The Palawan Scientist
Vol. 1, No. 1. pp. 44-54, 2004

ISSN No. 1656-4707

Standardization of Production Technique for Marinated Semi-Dried Fish

Violeta T. Bargoyo and Ria S. Sariego
WPU-Puerto Princesa Campus
Sta. Monica, Puerto Princesa City

ABSTRACT

A protocol for marinated semi-dried siganids production was developed through standardization of marinating procedure and shelf-life determination. The process involves soaking the fish in saturated brine solution for 15 minutes and in 1:3 vinegar-water ratio for 60 minutes. The processed fish has a shelf life of 3 days at room temperature, 2 months when refrigerated, and 51/2 months under freezing temperature.

Keywords: siganids, semi-dried fish, marinated fish, fish processing

INTRODUCTION

Palawan has numerous coastal villages where the major livelihood is fishing. There are areas without the benefit of electricity and therefore has no ice plants or cold storage facilities where the fish catch can be temporarily stored or frozen prior to shipment.

Because of the relative isolation of these areas, the fishermen are forced to sell their products at a very low price or just dry their product traditionally. A new high value added product is gaining popularity among the local fish processors in Puerto Princesa City as an alternative to traditional drying. This product is called marinated semi-dried fish or locally known as "lamayo". Raw materials for this product are rabbitfishes and halfbeaks which are abundant in the area throughout the year. However, quality of processed product varies with each fish processor. The products are also packaged poorly, limiting the product market to the locality.

Standardization of production technique and development of appropriate packaging would further boost this industry. It would expand the product market out of the province and will command high price for the product. It would also generate more employment for women and help many fishing families alleviate their standard of living.

METHODOLOGY

The study involved two parts: Part I dealt with the standardization of procedure for marinated semi-dried fish production and Part II involved shelf life determination and microbiological analysis of the standardized products.

Part I. Standardization of Marinated Semi-Dried Fish Production

Fresh siganids, *Siganus canaliculatus* locally known as danggit, samaral or bararawan were used as raw materials. The fish samples were split into butterfly fillets, eviscerated and cleaned.

The cleaned raw materials were soaked directly in 25% brine solution at varying time (15, 30, 45 and 60 minutes), after which, the fish were brushed and washed thoroughly in clean water. The fish were then soaked in varying levels of vinegar-water solution (1:1, 1:2, 1:3 and 1:4) at different soaking time, (15, 30, 45 and 60 minutes). Amounts of other ingredients used like garlic and pepper powders were constant in all treatments. After each treatment, the fish samples were air-dried for 15-30 minutes. They were then fried and subjected to sensory evaluation using the 9-point Hedonic Rating Scale. Scores were statistically analyzed using Analysis of Variance (ANOVA).

Part II. Shelf life Determination / Sensory Evaluation and Microbiological Analysis

A. Shelf Life Determination / Sensory Evaluation

The standardized products were packed using different packaging materials such as sealed polyethylene bags, vacuum-packed polyethylene bags and styrofoam tray covered with cling wrap. The packed samples were stored at room, refrigerated and freezing temperatures and were evaluated every other day, weekly and twice a month, respectively. A sensory panel evaluated the fried products using the Hedonic Rating Scale. Scores were statistically analyzed using analysis of variance (ANOVA).

B. Microbiological Analysis

B_1. Total Plate Count Determination

A 10^{-1} dilution was prepared using 30 grams macerated sample with 270 ml sterile diluent (0.1% peptone). Using a sterile pipette, 1 ml of the 10^{-1} dilution was transferred to a test tube with 9 ml of the sterile diluent to prepare the 10^{-2} dilution. The process was repeated until a 10^{-6} dilution of the sample was obtained.

One ml each of the diluted samples was transferred to duplicate petri dishes. These were mixed with approximately 15 ml of melted agar at 45°C temperature. The plates were then allowed to stand on a horizontal surface until the medium hardened.

The petri dishes were inverted and incubated at 35°C for 48 hours. Only those plates with 30-300 microbial colonies were counted and marked. Spreading colony was counted as a single colony. The arithmetic mean count was determined and the value obtained was reported as colony forming unit per gram (cfu/g).

B_2. Cultural / Morphological Characteristics and Gram Reactions of Microorganisms

Cultural characteristics of colonies such as form, margin, elevation and color were determined as seen on the agar surfaces of petri dishes. Morphological characteristics such as shape, arrangement of cells and special growth features were determined on the prepared smears in glass slides. Gram stain reaction of each colony was determined in a prepared fixed bacterial smear. The smear was stained with 0.5 to 1.0 ml crystal violet. It was then rinsed with water, treated with Lugol's iodine for 1 minute, after which it was decolorized with alcohol and again washed with water. The smear was then counter stained with safranin for 1 min, rinsed with water again then air dried. The stain reaction was then observed.

RESULTS AND DISCUSSION

Standardization of Production Process

The fried processed fish under different treatments were all acceptable to the panelists (Table 1). Mean hedonic rating scores ranged from 6.5 to 8.3. These scores are equivalent to "like slightly" to "like very much". The acceptability of the process can be attributed to the osmotic effect of salt and the flavor enhancing effect of acetic acid. The salt diffuses through the fish flesh by dialysis mechanism and water diffuses out due to osmotic pressure between the brine and the fish muscle (Hall, 1997). The water removed from the fish muscle is then replaced by the marinating solution when the fish is soaked in it. The acetic acid in vinegar together with the action of proteolytic enzymes causes partial breakdown of protein and release some free amino acids giving marinades its characteristic taste (Clucas and Ward, 1996).

Table 1. Panel mean scores of fried marinated semi-dried siganids soaked in concentrated brine and vinegar-water solutions in varying levels and time.

Soaking Time in Concentrated Brine Solution (min)	Soaking Time in vinegar-water solution (min.)	Mean Hedonic Rating Scores			
		1:1	1:2	1:3	1:4
15	15	7.2	7.2	7.2	6.9
15	30	7.4	6.9	7.4	7.6
15	45	7.8	7.1	7.6	7.7
15	60	7.5	7.5	8.3	7.2
30	15	7.4	7.8	7.8	7.4
30	30	7.6	8.0	8.1	7.5
30	45	7.5	7.3	7.5	7.0
30	60	7.2	7.3	7.5	7.2
45	15	8.3	7.3	7.9	7.6
45	30	7.9	7.3	8.0	7.6
45	45	7.8	7.8	8.1	7.3
45	60	7.7	7.9	7.9	6.5
60	15	8.0	7.4	7.1	7.1
60	30	7.8	7.5	7.1	6.7
60	45	8.1	7.2	7.4	7.4
60	60	8.1	8.2	7.9	7.7

Analysis of variance indicated a significant difference between samples at 5% level. The sample causing a significant difference was identified as the sample soaked in concentrated brine for 15 minutes and in 1:3 vinegar-water solution for 60 minutes. This implies that production of marinated semi-dried siganids required a considerably shorter period of soaking in concentrated brine solution and in lower concentration but longer period of soaking in vinegar-water solution to attain the desired flavor. This sample was rated by the panelists as "like very much".

Part II. Shelf life Determination and Microbiological Analysis

Results of sensory evaluation indicated that marinated semi-dried fish packed either in polyethylene bag or styrofoam tray cling wrap are still acceptable on the 3rd day of storage (Table 2). However, microbial load of samples is high indicating the perishability nature of fish, but the value is within the 1×10^5 cfu/g food microbial load limit set by BFAD (1990). On the fourth day of storage, all samples were noted to be objectionable. Aside from the undesirable change in fish flesh flavor and odor texture, there is growth of molds, making them unfit for consumption. This can be attributed to the influence of

temperature on the microbial and enzymatic (cathepsins) activities which is greatest in moist foods (ICMSF, 1980).

Table 2. Panel mean scores and microbial loads of standardized marinated semi-dried siganids packed in electric sealed polyethylene bag and styrofoam tray with cling wrap stored at room temperature (30 ± 2 °C).

Storage Period (days)	Sealed polyethylene bag		Styrofoam tray w/ cling wrap	
	Mean Score	Microbial load (cfu/g)	Mean Score	Microbial load (cfu/g)
1	7.3	$6.8X10^3$	7.5	$4.3X10^3$
3	7.0	$2.6X10^4$	6.5	$6.5X10^4$

Table 3. Panel mean scores and microbial loads of standardized marinated semi-dried siganids packed in polyethylene bag and styrofoam tray with cling wrap stored at refrigerated temperature (3.0-7.0 °C).

Storage Period (days)	Electric-sealed Polyethylene bag		Styrofoam tray w/ cling wrap		Vacuum-packed Polyethylene bag	
	Mean Score	Microbial load (cfu/g)	Mean Score	Microbial load (cfu/g)	Mean Score	Microbial load (cfu/g)
1	7.8	$1.7x10^3$	7.6	3.9×10^2	7.6	2.1×10^3
7	8.3	$6.1x10^3$	7.7	9.2×10^2	7.5	3.9×10^3
15	6.5	$4.9x10^4$	7	2.0×10^3	7.2	4.5×10^4
22	7.3	$6.6x10^4$	6.9	4.1×10^4	7.1	1.9×10^4
28	6.9	$7.8x10^4$	6.7	6.4×10^4	6.7	1.4×10^4
36	6.8	$6.1x10^4$			6.8	1.7×10^4
42	6.2	$6.8x10^4$			6.3	1.0×10^4
51	3.8	$1.7x10^5$			6.1	1.5×10^4
60					4.7	1.5×10^4

Samples stored at refrigerated temperature showed lengthened shelf life as compared to samples stored at room temperature. The result revealed that most refrigerated samples lasted from one to two months regardless of packaging material used. A shelf-life of 14 days for marinades stored at 0°C to 8°C with no specific species of fish mentioned had been reported by Clucas and Ward (1996). Their finding was surpassed by the result of this study. Based on panel mean scores, vacuum packed samples were unacceptable on the 60th day of storage while sample with cling wrap was still acceptable during that storage period. No distinct trend in microbial load was observed prior to the deterioration of sample quality. Samples packed in polyethylene bags however, exceeded the microbial standard limit set by BFAD (1990). These samples were objectionable on the 51st and 42nd day of storage, respectively. A long period of refrigerated storage common for many foods permits psychrophic population to reach many million

grams in only few days resulting in objectionable changes in color, taste and texture (ICSMF, 1980).

Table 4. Panel mean scores and microbial loads of standardized marinated semi-dried siganids packed in polyethylene bag and styrofoam tray with cling wrap stored at freezing temperature (-18.0 °C).

Storage Period (days)	Electric-sealed Polyethylene bag		Styrofoam tray w/ cling wrap		Vacuum-packed Polyethylene bag	
	Mean Score	Microbial load (cfu/g)	Mean Score	Microbial load (cfu/g)	Mean Score	Microbial load (cfu/g)
30	7.4	$3.4x10^3$	6.8	$5.6x10^3$	7.5	$3.2x10^3$
41	6.9	$2.4x10^4$	7.5	$6.0x10^4$	7.5	$5.1x10^4$
55	7.3	$4.2x10^4$	7.3	$3.1x10^4$	7.4	$5.6x10^4$
70	7.2	$3.1x10^4$	6.9	$1.4x10^4$	7.6	$3.5x10^4$
84	6.8	$6.2x10^4$	6.8	$5.1x10^4$	7.2	$2.7x10^4$
99	6.9	$4.5x10^4$	7.2	$1.7x10^4$	7.8	$1.3x10^4$
113	7.4	$6.3x10^4$	7.2	$1.9x10^4$	7.8	$1.7x10^4$
139	7.1	$1.7x10^4$	7	$1.2x10^4$	7.1	$4.8x10^4$
168	7.3	$1.3x10^5$	7.4	$3.4x10^3$	7.2	$1.02x10^3$

Samples stored at refrigerated temperature showed lengthened shelf life compared to samples stored at room temperature. The result revealed that most refrigerated samples lasted from one to two months regardless of packaging material used. A shelf-life of 14 days for marinades stored at 0°C to 8°C with no specific species of fish mentioned had been reported by Clucas and Ward (1996). Their finding was surpassed by the result of this study. Based on panel mean scores (Table 8), vacuum packed samples in both production process were both unacceptable on the 60^{th} day of storage while sample with cling wrap was still acceptable during that storage period. No distinct trend in microbial load was observed prior to the deterioration of sample quality. Samples packed in polyethylene bags however, exceeded the microbial standard limit of 1×10^5 colony forming unit per gram set by BFAD (1990). These samples were objectionable on the 51^{st} and 42^{nd} day of storage, respectively. A long period of refrigerated storage common for many foods permits psychrophic population to reach many million grams in only few days resulting in objectionable changes in color, taste and texture (ICSMF, 1980).

Panel mean scores and microbial loads of all frozen samples packed using the three packaging materials indicated that the samples were still acceptable after 51/2 months of storage (Table 4). Bacterial load of these samples were far below the microbial standard limit set by BFAD (1990).

A decreasing trend in microbial count of different samples was noted in the frozen samples. This proved the efficiency of the freezing process in slowing or stopping microbial growth. Low temperature reduces reaction rates wherein water in fish freezes and become bound, thus reducing water activity and bacterial

growth (Gartwaite as cited by Hall 1997). Deteriorative processes are likewise retarded at reduced temperature eventually stopping spoilage.

The sensorial evaluation of all samples decreased as storage period lengthened. The sensorial characteristics (color, odor, texture and flavor) changed with prolonged storage. It was observed that fish that was frozen gradually develop an off flavor and off odor which can be likened to boiled clothes, wet cardboard and cold tea (Mc Grill 1974 as cited by Hall 1997).

Cultural and Morphological Characteristics of Isolated Microorganisms

Cultural characteristics of the microorganisms isolated from the marinated semi-dried siganids were macroscopically determined while the morphological characteristics and gram reactions were microscopically observed. These microorganisms were not assessed whether they were initial flora of the raw materials or were obtained during catching, on board handling or processing. Microbial assessment of the raw materials during catching, on board handling and during retailing were beyond the scope of the study.

There were two mold colonies identified in samples stored at room and refrigerated temperatures. Both colonies have the same cultural characteristics but possessed different morphological characteristics (Tab. 5). Colony 1 had a non-septate hyphae with stolons and rhizoids whereas Colony 2 possessed septate hyphae with branched mycelium.

Table 5. Cultural and morphological characteristics of prominent molds isolated from marinated semi-dried fish stored at room and refrigerated temperatures.

Colony	Cultural Characteristics	Morphological Characteristics
1	Cottony white Abundant Raised from agar surface Circular in form	Non-septate hyphae With stolons and rhizoids Sporangiospheres arise at the nodes Sporangia black and large
2	Cottony white Raised from agar surface Circular in form	Septate hyphae Branched mycelium Heads tightly packed, globular and colored black

On the other hand, three kinds of prominent bacteria were isolated from samples stored at the same condition. The bacteria were all gram +, have the same form and elevation but possessed varying margin and color. They also have different morphological characteristics. (Table 6).

Table 6. Cultural, morphological characteristics and gram reactions of prominent bacteria isolated from marinated semi-dried siganids at refrigerated and room temperatures.

Cultural Characteristics				Morphological Characteristics	Gram Reaction
Form	Elevation	Margin	color		
Circular	flat	Entire	white	cocci, arranged in masses	gram +
Circular	Flat	Entire	cream	non-swelling rods	gram +
Circular	Flat	Entire	slightly lobate	long and slender rods	gram +

A contributory factor to the perishability of finfish is the composition of the native microflora which appears to be a function of water temperature to which the fish was harvested. Also, the quality and microbial load of fresh fish is affected by the harvesting method. Abusive handling at harvest will be detrimental to subsequent quality and shelf-life of fish (Martin 1994 as cited by Ward). Moreover, Hermes (1998) emphasized that quality control and quality assurance must be both applied from the moment fish is harvested until it lands on the plate of the consumer.

The salt used and the added spices in marinated semi-dried products cannot be disregarded as possible sources of the isolated microorganisms. Hermes (1998) further stated that some salt tolerant bacteria are found in many commercial salts. Also, it has been found that spices frequently contain excessive number of microorganism unless subjected to antimicrobial treatment (ICMSF 1980). The mold in spices may vary with the spice to some extent. Furthermore, spices are of interest to microbiologists because they may become moldy, contain large number of microorganisms, some exhibit antimicrobial activity and stimulate microbial metabolism.

During processing of fish samples, proper techniques (i.e. gutting and splitting) and good housekeeping (cleanliness) hygiene and sanitation of workers, materials and surroundings were observed. These processes ensured that the finished products were safe and produced under the set of Good Manufacturing Practices.

Packaging Marinated Semi-dried Siganids

Polyethylene bag and styrofoam tray with cling wrap were the suitable packaging materials for marinated semi-dried siganids stored at room temperature. Best packaging materials for the product stored at refrigerated temperature were the electric sealed polyethylene bag and vacuum packed polyethylene bags. The use styrofoam tray with cling wrap was noted not appropriate at this temperature even though the styrofoam displayed good support for the samples. The high

water transmission property of the cling wrap increased the moisture content of the samples promoting growth of microorganisms. At freezing temperature, vacuum packing proved to be the most appropriate packaging method. Vacuum packed products showed better keeping qualities and the method also offered a better visual effect due to close contact of the packaging materials to the product (Hermes 1998). Further, ice build up within the pack is also avoided. The use of styrofoam tray with cling wrap was noted not appropriate for frozen packed samples stored at prolonged freezing temperatures because the styrofoam become brittle at prolonged storage.

The protocol derived from the above results is stated below:

Production Protocol for Marinated-Semi Dried Siganids

Materials:

10 kg	siganids about 50-100 g each
1 kg	rock salt
30 g	white pepper
30 g	garlic powder
1 l.	vinegar
	potable water
	weighing scale
	basins
	strainers
	drying trays or racks
	chopping boards
	new toothbrush
	measuring cups and spoons
	knives
	packaging materials

Production Process:

1. Wash the fish thoroughly with clean water.

2. Split the fish into butterfly fillets. Eviscerate and remove adhering dirt
and blood. Wash.

3. Prepare the 25% brine solution by mixing 1 part salt into 3 parts water. Note that there will be salt crystal that will remain undissolved.

4. Soak the fish into the 25% brine solution for 15 minutes.

5. Remove the fish and brush off the remaining adhering dirt and blood.

6. Wash 2 times with clean water. Drain well.

7. Prepare the marinating solution by mixing 1 part vinegar to 3 parts water. Add 30 grams each of garlic powder and white pepper.

8. Soak the fish in marinating solution for 1 hour. Drain well.

9. Air dry the marinated fish for 15 to 30 minutes or until no solution is dripping from the fish flesh.

10. Pack in desired packaging materials.

    The product can be stored at room temperature for 3 days, at refrigerated temperature for 42 days and in freezer for 51/2 months using polyethylene bags as packaging materials.

LITERATURE CITED

Arroyo, P. 1982. The Science of Philippine Foods. Department of Fish Processing Technology. UP College of Fisheries, Q.C.

BFAD. 1990. Standard Limits in Food Microbiology.

Clucas, I. J. and Ward, A. R. 1996. Post-harvest Fisheries Development: A Guide to Handling, Preservation, Processing and Quality. Natural Resources Institute, U.K.

Gatchalian, M. M.1989. Sensory Evaluation Methods for Quality Assessment and Development. U.P. Diliman, Q. C.

Hall. G. M. 1997. Fish Processing Technology Chapman and Hall, N. Y.

Hermes, J. E. 1998. Fish Processing technology in the Tropics. Tawid Publications, Q. C.

ICMSF. 1980. Microbial Ecology of Foods. Vol. 2. Food Commodities. Academic Press Inc. Florida.

Pomeranz, Y. and Meloan, C. E. 1987. Food Analysis Theory and Practice. 2nd Ed. AVI Publ. Co. Inc.

Acknowledgment

Grateful thanks is extended to the Bureau of Fisheries and Aquatic Resources Regional Office-IVB for the financial assistance and to the WPU-PPC faculty, staff and students for their assistance as willing evaluators throughout the conduct of the study.