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

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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 pinnata) 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 value, but there were significant 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 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 \((0.39 \times 10^6 \text{ cfu/g})\) but increased the Lactic Acid Bacteria count \((2.93 \times 10^6 \text{ cfu/g})\). 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 preparation 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
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 batractus), 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 extend 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°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, aw, 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 (aw) measurement

Water activity (aw) 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 37°C. Lactic Acid Bacteria were similarly enumerated on de Man Rogosa Sharpe Agar (MRSA) after incubation for 48 hours at 37°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 significance. 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\(^*\)

<table>
<thead>
<tr>
<th>pH</th>
<th>Palm sugar (%)</th>
<th>Lane juice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.64±0.01</td>
<td>1.64±0.01</td>
</tr>
<tr>
<td>4</td>
<td>1.64±0.01</td>
<td>1.64±0.01</td>
</tr>
<tr>
<td>6</td>
<td>1.64±0.01</td>
<td>1.64±0.01</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Palm sugar (%)</th>
<th>Lane juice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.83±0.05</td>
</tr>
<tr>
<td>5</td>
<td>0.86±0.00</td>
</tr>
<tr>
<td>10</td>
<td>0.84±0.04</td>
</tr>
<tr>
<td>15</td>
<td>0.84±0.02</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Palm sugar (%)</th>
<th>Lane juice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36.35±0.75</td>
</tr>
<tr>
<td>5</td>
<td>30.77±1.43</td>
</tr>
<tr>
<td>10</td>
<td>31.16±1.01</td>
</tr>
<tr>
<td>15</td>
<td>30.59±1.27</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Palm sugar (%)</th>
<th>Lane juice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.84±0.72</td>
</tr>
<tr>
<td>5</td>
<td>15.85±0.67</td>
</tr>
<tr>
<td>10</td>
<td>16.26±0.59</td>
</tr>
<tr>
<td>15</td>
<td>17.33±0.61</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Palm sugar (%)</th>
<th>Lane juice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.18±0.21</td>
</tr>
<tr>
<td>5</td>
<td>1.69±0.05</td>
</tr>
<tr>
<td>10</td>
<td>1.87±0.13</td>
</tr>
<tr>
<td>15</td>
<td>2.12±0.07</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Palm sugar (%)</th>
<th>Lane juice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.85±0.22</td>
</tr>
<tr>
<td>5</td>
<td>2.15±0.29</td>
</tr>
<tr>
<td>10</td>
<td>1.39±0.06</td>
</tr>
<tr>
<td>15</td>
<td>0.94±0.11</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Palm sugar (%)</th>
<th>Lane juice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.95±0.18</td>
</tr>
<tr>
<td>5</td>
<td>5.74±0.26</td>
</tr>
<tr>
<td>10</td>
<td>5.29±0.50</td>
</tr>
<tr>
<td>15</td>
<td>3.81±0.77</td>
</tr>
</tbody>
</table>

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 (4.20) sample prepared by addition of 10% and 15% w/w salt combined with 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% salt 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% salt 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 Udo (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 10⁶ cfu/g (sample without palm sugar addition) to 2.05, 2.48 and 2.10 x 10⁶ 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.
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 10⁶ 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.

References


Arianti, L. 2004. Isolation and Identification of Halophilic Acid Bacteria from Wadi Betok (Anabas testudineus Bloch) which cultured in agaro media with NaCl 10%. (Isolasi dan Identifikasi Bakteri Asam Halofilik dari Wadi Ikan Betok (Anabas testudineus Bloch) yang Ditumbuhkan pada Media Agar dengan Konsentrasi NaCl 10%). Banjar, Indonesia : Universitas Lampung Mangkurat, BSc. thesis.


