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Fermentation of Indian Oil Sardines (*Sardinella longiceps*) Utilizing Lankauas (*Alipinia Pyramidata Blume*) as Enhancer and Catalyser

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Abstract. The utilization of *Lankauas (Pyramidata Blume merr)* in fermented Indian oil sardines (*Sardinella longiceps)* was conducted to determine its effect to fermentation period and the physical attributes of the fish sauce and bagoong. Three (3) different formulations were prepared and the amount of liquid hydrolysed from the formulation was observed daily. Likewise, the nutritional value or chemical constituents and halophilic yeast were determined. The raw materials are highly nutritious and have paramedic effect. Result of sensory evaluation was analysed through Friedman two-way analysis of variance by ranks (ANOVA). It was found out that the best treatment or formulation was Treatment 1 a mixture of fish with 25% salt and 5% of *lankauas* added per kilo of fish. Utilization of *lankauas* to *Sardinella longiceps* shortened the fermentation period to 60 days and improved the quality and enhanced the flavour and odour. Protein and mineral content of fish sauce (patis) and Fish paste (bagoong) was very high and exceeded the Philippine standard for quality of fermented fish. Products were safe from any pathogen bacteria. Halophilic yeast count increased with decreasing salt concentration.

Keywords: Fermented sardines with langkuas

1. Introduction

The Philippines is an important producer of fish in the world, ranking 13th among the 51 top fish producing countries in 1996, with its total production of about 1.8 million metric tons, or a share of 1.9 % to the total world catch of 94.625 million metric tons. Although not a dominant player in the national economy, fisheries is nevertheless an important sector, with its contribution of US$ 1.8 billion, 2.7% percent to the country's Gross National Product (GNP) of US$ 68.2 billion at current prices in 1998. It also provides employment to about 1 million or 5 % of the total labor force. Conservative estimate that only t10% of that part of the world's catch are used for direct consumption. In addition, there are substantial losses of processed products, (Information on Fisheries Management, 2000) [1]. Therefore, increased production of fishery by fisherman and processors is required to limit losses, many of which could be avoided by the addition of simple technology.

Fermentation of fish is one of the easiest and economical methods of processing fish and has a promising investment return. Quiason, S. and J. Ang, (1994) [2] stated that the typical problems encountered with the production of fermented fish paste include long fermentation time that usually takes from 3 weeks to 1 year. Presently, in the Philippine fermented industry is developing for a process that could shorten the fermentation period, safe and nutritious. These developments in the production of fermented fish may help the country increase its domestic and export industry. Fish paste or bagoong na isda is one of the fermented fish products in the Philippines. It is a well-known saline product obtained by partial fermentation of fish and widely produced and consumed in comparison with fish sauce (Mojica, Elmer-Rico E, et.al 2005) [3]. Fish sauce is one of the most popular fermented fish products used as a condiment in Southeast Asia (Tsai, Yung-

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Hsiang, et al, 2006) [4]. It is traditionally produced by mixing whole fish with salt at a ratio of 1:1–3:1, and fermented for 6–12 months or longer.

Furthermore, Saisithi, P., Kasemsarn, J., Liston, D., & Alexander, M. (1966) [5] define fish sauce (patis) as the supernatant, liquid substance floating on the surface of mixture resulting from proteolysis, which is the product of the breakdown of proteins of fish.

In this study, researchers utilized the Lankauas (*Alipinia Pyramidata Blume*) in fermented Indian oil sardines (*Sardinella longiceps)* to determine its effect to the fermentation period as well as to the physical attributes and chemical properties of the products.

Indian oil sardines or "tunsoy" in local name are abundantly caught not only in Bicol Region but all over the Philippines. These fish is not commonly used for direct consumption because of its being delicate, spiny and oily in appearance. Some villagers in Bicol Region say that if the fish is in season these fishes are abundantly caught every day. When fishes were not sold during these days, these were salted or dried. During rainy season when drying cannot be done sometimes they are just thrown or buried in the sand. Hence, the purpose of this study is to preserve and save the surplus catch of sardines or “tunsoy” by processing it into fermented fish or bagoong and patis.

Lankauas (*Alipinia Pyramidata Blume*) is commonly found in primary forest throughout the island and provinces in the Philippines (Quisumbing, 1978) [6]. Bureau of Plant Industry (BPI), Philippines (2012) [7], reported that Lankauas is found in Rizal, Laguna, Camarines, and Sorsogon Provinces in Luzon; in Panay; in Leyte; in Lanao and Agusan Provinces in Mindanao; and in Palawan, generally in old clearings. It is sometimes planted. It also occurs in the Himalayan region through Malaya to the Moluccas. The rhizomes are strongly aromatic. In Java, according to Ochse, this plant is frequently cultivated. The young rhizomes and the tender, undeveloped shoots are eaten as are also the flower buds and flowers. The rhizome is used as a condiment. Its flavor is similar to ginger, but much less pungent. It is also cooked with the sap of sugar cane or with honey and water to produce an intoxicating beverage. In the Philippines the juice of the rhizome is painted on *anan*, or *pano blanco*, a kind of skin disease. According to Guerrero as stated by BPI, Philippines the rhizomes are carminative and stimulative, and a decoction of the leaves is used for antirheumatic and stimulant baths) [7].

This plant is cultivated in Bicol Region because its rhizomes were used as flavour enhancer to salted siganid "kuyog”. This study aims to utilize the enzyme (protease) from the rhizomes of Lankauas (*Alipinia Pyramidata Blume*) not only enhances the flavour but shorten the fermentation period 30 t0 60 days.

2. Materials and Method

2.1. Experimental Design

Three treatments were assigned in Complete Randomized Design (CRD) with 3 replicates. Treatment 3 was the control without *lankauas*. Thirty (25%) of salt was added for every kilo of fish. Treatment 2 was added with 25% of salt and 5% of *lankauas* while Treatment 1 was mixed with 25% of salt and 10% of *lankauas*. A daily observation was conducted and amount of patis was measured and recorded. After every month the average of liquid or sauce was recorded to determine the effect of protease to the fish. All treatment was subjected to sensory evaluation to determine the right formulation. After determination of the right formulation of the ratio fish, salt and *lankauas* the process were again repeated using the standard formulation. The physico-chemical and microbiological properties of the different fermented were analyzed at Department of Science and Technology, Region V, Philippines. Moreover, sensory evaluation of the fish sauce and paste samples by selected panelist was conducted to determine the acceptability of taste, color, aroma, texture, consistency and saltiness using a 9-point Hedonic scale. The Friedman two-way analysis of variance by ranks ANOVA (Analysis Of Variance) was used in determining the significance of each sample.

2.2. Manufacturing Process

The fish were washed thoroughly to remove the foreign materials such as sands, stones, seaweeds, etc. The Lankauas (*Alipinia Pyramidata Blume*) was peeled, washed thoroughly, pressed and grounded. For

every kilo of fish, 25% salt and 10% of *lankauas* were added for Treatment 1, and 25% of salt and 5% of *lankauas* for Treatment 2. Treatment 3 (the control) was added with 25% of salt. The mixture was packed in plastic container with cover, and allowed to ferment for 4 weeks to 12 weeks. Each has the capacity of 12,000 ml or 12 litres. During the experimentation the amount of sauce developed in the mixture were observed and recorded from 1st to 90 day. Agitation of the mixture was made by stirring regularly with a wooden paddle. When the fishes were totally hydrolyzed the fish sauce or paste were extracted and filtered. The fermented fish paste and sauce were packed in clean bottles and sealed with plastic caps and sealers.

3. Results and Discussion

3.1. Effect of Languas Pyramidata (Blume) Merr in Fermented Indian Oil Sardines (Sardinella Longiceps)

Table 1: Percentage of Hydrolyzed Fish Flesh from One to Three Months

	30 days 12,000 ml/container (ml)					60 days 12,000 ml/container (ml)					90 days 12,000 ml/container (ml)				
T	R1	R2	R3	M	%	R1	R2	R3	M	%	R1	R2	R3	M	%
T1	3600	3,000	2,800	3,133	26%	7000	7200	7400	7200	60%	8,100	8,200	7,400	7,433	62%
T2	4,000	3,800	4,200	4,000	33%	7000	8000	8500	7883	66%	8,200	8,200	8,600	8,333	69%
T3	1,400	1,600	1,500	1,500	13%	3000	3000	2,800	2933	24%	3,200	3,100	3,100	3,133	26%

Table 1 shows the percentage of hydrolyzed fish flesh from 30 days to 90 days. The result shows that the Lankauas (*Alipinia Pyramidata Blume*) has great effect on the fermentation period in fermenting *Sardinella longiceps* because within 90 days almost all the flesh of the fish already hydrolyzed. Hence, *lankauas* not only enhance the quality of fish sauce "patis" and fish paste "bagoong" but also accelerate the fermentation period.

3.2. Sensory Qualities of the Fish Sauce "Patis" and Fish Paste "Bagoong"

The data was based from the result of sensory evaluation conducted by trained panellists. As shown in Table 2 both fish sauce "patis" and fish paste "bagoong" at 5% level of significance, flavour, odour and saltiness was significantly different because the computed value is higher than the tabular value. The utilization of *lankauas* has differential effect particularly in flavour; odour and saltiness but not in colour. The treatment which was moderately salty was Treatment 1. Furthermore, the control ranks third to all physical attributes. The most preferred Treatment as to flavour, odour, and saltiness was Treatment 2 while for colour was Treatment I. These findings show that utilizing *lankauas* at 5% level of concentration improves the quality of the products and enhanced the flavour.

Table 2: Summary of Freidman Two-Way Analysis of Variance by Rank on the Physical Attributes on Patis and Bagoong

Products	Flavour			Odour			Colour			Saltiness		
	X^2C	TV	C	X^2C	TV	C	X^2C	TV	C	X^2C	TV	C
Patis	6.993	5.99	S	8.164	5.99	S	5.98	5.99	NS	6.62	5.99	S
Bagoong	9.281	5.99	S	10.107	5.99	S	5.617	5.99	NS	7.557	5.99	S

^{X2} Computed X ^{TV} Tabular value ^{C} Conclusion

3.3. Standard Formulation

Based from the findings, the best treatment as to the effects on fermentation period was Treatment 2, which was added with 25% salt and 5% *lankauas*. This finding is also true to the general acceptability and physical attributes of the patis and bagoong. Therefore, the standard formulation in preparing fermented Sardinella longiceps utilizing the *lankauas* was to add 25% salt and 5% *lankauas* for every kilo of fish.

3.4. Chemical Analysis of Fish Paste "Bagoong"

The bagoong and patis were graded according to its chemical composition or to its nutrition values. According to the Pure Foods and Drug Laws of the Philippines stated by Espejo, Hermes (1998) [8], the

protein content of especial bagoong must be12% and the sodium chloride 20% for regular bagoong than 6% and not less than 20% respectively. For fish Sauce "patis" the protein content is eight 8% for special and 4% regular. The sodium chloride is 20-25% and 20% for special and regular fish sauce "patis" respectively.

Philippine Standards Association (PSA) cited by Basamin, S.V. and Napugan R.SJ patis classified as:

Local term	PSA EQUIVALENT
Especial (First extract)	First class (A
Extra (Second extract)	Second class (B)
primera (third & fourth extract	Popular (C)

Basamin, S.V. and Napugan R.SJ (1961) [9] reported that those patis with higher total nitrogen are considered the better grades. First Class (A) is rated as the highest, Second Class (B) as next and Popular Class (C) the lowest.

As shown in Table 3, the protein content of special fish paste 12.59 and 6.43 for regular. While the protein content of special and regular fish sauce "patis" is 12.75 and 4.18, respectively. These results explained that the protein content exceeded to the Philippine Standard for the protein content of the product, hence, utilizing *lankauas* in fermenting Indian Oil Sardines improves its nutritional value. While for sodium chloride is less than with the specified standard. However, according to Department of Science and Technology (DOST) if sodium chloride is lower than the standard it shows that it is still very good and acceptable than with the higher content because too much sodium will affect the entire acceptability of the product.

Table 3: The Proximate Analysis of Fish Paste "Bagoong"

Samples	Moisture	Protein	Salt (NaCl)	Crude fat	Ash
Special Bagoong	48.53	12.59	16.25	1.14	15.82
Regular Bagoong	58.09	6.43	11.73	3.5	12.21
Special Patis	---	12.75	12.50	---	12.82
Regular Patis	---	4.18	11.36	---	17.21

Sanchez (2008) [10] explained that bacteria isolated from the intestines of marine fishes are halophilic and these microorganisms can also be introduced in the addition of salt. The principal halophilic species isolated from solar salt are *Bacillus* and *Micro-coccus*, and *Micrococcus* in rock salt.

The Philippines standard for halophiles count in fermented fish should not be more than 20%. Based from the result as indicated in Table 4 only special fish sauce "patis" has conform to the Philippine standard which is 19.75, while special and the regular fish paste "bagoong"; and regular fish sauce "patis" has exceeded a little to the specified standard with the total count of 23.5, 24.5 and 23.25 respectively. The result may be correlated to the minimal amount of sodium chloride of the product as shown in the result of chemical analysis. Similarly, in the study done by Besas, J. R. and Dizon, E, I. (2012) [11] the total plate count and lactic acid bacteria count decreased with increasing salt concentration. Huss and Valdimarson (1990) [12]. Sodium chloride plays an important role in microbial growth and therefore influences the activity of their amino acids decarboxylase. High salt concentration also affects the growth of lactic acid bacteria since these type of bacteria are generally tolerant of moderate salt concentrations in the range of 10% to 18% (Sanchez, P. C.,2008) [10].

Table 4: Halophiles Yeast Count

Samples	Halophiles Yeast Count	Method Used
Special Bagoong	23.5	PNS 413.1990
Regular Bagoong	24.5	
Special Patis	19.75	
Regular Patis	23.25	

The main action of fermentation in fish paste production is partly liquefaction. Considerable moisture is extracted from the fish after salting because of high osmotic pressure. Proteins are hydrolyzed and dissolved in the brine as storage period continues [13]. Additional protein from *lankauas* may induce bond breakage thus leading to formation of more soluble contributed to the microbial load Although these findings did not show any evidence that less sodium chloride will have higher susceptibility to halophiles yeast because of the constituent of langkuas was added to the fermentation process. Although Huss and Valdimarson, (1990) concluded that Pathogens rarely multiply at high salt concentrations, efforts should be made to minimize microbial load in the product to protect consumer from food poisoning and food-borne microorganism and to make sure that the product be safe to eat.

4. Conclusions

Utilization of *langkuas* in fermentation of fish takes 30-60 days for the fish to be reduced to fish paste and sauce and produces aromatic flavor and odor. The more acceptable fermented Indian Oil sardines is when it added with *langkuas* is 25% of salt and 5% of *langkuas* for one kilo of fish during processing. Utilizing langkuas not only improves it nutritional value but it shorten the fermentation period. Hence, Langkuas (*Pyramidata Blume merr*) can be used as a catalyzer in fermentation of fish and other products.

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