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Traditional method of fish treatment, microbial count and palatability studies on spoiled fish

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ABSTRACT

Aims: To evaluate the microbial count and palatability acceptance of spoiled fish after treatment with traditionally used natural solution.
Methodology and results: To compare microbial count of spoiled fish before and after treatment with natural solution practiced by local people in Malaysia, 10 g of spoiled fish was respectively rinsed with 100 mL of 0.1% of natural solution such as *Averrhoa bilimbi* extract, rice rinsed water, rice vinegar, *Citrus aurantifolia* extract, salt, flour, and *Tamarindus indica* extract. Flesh of fish rinsed with rice vinegar was found to be able to reduce microbial count (CFU/mL = 0.37 X 10^7) more than 4.5 times when compared to spoiled fish (CFU/mL=1.67x 10^7). Spoiled fish that was treated with rice vinegar was prepared into a cutlet and fried. The cutlet was subjected to palatability acceptance study by a group of residents in Palm Court Condominium, Brickfields, Kuala Lumpur. The palatability study from the Cronbach alpha shown that the taste have the reliability of 0.802, the aroma has the reliability of 0.888, colour with the reliability of 0.772, texture or mouth feel have reliability of 0.840 and physical structure of the cutlet is 0.829.
Conclusion, significance and impact of study: Treatment of spoiled fish using rice vinegar as practice by local people traditionally shown a significant reduction in microbial count and the vinegar-treated fish could be developed into a product that is safe and acceptable by the consumer.

Keywords: spoiled fish, traditional treatment, rice vinegar

INTRODUCTION

Fish is one of the most important protein sources in human nutrition. Each year, tones of fish have been landed (FAO, 2007). Among these fish, there are also 6.82 million metrics tonnes of discarded or spoiled fish in Asia countries (Kelleher, 2005). According to Kelleher (2005), discarded or spoiled fish is the fish that before was taken to the shore or brought to the surface of vessel and are then thrown back to the sea which is already dead or dying or going to die. The fish is discarded for various reasons such as wrong species of fish, not preferable sized fish, fish that is not preferred sex, damaged fish, inedible or poisonous fish, spoilage of fish occurs rapidly, quotas or high grading, season, gear, prohibited fishing area and lack of space on the boat (Clucas, 1997).

According to Matthew and Hammond (1999), discarding has becoming a very serious issue currently especially to the health and environment. Discarded or spoiled fish producing pungent smell usually is caused by microbial action. Spoilt fish has indigenous bacteria and also enteric bacteria which could cause food borne illness in human. Indigenous bacteria are *Clostridium botulinum*, *Vibrio* spp., *Aeromonas hydrophila* and enteric bacteria are *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *and Staphylococcus aureus* (Lyhs, 2009). Moreover, since fish becoming more expensive, discarded or spoiled fish which was thrown back to the sea before are now being converted into edible food products such as Fish Protein Concentrates (FPC), Silage, Collagen Chitin, Chitosan, Fish pickles, Sausages, Surimi, Fish balls and etc (Raffi, 2011).

In Malaysia, it is a normal traditional practice in each household to rinse fish using natural solution to remove the smell (Hanieliza, 2010a; b). Many natural solutions such as rice water, tamarind extract, vinegar, salt, lime extract, flour are being used daily. However, there is no study done on the effectiveness of these solution in removing the smell and spoilage of fish by microbes. Therefore, we would like to explore the possibility of providing simple solution to reduce the number of microbes that causing spoilage of and convert the treated fish into a product that is acceptable by the society.

MATERIALS AND METHODS

Preparing fish flesh for treatment

For this research, *Nemipterus japonicus* or its local name ikan kerisi, one of the most popular fish in the East Coast of Malaysia and also highly perishable was used. The fish was obtained from the local market around Jeli, Kelantan area. To

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stimulate the spoiling process for the fish, fresh fish was bought and kept at room temperature for 24 h. After 24 h the fish internal organs was removed and the flesh was used for treatment.

Treatment of fish flesh

For control, 10 g of spoiled flesh and fresh flesh were rinsed with sterile water (Berkel, 2004). For treatment, 10 g of spoiled flesh was rinsed in 100 mL of 0.1% fresh and clean *Averrhoa bilimbi* extract, rice *v*inegar, *Citrus aurantifolia* extract, salt, flour, rice rinsed water and extract of *Tamarindus indica* for 1 min.

Microbial count for treated fish flesh

For microbial test, 10 g sample of control and treated flesh was blended with 100 mL 0.1% peptone water to achieve the final volume of 250 mL until a homogeneous suspension was obtained. pH of flesh suspension was adjusted with NaOH or HCL to be between around 5.5 to 7.6. The flesh suspension was centrifuged at 1,200 rpm for 30 min to concentrate the pellet. 1.0 mL of the supernatant was collected and serially diluted to 10^{-5}. Approximately 0.1 mL of each dilution was transferred into nutrient agar and spread using hockey stick. For microbial growth, the agar plate were inverted and incubated at 37 °C for 48 h (Downes and Ito, 2001).

Preparation of cutlet

For preparation of fish cutlet, 100 g of potato was boiled in 1.5 L water with ½ teaspoon of turmeric powder and 1 teaspoon of salt until the potato cooked well. At the same time, fish was filleted to remove bone and smashed. Other ingredients such as onion, ginger, pepper powder, were added into the smashed ingredients. Then all of the ingredients were mixed well until fine dough was obtained. The dough were divided into small pieces and then dipped into egg and bread crumbs before deep frying in cooking oil.

Cutlet microbial test

Cutlet microbial test was conducted as done for microbial test for fish flesh.

Cutlet protein test

Protein content of the fish cutlet was determined using Kjeldahl (Rhee, 2001). The method is divided into three parts which are digestion, distillation and titration.

Cutlet palatability

Cutlet palatability was done through distributing questionnaires to respondent. The sensory attributes that were tested are texture or mouth feel, smell/odour/aroma, taste, physical structure and colour (Luning and Marcelis, 2009). Each item and it's decription used in the fish cutlet palatability test is shown in Table 1.

RESULTS

Fish treatment

Flesh of fish rinsed with rice vinegar has less microbial count (CFU/mL = 0.37 X 10^7) compared to the fresh fish (CFU/mL = 0.50 X 10^7) and spoiled fish (CFU/mL=1.67x 10^7). Most of other treatments (Figure 1) were able to reduce the count of microbes compared to the spoiled fish (CFU/mL=1.67x 10^7) but still more that the fresh fish (CFU/mL = 0.50 X 10^7), flesh of fish rinsed with rice water has the microbial count of CFU/mL = 0.85 X 10^7, followed by fish rinsed with flour (CFU/mL=0.99 X 10^7), *Averrhoa bilimbi* extract (CFU/mL= 1.05 X 10^7), salt (CFU/mL=1.3 x 10^7). However, flesh of fish rinsed with *Citrus aurantifolia* extract (CFU/mL= 2.06 X 10^7) and *Tamarindus indica* extract (CFU/mL=3.08 X 10^7) have microbial count more than the spoiled fish. Rice vinegar has the ability to reduce a significant number of the microbes comparing with other treatments given to the fish. This is maybe due to the acidity nature of the vinegar.

Table 1: Item and its description that was used for the fish cutlet palatability test.

Item	Description
Taste 1	Do you like fish?
Taste 2	Have you ever eaten products made from fish?
Taste 3	Do you like food made from fish product?
Taste 4	Do you like cutlets?
Taste 5	Do you like fish cutlet?
Taste 6	Do you like the taste of the fish cutlet?
Aroma 1	Do you like the aroma of the cutlet?
Aroma 2	Does the aroma induce you to consume it?
Aroma 3	Is the cutlet aroma suitable for it?
Colour 1	Does the colour of the cutlet induces you to consume it?
Colour 2	Is the colour of the cutlet suitable?
Texture/mouth feel 1	Do you like the texture of the cutlet?
Texture/mouth feel 2	Is the cutlet juicy? Do you like it?
Texture/mouth feel 3	Is the cutlet is smooth and no any large bits of any ingredients?
Texture/mouth feel 4	Are the ingredients of the cutlet well blended?
Physical Structure 1	Is the cutlet soft?
Physical Structure 2	Is the physical structure of the cutlet smooth and no any large bits of ingredients?

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Physical Structure 3	Is the shape of the cutlet suitable?

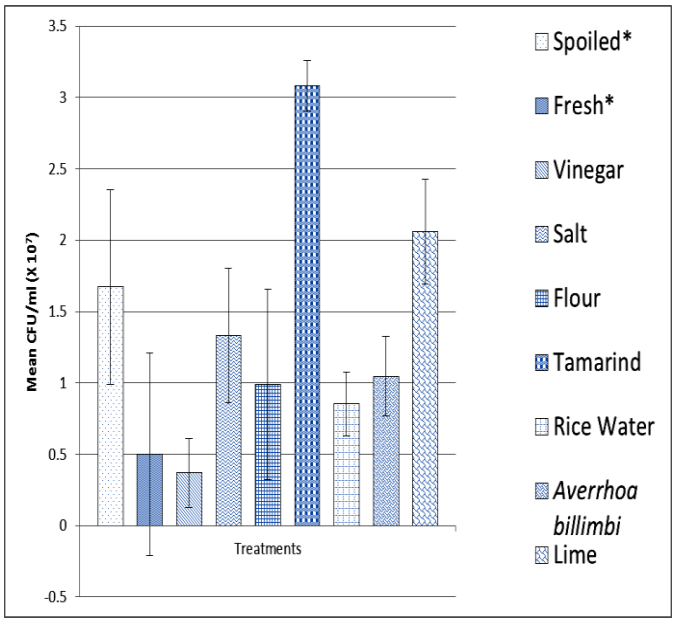

Figure 1: Microbial count of fresh, spoiled and treated fish flesh grown on nutrient agar at 37 °C for 48 h.

Cutlet microbial test

The mean of colony forming unit (Table 2) for spoiled fish cutlet (2.30 X 10^6 CFU/mL) is not significantly (p>0.05) lower than fresh fish cutlet (3.34 X 10^6 CFU/mL).

Table 2: The microbial count (CFU/mL) of fish cutlet prepared from flesh of fish treated with rice vinegar and fist cutlet prepared with fresh fish.

Fish Cutlet		CFU/mL	Std. Deviation	t-test value
Pair 1	Treated	2.30 X 10^6	.64031	P= 0.056
	Fresh	3.34 X 10^6	.92751	

Cutlet protein test

Protein content was analyzed for the spoiled fish and fresh fish cutlet using the Kjedhal method. The protein content of spoiled fish cutlet is 14.28% while for the fresh fish was 10.06% as shown in Table 3.

Table 3: Protein contents of the fish cutlet prepared from fresh and spoiled fish were determined using Kjeldahl (Rhee, 2001).

Flesh of fish (g)	Protein content (%)
Spoiled fish	14.28
Fresh fish	10.06

Cutlet palatability

Reliability

All variables tested ie. fish cutlet's taste, aroma, colour, texture/mouth feel and physical structure all have Cronbach alpha value is between 0.7 and 0.9. The Cronbach alpha for taste is 0.802, aroma is 0.888, colour is 0.772, texture/mouth feel is 0.840 and for physical structure is 0.829 as shown in Table 4.

Table 4: Cronbach alpha reliability test result for items used as variables in the palatability test of the fish cutlet.

Variables	Number of items	Cronbach alpha
Taste	6	0.802
Aroma	3	0.888
Colour	2	0.772
Texture/mouth feel	4	0.840
Physical Structure	3	0.829

Respondent's profile

Approximately 50 questionnaires were distributed for the palatability test of the fish cutlet produced from the rice-vinegar treated fish. From Table 5, there were 22 male (44%) and 28 female (56%) respondents for the test. The respondents mostly were from the age range of 13-20 (24 %). The least respondent is those from the aged 5-12 years old (6.0%).

Table 5: Respondent's demographic profile of the rice vinegar treated fish cutlet palatability test.

Background	Frequency (no)	Percentage (%)
Gender:		
- Male	22	44
- Female	28	56
Age		
- 5-12	3	6.0
- 13-20	12	24.0
- 21-30	7	14.0
- 31-40	10	20.0
- 41-50	8	16.0
- >50	10	20.0
Race		
- Malay	4	8.0
- Indian	33	66.0
- Chinese	13	26.0

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Religion			
-	Islam	6	12.0
-	Hindu	24	48.0
-	Buddha	10	20.0
-	Others	10	20.0

The majority of the respondents' races are Indians (66.0%) followed by Chinese (26.0%) and least was the Malays with percentage of 8.0%. The respondents' religion were mostly Hindu (48.0%), 10% were Buddhist, 12 % were Muslim and 20 % were other religion.

Descriptive analysis

As shown in Table 6 the respondents likes the colour of the cutlet the most (M= 4.70). The score other the other elements were all above mean value of 4. This is considered as overall good score for the fish cutlet.

Table 6: Descriptive analysis of the rice vinegar treated fish cutlet palatability test.

	N	Mean	Overall Mean	Std. Deviation
Taste 1	50	4.26		
Taste 2	50	4.90		
Taste 3	50	4.36	4.46	0.22607
Taste 4	50	4.36		
Taste 5	50	4.42		
Taste 6	50	4.44		
Aroma 1	50	4.10		
Aroma 2	50	4.06	4.13	0.09452
Aroma 3	50	4.24		
Colour 1	50	4.64	4.70	0.08485
Colour 2	50	4.76		
Texture 1	50	4.38		
Texture 2	50	4.16	4.32	0.10504
Texture 3	50	4.34		
Texture 4	50	4.38		
PhyStruct 1	50	4.38		
PhyStruct 2	50	4.48	4.45	0.06429
PhyStruct 3	50	4.50		
Valid N (listwise)	50			

DISCUSSION

Flesh of fish rinsed with rice vinegar has significantly less microbial count compared to the fresh fish and spoiled fish. Most of other treatments were able to reduce the count of microbes compared to the spoiled fish but still more that the fresh fish. However, flesh of fish rinsed with *Citrus aurantifolia* and *Tamarindus indica* extracts have microbial count more than the spoiled fish. Rice vinegar might have the ability to reduced microbial count due to present of acetic acid (Bell, 2002).

From the t-test conducted for fish cutlet for fresh and spoiled fish, 2 tailed value showed p=0.056. According to Schloesser (2000), the critical P level at p=0.05 (5%). Therefore, we can conclude that there is a statistically no significant difference between the mean of discarded fish cutlet and fresh fish cutlet. The microbial count in both discarded and fresh fish have no significant difference and therefore, the discarded fish cutlet is considered safe for consumption. Maximum recommended bacterial counts for marginally acceptable quality fish product is 10^7 CFU/g (Silva, 2002). Therefore, the cutlet could be marketed as frozen food in the market since the microbial count of the cutlet made from discarded fish is 2.30 X 10^6 CFU/g (Note: CFU/g = CFU/mL).

For the palatability analysis, after factor loading was carried out, most of the variables showed an acceptable range of reliability. Basically 0.7 or more reliability coefficients are considered adequate (Leech *et al*., 2005). According to Mokhtar *et al.* (2011), the reliability index of 0.90-1.00 is very high, 0.70-0.89 is high, 0.30-0.69 is moderate and 0.00-0.30 is low. Table 4 shows the reliability test for the survey conducted. We can conclude that all of the instruments from the questionnaire result have high reliability since the Cronbach alpha has the variable value above 0.7.

The protein content for the spoiled fish is higher may be because the vinegar acidity that was used to wash the spoiled fish affects the protein content of the cutlet. According to Silva (2002), the protein content in lean fish has an average of protein content of 15% to 20%. The protein content also varies with the species of fish. The percentage of protein content for the cutlet as shown in Table 3 is at acceptable level as frozen food product when comparing the protein content of fish ball as reported by Huda *et al*. (2010) which ranged between 7.54-9.89%. According to Nurnadia (2011), the fish used in this research, *N. japonicas,* with local name '*ikan kerisi'* has the protein content of 18.17±1.36%. The protein content in this cutlet maybe lower than the original fresh fish because some ingredients such as potato, onion and ginger may have affect the protein content of the cutlet. The cutlet is subjected to high temperature which could cause the protein in the fish to degrade. This may also be the reason of the less protein content when comparing to the normal fish (Silva, 2002). According to Labconco (2012), the result for protein test may be different from expected due to uneven digestion, sample problem or even the acidity or salt of the sample used.

CONCLUSION

From the present research, a product from spoiled fish was able to be produced for human consumption. The microbial test conducted shows that the spoiled fish cutlets (CFU/mL = 2.30 X 10^6) have almost same microbial count with the fresh fish cutlet (CFU/mL = 3.34 X 10^6). Rice vinegar (CFU/mL = 0.37 X 10^7) has the ability to reduce most of microbes in the spoiled fish compared to the other treatments using *Averrhoa bilimbi*, *Citrus aurantifolia* extract, salt, flour, rice rinsed water, and *Tamarindus indica* extract. The cutlet made from the spoiled fish has attracted the respondents for the organoleptic testing using questionnaire. Most of the respondents like the colour of the cutlet. From the Cronbach alpha result, all of the

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value is above 0.7 which is at satisfactory level. Therefore, spoiled fish can be used for human consumption after being treated with rice vinegar and turned into process product such as cutlet. This would avoid wastage of the spoiled fish where some places in the world the spoiled or discarded fish are thrown back into the sea.

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