International Food Research Journal 20(5): 2849-2855 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Quality of fermented fresh water fish (*Wadi Betok*) added with palm (*Arenga pinnata*) sugar and Lime (*Citrus aurantifolia*) juice

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Article history

Received: 5 November 2013
Received in revised form: 15 May 2013
Accepted: 18 May 2013

Keywords

Fermented fresh water fish
Palm sugar
Lime Juice

Abstract

The objectives of this study were to determine the physicochemical characteristics, sensory acceptability and microbial quality of fermented fresh water fish (*Betok-Anabas testudineus* Bloch) locally known as *Wadi Betok*, which was prepared by using 15% w/w salt and addition of palm (*Arenga pinata*) sugar (0, 5, 10 and 15% w/w) and lime (*Citrus aurantifolia*) juice (0, 2, 4 and 6% w/w). An interaction effect of combination of palm sugar and lime juice added was observed and no significance different were found in a_w value, but there were siginificant different ($P < 0.05$) in pH value, salt, protein, moisture and ash contents and TVB-N value among those treatments. The addition of 15% palm sugar and 6% lime juice had a_w 0.85, moisture content 30.01% and protein content 23.42%. Sensory evaluation showed that samples prepared using 15% palm sugar and 6% lime juice were acceptable by panelists although combination of 15% palm sugar and 4% lime juice had a higher sensory score. The addition of 15% palm sugar and 6% lime juice reduced the TPC (0.39×10^6 cfu/g) but increased the Lactic Acid Bacteria count (2.93×10^6 cfu/g). It can be concluded that addition of 15% palm sugar and 6% lime juice in preparation of salted fermented fresh water fish obtained the best sample from its physicochemical characteristic, microbial quality and acceptability by the panelists.

Introduction

In South Kalimantan, traditional method of preserving surplus fish which has been practiced for years are salting and fermentation process and this is due to unavailable low temperature storage facilities especially in cottage level industries. The fermented fresh water fish in South Kalimantan or locally known as *Wadi Betok* are produced at cottage level and usually prepared from fresh water fish Climbing Perch (*Anabas testudineus* Bloch) or "walking fish" with local name *ikan Betok* or *ikan Papuyu*. The whole fresh water fish after evisceration, descaled, washed in running tap water, drained and then were layered in fermentation bowl and each layer was covered with coarse salt (30 – 100% w/w) and sealed tightly before fermented at ambient temperature for 7 days to 4 months (Basrindu, 1987; Rahayu *et al.*, 1992, Arianti, 2004; Petrus, 2009). Petrus *et al.* (2013) in their study found a wide variations of salt (5 – 100% w/w) had been used in production of fermented fresh water fish at cottage level industries and especially the high amount of salt added (> 15% w/w) could limited the consumers acceptance. Therefore they suggested to add some other ingredients such as palm sugar to reduce the salty taste as at present the cottage fermented fresh water fish producers added salt only during its preparation. The sensory test by untrained panelists showed that fermented fish added with 15% w/w salt during preparation had the highest organoleptic properties score, although some panelists detected a salty taste.

According to Köse (2010) in some western European countries in preparation of traditional fermented fish products, fresh fish were first marinated in a brine solution containing 6 – 18% salt and 0.3 – 2% vinegar (acetic acid) and some were marinated in lime juice after mixed with spices (ceviche) or salmon in solution of lemon juice with onion and tomato (lomi lomi). The addition of other ingredients such as vinegar, lemon juice, lime juice, onion, tomato were aimed to enhance the end products flavour and taste as well as together with salt play an important role as preservative agent. Organic acids in lemon or lime juice will also decrease pH value of fish flesh and together with salt added became a selective agent for microbial growth during fermentation process.

Huda (2012) reported that in Malaysia the

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fermented fresh water fish was also found and known as *Pekasam*, and this product was prepared using eviscerated and washed tilapia (*Oreochromis mossambica*), spotted gourami (*Trichogaster trichopterus*), catfish (*Clarias batracus*), java barb (*Puntius javanicus*), and snake head (*Channa striatus*). These fish were then mixed with salt and tamarind and packed in a sealed container for 2 – 3 days. After the first stage of fermentation the excessive salt was washed with water from salted fermented fish before mixed with roasted rice powder and brown sugar and kept in a sealed container for another 2 – 3 weeks before harvested as *Pekasam*.

Yeoh and Merican (1978), Yuen *et al.* (2009) and Mohamed *et al.* (2012) noted that palm sugar and tamarind or lime juice had also been used in preparation of fish sauce such as *Budu* in Malaysia. While Basrindu (1987) studied on the effect of salt and palm sugar on the preservation of *Betok* fresh water fish and found that addition of 15 – 20% salt and 30 – 40% palm sugar could extent the shelf life of *Betok* up to 30 days.

However, there was no information available on the utilization of combination of palm (*Arenga pinata*) sugar and lime (*Citrus aurantifolia*) juice in fermentation of salted fresh water fish (*Betok*). Therefore the aims of present study were to find out the physicochemical characteristics, sensory acceptability, and microbial quality of fermented fresh water fish (*Anabas testudineus* Bloch) or Wadi Betok prepared by addition of palm sugar and lime juice.

Materials and Methods

Samples for laboratory analysis

Fresh fish (*Anabas testudineus* Bloch), palm (*Arenga pinata*) sugar and lime (*Citrus aurantifolia*) fruits used in this study were purchased from local market in Banjar Baru. The preparation of fish fermentation in this study 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 (15% w/w) and palm sugar (0, 5, 10 and 15% w/w) combined with lime juice (0, 2, 4 and 6% w/w) respectively as treatments in the experiment, then sealed tightly before fermented at ambient temperature for 7 days. Each fermentation bowl containing approximately 15 - 20 fish.

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.

Experimental design

Completely Randomized Design was used as experimental design for this study and consist of two factors namely concentrations of palm sugar: 0 (control), 5, 10 and 15% w/w and lime juice 0 (control), 2, 4, and 6% w/w respectively. Three replications of experiment were carried out and the parameters were also measured in three replicates for pH, a_w, moisture , protein , fat and ash contents, TVB-N , sensory properties : colour, aroma, texture and taste, TPC and LAB count.

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 (a_w) 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 colour, aroma, texture and taste

were evaluated on 7 point 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 (15 g) 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 37ºC. 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 two ways 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 Discussions

Physicochemical characteristics of Wadi Betok samples

The addition of different concentrations of palm (*Arenga pinata*) sugar and lime (*Citrus aurantifolia*) juice during preparation of fermented fresh water fish affected significantly ($P < 0.05$) on pH value , salt , moisture, protein, ash contents and TVB-N value; but did not affected its aw value.

The pH value of samples were in the range of 5.95 – 6.33 (Table 1) and a slight increase of pH value was observed with an increased amount of palm sugar and lime juice added during preparation. This slightly higher pH values were possibly due to insufficient amount of organic acids in lime juice to act together with salt to reduce pH of samples during fermentation.According to Falade *et al*. (2003) lime juice contained 29.40% ascorbic acid,4.12% citric acid and 4.19% total organic acid, where as weak acids only a part of it will be ionized, while Desniar *et al.* (2009) noted that during fermentation process, salt were not completely ionized into Na^+ and Cl^-, therefore the pH value were not decreasing significantly although lime juice was added.

Some workers also reported the pH value of early stage of their fermented fish samples were in the range of 6.14 – 6.70 (El Hag *et al*., 2012 a,b) and Desniar *et al*. (2009) observed pH value of peda samples (Indonesian fermented fish) was 6.0 after 6 days of fermentation. While Ezeama and Udoh (2012) reported that pH value of samples prepared from catfish by addition of 10% and 15% salt were 5.62 and 5.46 respectively

The a_w values were relatively similar for all samples and this indicated that the addition of palm (*Arenga pinata*) sugar and lime (*Citrus aurantifolia*) juice up to 15% and 6% respectively together with 15% salt during preparation did not act properly as humectant. Purnomo *et al.* (1983), Purnomo (1986) and Purnomo (2012) noted that palm (*Cocos nucifera*) sugar or known as coconut sugar together with salt if mixed in minced meat during preparation of spicy dried meat (dendeng giling) could reduced significantly a_w value of end products. Furthermore, it was reported that a different quality of coconut sugar added during spicy dried meat preparation also affected differently in reducing a_w and moisture content of end products. However, the dry curing in this study was carried out by rubbing mixture of coarse salt, palm sugar and lime juice on the surface of whole fish samples, and it is assumed that such condition could reduce the flow rate of mixture to penetrate completely into fish flesh, hence the mixture gave only a slight effect on the decreasing either pH and a_w values of end products.

Salt content of samples were between 3.56 and 6.08%, and an increased of salt content 3.61, 4.79 and 5.96% were observed in samples with addition of 5, 10 and 15% palm sugar and 6% lime juice respectively (Table 1). However, the addition of palm sugar only showed an increase of salt content in sample prepared by addition of 15% palm sugar (5.43%). A fluctuating salt contents were also observed in this study and it is probably due to coarse salt used and dry cured method by just rubbing coarse salt on the fish surface,and therefore penetration of salt into fish flesh were affected. Desniar *et al.* (2009) reported that in their products which fermented for 6 days and addition of 30% salt during preparation was 5.30%.

The addition of palm sugar and lime juice significantly ($P < 0.05$) affected the moisture content of samples, and a slightly increased of moisture content was observed in sample prepared by addition of 15% palm sugar and 6% lime juice (30.01%) compared to the one added with 15% palm sugar only (29. 50%). It was possibly due to the moisture content of lime

Table 1. Physicochemical properties of Wadi Betok prepared using combination of palm sugar and lime juice*

pH

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	5.95^{a} ±0.13	5.98^{ab}±0.10	6.08^{ab}±0.10	6.13^{ab}±0.10
5	6.10^{ab}±0.14	6.10^{ab}±0.14	6.03^{ab}±0.13	6.15^{abc}±0.13
10	6.15^{abc}±0.13	6.15^{abc}±0.06	6.33^{c} ±0.22	6.18^{bc} ±0.05
15	6.08^{ab}±0.17	6.05^{ab}±0.06	6.18^{bc}±0.13	5.98^{ab}±0.05

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

a_w

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	0.83^{a}±0.05	0.83^{a}±0.05	0.84^{a}±0.04	0.84^{a}±0.04
5	0.86^{a}±0.00	0.85^{a}±0.04	0.85^{a}±0.03	0.86^{a}±0.04
10	0.84^{a}±0.04	0.85^{a}±0.03	0.85^{a}±0.03	0.85^{a}±0.03
15	0.84^{a}±0.02	0.84^{a}±0.02	0.84^{a}±0.03	0.85^{a}±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).

Salt content

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	4.48^{abcd}±0.79	3.61^{a} ±1.33	3.94^{ab} ±0.83	3.56^{a} ±0.54
5	4.50^{abcd}±0.50	4.45^{abcd}±0.80	5.35^{bcd} ±0.70	3.61^{a} ±1.42
10	4.00^{ab} ±1.02	6.08^{d} ±1.47	5.74^{cd} ±1.43	4.79^{abcd}±0.81
15	5.43^{bcd} ±0.76	4.70^{abcd} ±0.49	4.23^{abc} ±1.29	5.99^{d} ±0.81

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

Moisture content

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	36.35^{g} ±0.75	33.14^{def}±1.06	32.15^{cde}±1.98	34.64^{fg} ±1.24
5	30.77^{abc}±1.43	34.46^{fg} ±1.32	34.49^{fg} ±0.85	34.06^{ef} ±1.93
10	31.16^{abcd}±1.01	33.85^{ef} ±1.24	30.36^{abc} ±1.37	34.07^{ef} ±1.03
15	29.50^{a} ±1.27	34.45^{fg} ±1.60	32.10^{bcde}±1.31	30.01^{ab} ±1.14

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

Protein content

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	13.84^{a} ±0.72	15.47^{b} ±0.52	15.58^{b} ±0.71	16.18^{bc}±0.57
5	15.85^{b} ±0.67	16.17^{bc}±0.54	17.09^{cd}±0.57	17.85^{de}±0.52
10	16.26^{bc}±0.59	18.18^{e} ±0.68	20.30^{g}±0.66	21.14^{gh}±0.80
15	17.33^{de}±0.61	19.34^{f}±0.62	21.73^{h}±0.58	23.42^{i}±0.66

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

Fat content

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	1.18^{a} ±0.21	1.22^{a} ±0.13	1.36^{ab}±0.12	1.56^{bc}±0.09
5	1.69 ^{cd}±0.05	1.76 ^{cd}±0.08	1.83 ^{d} ±0.06	2.21^{ef}±0.19
10	1.87^{d} ±0.13	2.20^{ef}±0.18	2.61^{g}±0.11	3.00^{h} ±0.17
15	2.12^{e}±0.07	2.39^{f}±0.07	3.32^{i}±0.16	3.33^{i}±0.30

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

Ash content

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	2.88^{j} ±0.22	2.64^{i} ±0.12	2.42^{h} ±0.05	2.48^{hi}±0.09
5	2.15^{g}±0.29	1.79^{f}±0.18	1.56^{e} ±0.08	1.47^{de}±0.07
10	1.38^{de}±0.06	1.30^{cd}±0.07	1.03^{ab}±0.13	1.11^{bc}±0.13
15	0.94^{ab}±0.11	1.02^{ab}±0.15	0.86^{a}±0.09	0.97^{ab}±0.15

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

TVBN (meq/100 g)

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	5.95^{a} ±0.18	5.75^{b} ±0.20	4.55^{bc} ±0.25	4.29^{bc} ±0.19
5	5.74^{cd}±0.26	4.34^{cd}±0.26	3.79^{cd}±0.66	3.65^{de}±0.34
10	5.29^{de}±0.502	2.89^{de}±0.60	4.14^{de}±0.83	3.96^{e} ±0.78
15	3.81^{f}±0.77	3.19^{f}±0.34	3.19^{f}±0.50	1.69^{f}±0.38

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

juice added as well as the hygroscopic characteristic of palm sugar which could absorbed the surrounding water vapour affected its moisture content. Irianto and Irianto (1998) noted that moisture content of peda (fermented mackerel (*Rastreligger negletus*) fish) in Java was 40%.

It is interesting to note that increasing amount of palm sugar and lime juice added during preparation of *Wadi Betok*, will also increase protein content of end products significantly ($P < 0.05$). The addition of 15% palm sugar and 6% lime juice increased the protein content of samples from 13.84% (without addition of palm sugar and lime juice) to 23.42%. It is believed that addition of palm sugar (15%) and lime juice (6%) could prevent salt penetrate completely into fish flesh and resulted slower driven of water with all soluble nutrients out from fish flesh, thus a higher protein content was observed. Irianto and Irianto (1998) also observed similar protein content of 28% in peda. According to Anihouvi *et al*. (2006) protein content of lanhouin a fermented fish product of Republic Benin was in the range of 24.6 – 26.5%, while Achinewu and Oboh (2002) also reported an increased protein content from 16% to 18% due to fermentation process.

Fat content of samples were affected significantly by addition of palm sugar and lime juice and the highest fat content observed was in samples prepared by addition of 15% palm sugar and 6% lime juice (3.33%). According to Windarwati (2006) palm sugar containing 0.57 - 4.6% (dry basis) fat and 5.60 – 9.27% (wet basis) moisture content. It is believed that fat content of palm sugar may contribute to the increased of fat content of samples as fat contents of samples prepared by addition of 5, 10, and 15% palm sugar were observed also higher compared to the one without palm sugar addition.

The addition of palm sugar and lime juice significantly ($P < 0.05$) affected ash content of samples, and it was observed that the addition of palm sugar decreased the ash content of sample without palm sugar addition i.e. 2.88% to 2.15, 1.38 and 0.94% of samples added with 5, 10 and 15% palm sugar, respectively. These results were different to the one reported by Ezeema and Udoh (2012) where ash content of salted and fermented catchfish (*Clarias buthupogon*) in Nigeria was in the range of 11.55 – 13.72% if 10% and 15% salt added during fermentation and Oetterer *et al.* (2003) noted that increased in salt content due to the presence of salt in sardine muscle.

TVB-N values of samples were also affected by addition of palm sugar and lime juice ($P < 0.05$) , the

Table 2. Sensory acceptability of panelists on *Wadi Betok* Modificaton*

Colour

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	4.13^{a} ±1.68	4.40^{ab} ±1.33	4.20^{a} ±1.40	5.40^{cde}±1.45
5	4.73^{abc}±1.14	4.80^{abc}±1.32	4.30^{a} ±1.29	5.47^{de}±1.14
10	4.57^{ab} ±1.19	5.90^{e} ±0.88	4.73^{abc}±1.20	4.47^{ab} ±1.46
15	4.10^{a} ±1.40	5.07^{bcd}±1.11	5.97^{e} ±0.85	5.97^{e} ±0.89

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

Aroma

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	4.07^{a} ±1.44	4.03^{a} ±1.33	5.17^{cde}±1.32	5.00^{bcd}±1.49
5	4.60^{abc}±1.38	5.13^{cd} ±0.90	4.43^{abc}±1.48	4.53^{abc}±1.43
10	5.43^{def}±1.19	5.93^{f} ±0.98	4.70^{abc}±1.24	4.43^{abc}±1.36
15	4.50^{abc}±1.33	4.33^{ab} ±1.37	5.93^{f} ±0.87	5.83^{ef}±0.87

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

Texture

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	4.20^{a} ±1.73	4.37^{ab}±1.83	4.23^{a} ±1.70	5.40^{cd}±1.38
5	4.47^{ab} ±1.25	4.63^{ab} ±1.25	4.77^{abc}±1.30	5.07^{bc} ±1.34
10	4.37^{ab}±1.30	6.10^{de}±0.76	4.33^{ab}±1.15	4.43^{ab}±1.38
15	4.70^{abc}±1.42	4.77^{abc}±1.28	6.33^{e} ±0.61	5.87^{de} ±0.97

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

Taste

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	4.40^{ab} ±1.35	5.10^{bcd}±1.60	4.73^{abc}±1.53	4.50^{ab}±1.43
5	4.87^{abc}±1.17	4.50^{ab} ±1.38	4.33^{ab} ±1.63	5.40^{cde}±1.16
10	4.73^{abc}±1.41	5.83^{de} ±0.83	4.67^{abc}±1.37	4.23^{a} ±1.55
15	5.43^{cde}±1.57	4.73^{abc}±1.41	5.93^{e} ±1.11	5.90^{e} ±0.84

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

addition of palm sugar was observed decreasing the TBV-N value from (5.95 meq/100 g) sample without palm sugar to 3.81 meq/100 g (sample with 15% palm sugar).

However, the addition of lime juice together with palm sugar also affected the TVB-N value of samples and it is interesting to note that the increasing amount of palm sugar and lime juice added also decreased TBV-N values. The TBV-N value 4.29 meq/100 g was observed in sample prepared with 6% lime juice only and it was then decreasing to 3.65 – 1.69 meq/100 g if samples prepared with addition of 5 – 15% palm sugar and 6% lime juice (Table 1). It is assumed that addition of palm sugar together with salt and lime juice could prevent the growth of spoilage microorganisms. Desniar *et al.* (2009) also reported that TVB-N values of *peda* also decreasing due to the prevention of spoilage microorganisms by addition of salt during fermentation process. A similar results was also reported by Nayeem *et al.* (2010) where TBV-N values of traditional *chepta suki* (semi fermented and salt dried fish of Bangladesh) was in the range of 1.12 – 3.12 mg/100 g, and these values are still acceptable for fish products. According to Ndaw *et al*. (2008) TVB-N values are affected by species, age and sex of fish, location and time of catching of fish.

The differences of physicochemical characteristics of fermented fresh Betok compared to other reports are possibly due to different fish species, size of fish as raw material, sanitation and hygiene, quality of additional ingredients, climate condition during fish catching, fermentation time and temperature and some other factors. According to Visessanguan *et al.* (2004) protein and fat as well as other characteristics were different between fish species.

Sensory evaluation of Wadi Betok samples

High scores for colour, aroma and taste (5.97, 5.93 and 5.93) were given by the panelists for samples prepared by addition of 15% palm sugar and 4% lime juice (Table 2.). While the sensory evaluation of texture scores by 30 panelists were showed in Table 2 and the addition of palm sugar (15%) and lime juice (6%) was observed gave a higher score (5.87) compared to sample without palm sugar and lime juice (4.20). However, sample prepared by addition of 15% palm sugar and 4% lime juice was scored 6.33 which means this sample was the most preferable by the panelists from texture point of view. High scores for colour, aroma and taste (5.97, 5.93 and 5.93) were also given by the panelists for samples prepared by addition of 15% palm sugar and 4% lime juice (Table 2.). These findings indicated that addition of palm sugar and lime juice together with salt during fermentation could improve the sensory attributes of end products, and although addition of 15% salt and 4% lime juice was the most preferable one, but statistically panelists score for sensory attributes of this sample was not different (P > 0.05) with 15% salt and 6% lime juice sample.

Nwabueze and Nwabueze (2010) reported that fermentation process could enhance the sensory attributes of fermented *Heterotis niloticus* in Nigeria, while Ezeama and Udoh (2012) also observed that addition of 10% and 15% w/w salt combined with red pepper (1% w/w) and garlic powder (1% w/w) was the most preferable sample

Microbial quality of Wadi Betok samples

Total microbial and lactic acid bacteria counts of fermented fresh fish samples prepared by addition of different concentrations of palm sugar and lime juice were presented in Table 3. The addition of palm sugar in preparation of *Wadi Betok* increased the amount of TPC from 1.58 x 10^6 cfu/g (sample without palm sugar addition) to 2.05, 2.48 and 2.10 x 10^6 cfu/g (samples with addition of 5, 10 and 15% palm sugar). This condition is possibly due to the different initial microbial quality of fresh fish and palm sugar used.

Table 3. Microbial quality of Wadi Betok Modification*

Total Plate Count (TPC) (10^6 cfu/ g)

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	1.58^{gh} ±0.34	1.23^{ef} ±0.21	0.77^{bcd}±0.18	0.57^{ab} ±0.22
5	2.05^{i} ±0.19	1.20^{ef} ±0.08	0.89^{cd} ±0.06	0.58^{ab} ±0.05
10	2.48^{j}±0.22	1.23^{ef} ±0.10	1.05^{de} ±0.10	0.71^{bc}±0.12
15	2.10^{i} ±0.16	1.68^{h} ±0.13	1.38^{fg}±0.10	0.39^{a} ±0.04

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

Lactic Acid Bacteria (LAB) (10^6 cfu/g)

Palm sugar	Lime juice (%)			
(%)	0	2	4	6
0	2.90^{d} ±0.14	0.98^{c} ±0.65	0.19^{a} ±0.01	0.08^{a} ±0.02
5	1.53^{cd}±0.02	0.18^{a} ±0.02	0.02^{a} ±0.00	2.36^{d} ±0.15
10	2.80^{d} ±0.16	0.22^{a} ±0.02	2.65^{d} ±0.24	1.45^{c} ±0.19
15	0.16^{a} ±0.02	0.27^{ab}±0.02	0.91^{bc}±0.53	2.93^{d} ±0.10

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

Purnomo (1986) and Ho *et al.* (2008) reported that inconsistent quality of palm sugar was due to the poor sanitation and hygiene in traditional processing and also unstable quality of sap juice (nira) as palm sugar raw material.

LAB counts for samples without addition of palm sugar and lime juice was 2.90 x 10^6 cfu/g, and the addition of palm sugar (5, 10 and 15%) slightly decreasing the amount of LAB compared to LAB counts from samples added with lime juice (2, 4 and 6%) only (Table 3). It is believed that organic acids in lime juice made an unfavourable condition for LAB to grow, and according to Aibinu *et al.* (2007) and Onyeagba *et al.* (2004) undiluted lime juice were found quite effective as antimicrobial agent. However the addition of 15% salt combined with 6% lime juice reduced the total microbial count but increased the LAB counts,and this possibly due to the role of salt, palm sugar and lime juice in preventing spoilage microbial growth but promoting the LAB growth.

Conclusions

Incorporation of palm sugar and lime juice together with 15% salt affected the physicochemical characteristics, sensory acceptability and microbial quality of fermented fresh water fish (*Wadi Betok*). The addition of 15% salt, 15% palm sugar and 6% lime juice during preparation of *Wadi Betok* showed acceptable sensory attributes with highest protein and Lactic Acid Bacteria content and lower moisture content, TVB-N value and microbial count, although addition of 4% instead of 6% lime juice also resulted a slightly higher panelists acceptability. It can be concluded that addition of 15% palm sugar together with 6% lime juice could improve the quality traits of *Wadi Betok.*

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