchased from fish fermentation cottage industries in Banjar regency, South Kalimantan and the producers were chosen using purposive random sampling before the samples were purchased for laboratory analysis. The preparation of fish fermentation in these sites were fresh fish with relatively same size approx.10.29 cm (weight : 13.48 g) after evisceration, descaled, washed in running tap water, drained and layered in fermentation bowl (size: 18.7 cm length and 20.0 cm diameter) and each layer was covered with coarse salt and sealed tightly before fermented at ambient temperature for 7 days.

Samples preparation for sensory evaluation

Fermented fish samples which will be used for sensory evaluation were soaked in tap water for about 30 minutes to reduce the salt content before deep fried at ±175^{o}C for 6 minutes and for evaluation one whole deep fried fish was served to the panelists.

pH measurement

The pH of Wadi Betok were measured by using digital pH meter following the method as described by Afrianto and Livyawati (1989) . Homogenates were prepared by blending homogenously 5 g of sample with 10 ml distilled water and the pH of homogenates was recorded by immersing glass electrode of digital pH meter (Hanna model HI 98107 - USA).

Water activity (aw) measurement

Water activity (a_w) of Wadi Betok samples were determined using water activity meter (Rotronic Hygroscope DT (RHDT) - Thomas Scientific - HP23 - AW SET, USA) according to the method of Purnomo (1995).

Proximate composition

The salt, moisture, protein, fat and ash contents of Wadi Betok samples were determined according to standard methods of AOAC no 976-18; 950-46; 992-15; 991-36 and 920-163, respectively (AOAC, 2000).

TVB-N measurement

TVB-N of Wadi Betok samples were determined according to procedure of Conway as stated in the manual of Siang and Kim (1992).

Sensory evaluation

Sensory evaluation was conducted with 30 untrained panelists, and deep fried Wadi Betok samples were served to the panelists. The sensory attributes such as texture, aroma, taste and colour were evaluated on 7 point descriptive scale in hedonic scale scoring method (1 = extremely undesirable and 7 = extremely desirable) as described by Soewarno (1985).

Microbial quality

Total Plate and Lactic Acid Bacteria Counts of Wadi Betok samples were determined by the methods described by Fardiaz (1989). Samples of Wadi Betok fillet (15g) were taken aseptically and homogenized in sterile 0.1% (w/v) peptone solution containing 10%(w/v) NaCl (85 ml) for 1 minute. The homogenate was serially diluted and used for microorganisms enumeration. Total bacterial counts were determined on Standard Plate Count Agar (PCA Oxoid) after incubation for 48 hours at 37º C. Lactic Acid Bacteria were similarly enumerated on de Man Rogosa Sharpe Agar (MRSA) after incubation for 48 hours at 37º C. The populations of bacteria were expressed as 10^6 cfu/g.

Statistical analysis

Data obtained were analysed by using one way Analysis of Variance (ANOVA) and differences among means were determined by Duncan Multiple Range test using SPSS version 15 (Chicago, Illinois, USA), and 95% confidence level was used as indicator for statistical significancy. Data were presented as means ± standard deviation and each analysis were replicated three times for proximate analysis, sensory evaluation and microbial counts.

Results and Discussion

Physicochemical characteristic of Wadi Betok

The survey of location was taken at the centre of *Wadi Betok* processing units i.e 3 districts namely Astambul, Gambut and Kertak Hanyar and then a purposive sampling was carried out to obtain samples. The survey results showed that variation of salt concentrations utilized during *Wadi Betok* preparation were 5, 10, 15, 25, 50, 75 and 100% (w/w) respectively. The physicochemical characteristics of those samples were presented in Table 1.

Table 1. showed that utilization of 25% and 50% salt during preparation of samples slightly reduced the pH value and it showed that utilization of salt had relatively small impact, and this could be affected by the short fermentation time i.e. only 7 days at ambient temperature. The range of pH value of *Wadi Betok* samples was 6.74 - 7.87. According to Desniar *et al*. (2009) pH value of peda samples after 6 days of fermentation was 6.0, while Bahri *et al*. (2006), Duman *et al*. (2007), El Hag *et al*. (2012)a and El Hag *et al*. (2012)b noted similar pattern of slightly decrease pH values in their fermented fish samples during the early stage of fermentation. El Hag *et al*. (2012)a also recorded that pH values at the starting of fermentation were in the range of 6.14 - 6.70, and El Hag *et al*. (2012)b found that utilization 25% salt during preparation of fermented *Debs* sp. (*Lebeo* sp.) after 4 days of processing decreased pH value of products to below 7.00.

The a_w value of products started decreasing in samples added with 25% salt (0.75) and slightly increased in samples added with 50% salt (0.77). Desniar *et al*. (2009) reported that a_w of Peda samples prepared using 50% salt was 0.75. The addition of salt during preparation of *Wadi Betok* will affect the shelf life of end products and it might be due to the role of salt with sodium chloride (NaCl) as major component reduced a_w values.

Moisture content of *Wadi Betok* samples were relatively similar and this is possibly due to the inact fermented fish are still soaked in its brine and no other processing steps were applied before the samples were taken for laboratory analysis. Moisture

Table 1. Physicochemical characteristics of Wadi Betok from South Kalimantan, Indonesia*

No of sample	Salt Concentration (% w/w)	Physicochemical characteristics							
		pH	a_w	Salt content (%)	Moisture content (%)	Protein content (%)	Fat content (%)	Ash Content (%)	TVB-N (mgN/100g)
1	5	7.63^{d}±0.06	0.93^{e}±0.01	2.386^{a}±0.049	33.38^{ab}±0.14	11.50^{a}±0.20	2.66^{e}±0.10	1.50^{a} ±0.07	17.57^{g}±0.40
2	10	7.87^{e}±0.04	0.89^{d}±0.01	3.262^{b}±0.039	33.29^{a} ±0.18	12.33^{b}±0.27	2.15^{c}±0.07	1.56^{ab}±0.07	13.80^{f}±0.39
3	15	7.53^{d}±0.16	0.84^{c}±0.03	5.514^{c}±0.196	33.45^{ab}±0.14	16.52^{f}±0.37	2.28^{d}±0.13	1.63^{b} ±0.06	11.16^{e}±0.29
4	25	6.89^{b}±0.07	0.75^{a}±0.02	6.422^{d}±0.157	33.55^{b} ±0.16	15.43^{e}±0.30	1.83^{b}±0.09	2.12^{e} ±0.08	9.25^{d}±0.88
5	50	6.74^{a}±0.11	0.77^{b}±0.02	8.512^{e}±0.116	35.32^{e} ±0.16	16.42^{f}±0.26	1.56^{a}±0.16	1.17^{cd}±0.10	7.83^{c}±0.75
6	75	7.18^{c}±0.09	0.76^{ab}±0.01	9.313^{f}±0.079	34.53^{d} ±0.29	14.29^{d}±0.28	1.46^{a}±0.06	1.95^{d} ±0.09	6.72^{b}±0.51
7	100	7.57^{d}±0.13	0.76^{a}±0.01	13.680^{g}±0.168	34.22^{c} ±0.27	13.74^{c}±0.08	1.81^{b}±0.08	1.85^{c} ±0.03	4.99^{a}±0.55

*Results are means ± standard deviation (n = 3); Means within the same column followed by same superscript are not significantly different (P<0.05).

content of *Wadi Betok* after 7 days of fermentation were in the range of 33.29% - 35.32%, while Desniar *et al*. (2009) noted that the moisture content of Peda samples with 30% , 40% and 50% salt added were 52.71%, 53.33% and 53.94% respectively. Irianto and Irianto (1998) reported that moisture content of Peda in their samples was 40% and according to Majumdar and Basu (2010) utilization of salt in fermentation involved an osmosis process where diffusion of salt into fish flesh are responsible for lowering moisture content as well as a_w values and simultaneously increase salt and ash contents of final products. El Hag *et al*. (2012)b also noted that moisture content of salted fermented *Debs* sp. (*Lebeo* sp.) was varied compared to other researchers founding and it was possibly caused by different fermentation methods, time and amount of salt added during curing.

The protein content started increase at utilization of 15% salt during preparation, and a similar pattern was also showed for ash content (Table 1). On the contrary salt content in samples increased as salt concentration added was also increased, however with higher amount of salt added during preparation of fermented fish, protein content of samples were decreasing again at utilization of 75% - 100% salt during preparation. In these samples the protein content ranging from 11.50% - 16.52% and it is possibly due to the fact that samples were obtained after only 7 days fermentation where probably protein degradation were not yet occured. Ndaw *et al*. (2008) also found a similar protein content (16.20%) in their fermented sardine samples and a similar pattern was also found by Desniar *et al*. (2009) in their Peda samples where increased of salt added (30 - 50%) also slightly increased its protein content (20.15% - 21.54%). While Nevry *et al*. (2011) reported that protein content of Adjuevan a traditional fermented fish of Ivory (Africa) purchased from three different processors were in the range of 24.21% - 26.81%. Stefánsson and Gudmunsdóttir (1995) reported that the soluble nitrogen fraction of total content during ripening of salted herring were increased and hence affected peptides and free amino acids of the product. According to Lawrie (1998) during storage crude protein content could decreased and this was due to some changes such as "Maillard reactions" or by an attack of myoglobin by bacteria and it was related to changes in pH. Hamm (1994) reported that fish muscle protein could swell by the presence of salt and protein denaturation might occured if salt concentration increased during fish fermentation process.

Fat content of samples found start decreasing in samples where 25% salt was added during preparation (1.83%) and further decreasing was found in samples with higher amount of salt added 50% (1.56%) and 75% (1.46%). A similar fat content was found in Peda samples i.e. 1.25% - 1.37% as reported by Desniar *et al*. (2009). El Hag *et al*. (2012)a also recorded a decrease in crude fat and ether extract in salted Kawara (*Alestes* sp.), and they assumed that it was due to leaching process from fish muscle in correlation with salt penetration into fish muscle.

While ash content started increasing in samples with 25% salt added during preparation (2.12%), but in samples with 50% - 100% salt added during samples preparation showed a decreasing ash content of end products (1.85% - 1.95%). These findings were much lower than ash content of Peda (15.96% - 16.90%) as reported by Desniar *et al*. (2009). Taorem and Sarojnalini (2011) noted that whole fish include bones and scales used as raw material in fermentation process together with minerals in salt could increase the ash content of end products.

On the contrary, Total Volatile Base Nitrogen (TVB-N) values of samples start decreasing in samples with 10% salt added during preparation (13.98 mg/100g) and 50% salt (7.82 mg/100g) and continued decreasing in higher amount of salt added as shown in Table 1. Desniar *et al*. (2009) reported the TVB - N values of Peda added with 30% salt and 50% salt during preparation were 18.42 mg/100g and 16.78 mg/100g respectively. El Marrakchi *et al*. (1990) noted that TVB-N was not really suitable for evaluating the changes during storage, but more useful for assessing the rate of deterioration of end product. TVB - N values were affected by fish species, sex and age, location and season of catching. While Babu *et al*. (2005) noted that TVB-N levels indicated the continues volatile bases production due to protein breakdown by microbial activity.

The other possible reason that concentrations of salt added during preparation just had a slightly impact on physicochemical characteristics of Wadi Betok was probably due to slow penetration of salt into fish flesh. This condition was caused by fish skin which could retard the penetration of salt as dry salting method was carried out in these cottage level producers. Anihouvi *et al*. (2011) reported that fermentation time for 4 days with ripening time of 8 hours were needed for Lanhouin a traditional fermented fish products to reach an acceptable levels of salt concentration, Total Viable Load and histamine content.

Sensory acceptability of Wadi Betok

The acceptability of *Wadi Betok* samples were determined using a sensory evaluation test (1:

Table 2. Sensory acceptability of panelists on *Wadi Betok* samples from South Kalimantan, Indonesia*

No. of samples	Salt concentration added (% w/w)	Score of sensory acceptability (1: dislike very much and 7 : like very much)			
		Texture	Aroma	Taste	Colour
1	5	2.21^{a}±0.66	2.21^{a}±0.66	3.24^{a}±0.97	3.11^{a}±1.10
2	10	3.09^{b}±0.78	3.02^{b} ±0.74	4.11^{b} ±0.69	3.80^{b}±0.61
3	15	5.90^{e}±0.57	5.89^{e} ±0.53	5.93^{e} ±0.49	5.64^{d}±0.58
4	25	5.25^{d}±0.45	5.01^{c} ±0.69	5.59^{de}±0.66	5.53^{d}±0.44
5	50	5.76^{e}±0.75	5.45^{d} ±0.29	4.99^{c} ±0.57	4.75^{c}±0.62
6	75	5.68^{e}±0.96	5.63^{de}±0.37	5.35^{cd}±0.73	5.38^{d}±0.77
7	100	4.75^{c}±0.73	3.27^{b} ±1.08	3.19^{a} ±0.94	5.42^{d}±1.00

*Results are means ± standard deviation (n = 3); Means within the same column followed by same superscript are not significantly different (P<0.05).

Table 3. Microbial quality of *Wadi Betok* samples from South Kalimantan, Indonesia*

No of sample	Salt concentration added (%w/w)	Total Plate Count (TPC) (10^6 cfu/g)	Lactic Acid Bacteria (LAB) (10^6 cfu/g)
1	5	2.44^{b}±0.65	2.13^{d} ±0.26
2	10	2.35^{b}±0.71	1.59^{c} ±0.16
3	15	0.25^{a}±0.06	0.18^{ab}±0.03
4	25	0.21^{a}±0.05	0.11^{a} ±0.07
5	50	0.03^{a}±0.01	0.05^{a}±0.04
6	75	0.03^{a}±0.01	0.05^{a}±0.03
7	100	0.02^{a}±0.01	0.293^{b}±0.03

*Results are means ± standard deviation (n = 3); Means within the same column followed by same superscript are not significantly different (P<0.05)

extremely undesirable and 7 : extremely desirable) and the results are presented in Table 2.

The untrained panelists gave highest score for texture, aroma, taste and colour of samples prepared by utilization of 15% salt and it is possibly that by utilization of 15% salt already could give a texture, aroma, colour and a sligthly salty taste which were acceptable to the panelists. Stefánsson and Gudmunsdóttir (1995) noted that during ripening of salted herring there was a gradual increase of either peptides and free amino acids, and these compounds are believed play an important role of organoleptic properties of end products. Nwabueze and Nwabueze (2010) recommended fermentation process in fish preservation as it could enhance the flavour of end products and make it more acceptable to consumers.

Microbial quality of Wadi Betok

The total microbial and Lactic Acid Bacteria (LAB) count of Wadi Betok samples purchased from cottage industries in South Kalimantan were presented in Table 3.

The total microorganism count using TPC method showed that by utilization of 15% gave a significant (P<0.05) decreased number of microorganisms from 2.44 x 10^6 cfu/g (addition of 5% salt) to 0.25 x 106 cfu/g (addition of 15% salt). Further decreasing number of microorganisms was observed by adding 100% salt (0.02 x 10^6 cfu/g). Desniar *et al*. (2009) reported that microorganisms number in peda samples after 6 days fermentation with addition of 50% salt during preparation was 4.3 x 10^3 cfu/g, and after 14 days was 3.3 x 10^4 cfu/g.The difference of microorganism numbers between peda and Wadi Betok are possibly due to the different number of microorganisms in fresh fish, quality of salt used, fermentation time, sanitation and hygiene during processing. While Taorem and Sarojnalini (2012) reported that microorganism numbers observed in Ngari a traditional fermented whole fish in India were in the range of 10^4 - 10^6cfu/g. It is interesting to note that Lactic Acid Bacteria (LAB) content decreasing with higher amount of salt added, and it is believed that the fermentation time were not suitable yet for LAB to grow. However the addition of 100% salt increased the number of LAB up to 0.29x 10^6 cfu/g, Thapa *et al*. (2004) also reported similar number of LAB (10^4 - 10^7 cfu/g) in their traditional fermented fish samples (Ngari, Hentap and Tungtap) of North India.

The range of TPC in this study was 0.02 - 2.44 x 10^6 cfu/g and it is similar to the one reported by Nevry *et al*. (2011) in Adjuevan traditional Ivorian fermented fish (1.2 x 10^6 cfu/g). Salt levels used during preparation not only act as preservative but also reduced a_w and moisture content of end products, where this reduction will produce an unfavourable condition for the microbial life.

Conclusion

In fact, salt concentration used in traditional fermentation of *Wadi Betok* (*Anabas testudineus* Bloch) in South Kalimantan were 5, 10, 15, 25, 50, 75 and 100% (w/w) respectively. Consequently physicochemical characteristics and sensory evaluation scores as well as the amount of microorganisms widely varies between samples. This indicate that there is no standard operating procedure available in traditional fermentation process of fish in this area, and therefore those parameters measured are affected by level of salt added.

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