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QUALITY ANALYSIS OF ANCHOVIES, *Stelophorus commersonii* DRIED IN DRYING RACKS

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Abstract: Traditional drying method may result poor quality of dried fishes. Thus, an appropriate and hygienic practice was proposed for drying the anchovies. Solar drying racks with tunnel structure roofs of four different materials; mosquito net, black net, aluminium sheet and plastic were constructed. Meanwhile traditional open sun drying method acted as controls. All samples were subjected to microbiology and biochemical analysis in order to reveal an improved drying method. Total bacteria counts were significantly ($p<0.05$) low in anchovies dried from black net and plastic at amount of 2.58±0.53 and 2.84±0.76 $\log_{10}$CFUg^{-1}, respectively compared to the anchovies dried in mosquito net (4.90±0.11 $\log_{10}$CFUg^{-1}). The accumulation of free fatty acid (FFA) in anchovies dried traditionally were recorded at 9.74±1.18 % oleic acid and showed a significantly ($p<0.05$) higher amount compared to anchovies dried in all types of roof material of the drying racks. TBARS value were significantly ($p<0.05$) low in both anchovies dries under aluminium sheet and plastic roof materials which were recorded at 2.16±0.03 and 3.22±0.14 mg MA 100g^{-1}, respectively compared to other methods. Meanwhile histamine content in anchovies dried under mosquito net and plastic did not reach the limit of acceptability (50 mg 100g^{-1}). A drying wooden frame racks with tunnel structure plastic roof were considered an improved solar drying practice for anchovies.

Keywords: Anchovies, drying racks, free fatty acids, histamine, total bacteria count

Introduction

Fish drying especially anchovy generate incomes for local communities of Setiu, Terengganu for their livelihood. Fish drying is a physical process, where the fish is exposed to air and the surface area become dry due to the evaporation of moisture from the surface into the air by the process of convective mass transfer (Doe, 2002). Drying of fish results of prolonging shelf-life and producing desired flavours and texture that has been widely practised by many societies and communities (Doe, 2002). After catch, anchovies were immediately washed and boiled in 10% brine solution at approximately two minute (Doe, 2002). Local artisanal fisherman uses the natural sunlight energy to dry the anchovies which are spread on the bamboo mat that lay on the ground.

This method, however, contaminates the anchovy by exposing it to dust, excreta from birds and animals and subjects it to destruction by birds, blowflies' larvae and animals or rain and wind (Alam, 2007; Reza *et al*., 2005). Further, direct sunlight exposure may destroy light sensitive nutrients in the fish. In addition, drying fish at open sun may risk of bacteria and molds contamination (Alam, 2007). The growth of bacteria may facilitate the chemical and enzymatic actions that deteriorate the fish flesh through physical and biochemical changes (Mustapha *et al*., 2014). Therefore, the quality of the product is low and sold at lower price.

Drying racks offers air circulation below the fish, reduce the incidence of fly larvae infestation and more convenient to gather up the fish for storage. A simple and hygienic wooden frame racks with tunnel structure roofs were proposed for fish drying.

The aims of this study is to assess the microbiology and biochemical quality of anchovies dried in four different materials of tunnel structure roofs; mosquito net, black net, aluminium sheet and plastic and compared with the traditional open sun fish drying method. This study revealed the improved solar drying practice with high nutritional quality for consumer consumption of dried anchovies.

Materials and Methods

Sample Preparation

Anchovies were harvested from Setiu coastal zone using anchovy's purse seine equipped with steamer for immediate boiling processing. All samples were transferred to the laboratory in chilled condition before randomly divide into five different treatments. Anchovies traditionally dried by thin scattering on mat that lay on the ground under open sun were categorised as controls. Drying racks were constructed a meter height, wooden frame with four different types of roof materials; mosquito net, black net, aluminium sheet and plastic. Samples at even amount were thin scattering on slanted wire-mesh tray. Drying occurs approximately 8-16 hours at temperature of 32-38°C and relative humidity of 45-75%.

Nutritional Analysis of Dried Anchovies

Moisture, protein, lipid, ash and salt content were analysed according to the AOAC (2000).

Microbiological Analysis of Dried Anchovies

Approximately, 10±0.1g of samples were weighed and put into sterile filter bag with 90ml of Maximum Diluent Recovery (MRD) (MERCK, Germany) before homogenized by using homogenizer (Interscience, France). An appropriate serial dilution were done before spread plating accordingly on selective agar at instructed incubation hours. Total bacteria count was determine using plate count agar (PCA) after incubation at 30°C after 48 hours. Samples were spread plate on McConkey agar before incubated at 35°C for 18-24 hours for total coliform determination.

Yeast and mold count were determined on Rose Bengal agar base after incubated at 25-30°C for 5 days. Enterobacteriaceae and *Pseudomonas* sp count were determine using violet red bile glucose agar and cetrimide agar, respectively and incubated at 35°C for 18-24 hours. Plate of serial dilution at the countable range of 30-300 colonies was selected as the reliable counts. Microbiological counts were expressed as log colony forming units per gram of samples ($\log_{10}$CFU g^{-1}).

Biochemical Analysis of Dried Anchovies

Free fatty acid (FFA) of dried anchovies were analysed according to method by Kirk and Sawyer (1991), and Karim (2011). Approximately 2.0± 0.1 g of anchovies with 40 ml of chloroform were homogenised (IKA T18 digital ULTRA TURRAX, Germany) before filtered through a No 541 Whatman filter paper. Final volume was made up to 40 ml with chloroform. 25ml of chloroform extracts were mixed with 25 ml of neutral ethanol and 1.0ml of 1 % phenolphthalein solution. The extracts were neutralised by titration against NaOH solution until a pink colour persisted for 15s. FFA content was calculated as in Equation 1, which acid values were defined as the number of mg KOH not NaOH required to neutralise the free fatty acid in 1 g of sample;

FFA (% oleic acid) =

$$\frac{(V\,(ml)\,XNX\,0.282}{W\,(g)}\,X\,100 \quad \text{Equation 1}$$

Where,
V = Titration (ml) of NaOH, N = Normality of NaOH and W = fish muscle (g) per 25 ml subsample.

Thiobarbituric acid reactions substance (TBARS) were analysed according to method by Karim (2013). The absorbance (A) were measured using spectrophotometer at λ= 538 nm. TBARS value was expressed as mg malonaldehyde (MA) $100g^{-1}$. Meanwhile, histamine level in each treatments were analysed using a rapid colorimetric determination (Patange *et al*., 2005). The concentration of histamine in samples was obtained from the standard curve for the corresponding absorbance measured at 496 nm by regression analysis. Histamine value was expressed as mg $100g^{-1}$.

Statistical Analysis

The entire experiments were replicated three times. Data were analysed using one way ANOVA. Significant differences among the treatments were determined using Tukey test at 0.05 level of probability. The correlation between the variables were analysed using Pearson's correlation test. The variables were bivariately normally distributed. Test of normality were at $p>0.05$. All statistical analysis were done using the IBM SPSS Statistic software (Version 20).

Results and Discussion

Nutritional Value of Dried Anchovies

Moisture content of anchovies showed no significant different among the controls, mosquito net and plastic treatments ($p>0.05$) (Table 1). Generally, dried anchovies from Malaysia east coast were recorded 10-28%. This showed an agreement with various authors documented that the moisture content of boiled-dried anchovies from Korean coast were approximately 20-28% (Lee *et al*., 1986; Kim *et al*., 2001; Heu & Kim, 2002). However, Dewi (2002) documented the Indonesian dried salted anchovies showed a slightly higher amount of moisture content (31.70±0.66%). Current studies showed the protein content of anchovies dried in all types of drying racks were significantly higher ($p<0.05$) compared to the open sun-drying (57.69±2.09%). This may explain that drying under direct sunlight may destroy the nutritional values of fish protein. These are due to the losses in availability of lysine and other essential amino acid (Johnson & Ndimele, 2011). Previous study by Lee *et al*. (1986), Kim *et al*. (2001), and Heu and Kim (2002) stated that protein content of anchovies from Korean sea was found in range of 45-58%. Similar amount was also recorded for Indonesian dried anchovies (49.62±1.49%) (Dewi, 2002). Interestingly, current studies also revealed that fish drying in racks are potentially increase the protein content up to range of 61-78% compared to the traditional method.

Meanwhile, lipid content of anchovies dried under black net and plastic (3.64±0.13 and 3.73±0.21%, respectively) were significantly lower ($p<0.05$) compared to other treatments (Table 1). Ash content in controls (23.97±0.55%) and anchovies treated under plastic roof (22.70±0.39%) were higher compared to other treatments ($p<0.05$). Current studies also showed a similar range of ash content in dried anchovies collected from Korean seas between 14 and 18% (Lee *et al*., 1986; Kim *et al*., 2001; Heu & Kim, 2002). However, Dewi (2002) documented that ash content in Indonesian dried salted anchovies are low (11.64±0.14%). Meanwhile, anchovies dried in open sun drying showed a similar amount of salt content to the anchovies dried under all types of drying racks ($p>0.05$) (Table 1). Philippine National Standard (2015) stated that dried anchovies shall comply 15% of salt content. Meanwhile Food Standard Australia New Zealand (2010) documented that salt content in dried anchovies is 31.40%. Recent studies by Kung *et al*. (2015) stated dried flying fish contains 1.38-7.60% of salt, which had an agreement with current studies (in range of 2-4%). Ames *et al*. (1991) stated salt content of approximately 10% can prevent infestation of insect and reduce losses to less than 10% during drying process.

Table 1: Nutritional value of anchovies dried in drying racks covered with four different materials; mosquito net, black net, aluminium sheet and plastic; with traditional open-sun drying as control

Drying racks materials	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Salt
Control	10.66±0.45[a]	57.69±2.09[a]	5.50±0.17[a]	23.97±0.55[a]	3.26±0.04[a,b]
Mosquito net	11.50±0.38[a]	78.50±1.97[b]	6.26±0.19[b]	21.13±0.86[b]	2.53±0.12[a]
Black net	27.04±0.61[b]	61.89±1.65[a,d]	3.64±0.13[c]	18.35±0.25[c]	2.71±0.58[a]
Aluminium sheet	24.57±0.88[c]	71.37±0.88[c]	6.53±0.13[a,b]	18.56±0.12[c]	3.63±0.29[b]
Plastic	11.05±0.07[a]	63.80±1.21[d]	3.73±0.21[c]	22.70±0.39[a]	2.63±0.30[a]

*Values are mean±stdev. *n*=3.**Different superscripts (a,b,c) in the same column indicate significance different ($p<0.05$) of specified nutritional value to the treatments.

Microbiological Quality of Dried Anchovies

Anchovies treated under mosquito net showed the highest total amount of bacteria (4.90±0.11 $\log_{10}$CFUg^{-1}) compared to other treatments ($p<0.05$) (Table 2). Total coliforms were higher in controls but not significantly different ($p>0.05$) to other treatments (Table 2). However, the coliform amount were not exceed the limit of acceptability level and were found negligible in dried anchovies of mosquito net drying racks. Malaysia Food Act 1983 (ACT 281) and Regulation (2014) documented the microbiological standard for fish products are 6.00 $\log_{10}$CFU g^{-1} for total plate count and 1.70 $\log_{10}$CFU g^{-1} for coliform count. Philippine National Standard (2015) initiates a lower level of microbiological requirement criteria for dried anchovies. The aerobic plate count acceptable level are at 5.00 $\log_{10}$CFU g^{-1} meanwhile the total coliform are at 1.00 $\log_{10}$CFU g^{-1}. Current studies showed a compliment for total bacteria count of both standard requirements for all treatments including controls. However, coliform counts for controls reach the acceptable level for consumption according to both standard requirements. Kung *et al*. (2015) documented that dried flying fish recorded total bacteria count in range between 4.51±0.41 and 8.18±0.27 $\log_{10}$CFU g^{-1} higher than the current studies.

Meanwhile, yeast and mold showed no significant ($p>0.05$) different in all treatments (at range of 2-3.3$\log_{10}$CFU g^{-1}). With regards to the yeast and mold count, Philippine National Standard (2014) indicate the limit level are at 3 $\log_{10}$CFU g^{-1}. Anchovies dried in both under the aluminium sheet and plastic are not comply with the requirement in regards to the yeast and mold limit level. In addition, Enterobacteriaceae and *Pseudomonas* sp. were recorded at 1.00±0.00 $\log_{10}$CFU g^{-1} in anchovies dried in open sun drying and were scarcely detected in anchovies treated in all types of drying racks (Table 2).

Biochemical Quality of Dried Anchovies

The accumulation of free fatty acid (FFA) in sample treatments under the drying racks showed a significantly lower ($p<0.05$) amount compared to the controls (Table 3). TBARS value in anchovies treated under aluminium sheet and plastic showed a significant ($p<0.05$)

Table 2: Microbiology quality of anchovies dried in drying racks covered with four different materials; mosquito net, black net, aluminium sheet and plastic; with traditional open-sun drying as control

Drying racks materials	Total bacteria count (log_{10}CFU g^{-1})	Total coliform (log_{10}CFU g^{-1})	Yeast and mold (log_{10}CFU g^{-1})	Enterobacteriaceae (log_{10}CFU g^{-1})	*Pseudomonas* sp (log_{10}CFU g^{-1})
Control	3.50±0.04[a]	1.80±0.17[a]	2.56±0.07[a]	1.00±0.00[a]	1.00±0.00
Mosquito net	4.90±0.11[b]	ND	2.81±0.71[a]	ND	ND
Black net	2.58±0.53[a]	1.70±0.00[a]	2.55±0.00[a]	ND	ND
Aluminium sheet	NA	1.70±0.00[a]	3.26±0.10[a]	1.00±0.00[a]	ND
Plastic	2.84±0.76[a]	1.70±0.00[a]	3.22±0.45[a]	ND	ND

*Different superscripts (a,b,c) in the same column indicate significance different ($p<0.05$). **ND indicates no bacteria growth (colonies) detected.

lower level compared to other treatments (Table 3). Dewi (2002) documented TBARS value of Indonesian dried salted anchovies were 0.82 mg MA $100g^{-1}$ and these are approximately 2-6 fold lower than found in Malaysian anchovies. Fu *et al*. (2015) stated drying which eliminate the moisture content enhanced the contact between fat and oxygen; these may promote the lipid oxidation. Therefore, TBARS value is more reliable and meaningful to assess the fat quality of heated samples (Fu *et al*., 2015; Siddaiah *et al*., 2001). Interestingly, only anchovies dried under mosquito net and plastic were not exceeding the limit of acceptability of histamine level (50 mg $100g^{-1}$). The histamine level in anchovies dried under mosquito net and plastic were recorded at 42.16±3.55 and 45.99±9.84mg $100g^{-1}$, respectively. Histamine level of dried anchovies collected from Vietnam were approximately 19.82 mg $100g^{-1}$ meanwhile Indonesian anchovies were recorded at 2.10 mg 100 g^{-1} (Food Standard Australia New Zealand, 2010). Lin *et al*. (2014) documented dried mahi-mahi had higher histamine content (68.15±0.00 mg $100g^{-1}$) compared to the mahi-mahi fillet (0.13±0.00 mg $100g^{-1}$). These indicate the drying process possibly increased the histamine level. However, with emphasising on the both mosquito net and plastic material of drying racks used, both showed a significant acceptable level of histamine content.

The correlation between variables were analysed using Pearson's correlation test at $p<0.05$ (Table 4). TBARS value showed a significant ($p<0.05$) positive correlation to the bacteria count in dried anchovies. Meanwhile, histamine value showed a significant ($p<0.05$) negative correlation to the bacteria count. There had been documented elsewhere, the bacteria count analysis are important in verify seafood product quality assessment. These may suggest that TBARS value is a good indicator for quality assessment for dried anchovies.

pH and Colour of Dried Anchovies

Drying anchovies under aluminium sheet showed a significant ($p<0.05$) higher of pH values compared to the anchovies of other treatments (Table 5). The anchovies had a slightly black appearance. Neither controls

Table 3: Free fatty acid (FFA), thiobarbituric acid reaction (TBARS) and histamine level of anchovies dried in drying racks covered with four different materials; mosquito net, black net, aluminium sheet and plastic; with traditional open-sun drying as control

Drying racks materials	FFA (% oleic acid)	TBARS (mg MA $100g^{-1}$)	Histamine (mg $100g^{-1}$)
Control	9.74 ± 1.18^{a}	3.99 ± 0.05^{a}	$60.72\pm5.25^{a,b}$
Mosquito net	1.27 ± 0.60^{b}	4.58 ± 0.07^{b}	42.16 ± 3.55^{a}
Black net	0.67 ± 0.23^{b}	3.92 ± 0.10^{a}	69.81 ± 2.92^{b}
Aluminium sheet	0.76 ± 0.13^{b}	2.16 ± 0.03^{c}	$67.17\pm12.47^{b,c}$
Plastic	0.67 ± 0.00^{b}	3.22 ± 0.14^{d}	$45.99\pm9.84^{a,c}$

*Different superscripts (a,b,c) in the same column indicate significance different ($p<0.05$).

nor drying in racks showed significant effects ($p>0.05$) towards a* value. However, b* value from anchovies treated under aluminium sheets showed a significant ($p<0.05$) lower amount compared to controls (Table 5). Total colour of anchovies treated under black net had a significantly ($p<0.05$) lowest amount compared to other treatments (Table 5). The decreasing whiteness maybe occurred due to the drying process. Previous studies by Heu & Kim (2002) documented that the L* of boiled-dried anchovies caught from Korean sea were in range between 44.45±0.17 and 60.98±0.12. These showed a less whiteness value compared to Malaysia anchovies. Therefore, Malaysian anchovies might have a slightly higher preference in terms of appearance. In addition, the Philippine National Standard (2015) also categorised a whitish or bluish or yellowish of anchovies were Grade A meanwhile an off-colour were categorised as Grade B. Regards to the current study, all treatments except anchovies treated under mosquito net are categorised as Grade A.

Table 4: Correlation of quality characteristics

Correlation categories	1	2	3	4
1. Total bacteria count		-.757**	.89	.732
2. Histamine value	-.757**		.116	-.369
3. Free fatty acid value	-.089	.116		.282
4. Thiobarbituric acid reactions value	.732**	-.369	.282	

*p,0.01

Table 5: pH and colour of anchovies dried in drying racks covered with four different materials; mosquito net, black net, aluminium sheet and plastic; with traditional open-sun drying as control

Drying racks materials	pH	L*	a*	b*	Total colour
Control	6.57±0.01[a]	65.56±2.05[a]	-0.19±0.20[a]	17.67±1.60[a]	67.91±2.13[a]
Mosquito net	6.53±0.01[a]	60.62±2.69[a]	-0.25±0.38[a]	13.94±2.47[a,b]	62.22±3.17[a]
Black net	6.51±0.02[a]	54.73±0.49[b]	-0.27±0.06[a]	16.64±0.68[a,b]	57.21±0.28[b]
Aluminium sheet	7.18±0.12[b]	59.87±5.68[a]	0.24±0.63[a]	12.89±1.67[b]	61.28±5.30[a]
Plastic	6.53±0.09[a]	61.45±0.66[a]	0.11±0.43[a]	15.46±1.40[a,b]	63.31±0.96[a]

*Different superscripts (a,b,c) in the same column indicate significance different ($p<0.05$).

Conclusion

A drying wooden frame racks with tunnel structure plastic roof were considered an improved solar drying practice for anchovies. The microflora accumulate in dried anchovies were low and spoilage indicator such as FFA, TBARS and histamine were minimal. The appearance of dried anchovies is acceptable for consumer preferences. In addition, it is also suggested that TBARS value is a good indicator in quality assessment for dried anchovies.

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