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Lactic Acid Bacterial Fermentation of *Burong Dalag*[1]

C. A. ORILLO AND C. S. PEDERSON[2]
University of the Philippines, College of Agriculture College, Laguna, Philippines

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The fermented food, *burong dalag*, prepared in many Filipino homes, was studied to determine the nature of the microbiological and chemical changes that occur during fermentation. This is a lactic acid bacterial fermentation in which the species *Leuconostoc mesenteroides*, *Pediococcus cerevisiae*, and *Lactobacillus plantarum* played the major acid-producing role. The *p*H was lowered to below 4.0, and about 0.9% acid as lactic acid was attained in 1 week. It was essential to keep the product covered well to exclude air and subsequent growth of yeasts and mold.

Fermentation provides an inexpensive method of preserving foods for future use. Its one great disadvantage is the lack of available scientific information regarding the nature of the processes. Fish are the most plentiful sources of protein available to the Orientals. They offer one of the best possibilities for raising the annual protein level of the diets of these peoples; however, they are subject to rapid deterioration in the warm, humid climate of the South East. Considerable quantities of fish are preserved by salting and drying and by preparing fish sauces, Hesseltine (1). Salting and drying necessitate an eventual soaking and desalting with a subsequent loss of soluble food substances. The high-salt content of fish sauces limits their use to small amounts; they are, therefore, relatively unimportant nutritionally.

Van Veen (3) described the product *phaak* or *mamchao*, prepared in Cambodia, consisting of salted fish kneaded with glutinous rice and treated with a native yeast. Van Veen stated, "It would be worthwhile to know whether bacterial action is important in the preparation of these products."

The Filipinos in some areas prepare a food consisting of fermented fish and rice colored with *angkok*, which is a deep red to purple colored rice grain produced by growing *Monascus purpureus* Winter on cooked rice. There are several similar blends incorporating fish or shrimp. One product, prepared with the fish *dalag*, is called *burong dalag*, whereas another product prepared with shrimp is called *burong hipon*.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1631.

[2] Visiting Professor (July 1, 1965 to August 31, 1967) from New York State Agricultural Experiment Station, Cornell Univ., Geneva, N.Y. 14456.

Since the product *burong dalag* becomes acid during fermentation, it was believed that lactic acid bacterial fermentation occurs. Considerable variation was observed among such products on the market. Some were acid and others were acid, alcoholic, and musty, indicating a mixed bacterial, yeast, and mold fermentation. The latter were considered inferior in quality.

MATERIALS AND METHODS

The changes in microbial flora and in the salt concentration and acidity of the liquid were determined daily with two preparations during the active bacterial fermentation of two rice fish blends. The first preparation contained 33.5% of prepared cut fish, 3½% salt, 19% washed rice, and 44% water. The second contained 28.4% fish, 2.8% salt, 21.3% rice, and 47.5% water. The salt was sprinkled over the prepared fish and then allowed to stand overnight. The following morning, the rice was cooked and cooled and then blended with the fish and *angkok*. This blend was packed tightly into a jar and covered with a plastic bag containing enough water to exclude air. The two preparations were allowed to ferment 7 and 10 days, respectively. The free liquid produced when the salted fish stood overnight was discarded in the first preparation but was added to the blend in the second preparation.

At intervals during fermentation, samples were removed for determination of total acid, *p*H, and salt content, and for bacteriological analysis using the procedures described by Pederson and Albury, (2). The samples, consisting of the moist rice and fish, were mixed with the first dilution of water in a Waring Blendor. For microbiological examination, proper dilutions of the blended sample were plated by using a tryptone, glucose, yeast extract-agar containing A and B buffer salts. Plates were incubated for 2 days at room temperature, usually at approximately 30 C. After the colonies were counted, usually 25 representative colonies from each plating were isolated for identification.

After the fermentation was complete, portions of

TABLE 1. *Changes in bacterial flora during fermentation of the first preparation of burong dalag*

Time (days)	Total count × 10^6	Estimated no. each species of bacteria × 10^6				
		L. mesenteroides	*Streptococcus and Micrococcus*	*P. cerevisiae*	*L. plantarum*	Yeast species
0	7					
1	2,000	1,680	320			
2	2,000	880	1,120			
3	1,080	376	188	376	140	
4	1,400	112		560	728	
5	3,000			360	2,640	
7	1,700			340	1,156	132

TABLE 2. *Development of acid and changes in bacterial flora during fermentation of the second preparation of burong dalag*

Time (days)	pH	Total acid as lactic acid	Total bacterial count × 10^6	Estimated no. of each species of bacteria × 10^6			
				Aerobic species	*S. faecalis*	*P. cerevisiae*	*L. plantarum*
		%					
1	6.72	0.01	0.32	0.32			
2	4.50	0.37	670		670		
3	5.10	0.34	650		364	286	
4	4.40	0.63	880		220	555	105
5	4.00	0.89	760			544	116
6	4.05	0.90	600			108	492
8	4.10	0.92	450			80	370
10	3.95	0.91	550				

the rice and the rice-fish combination were removed for protein analysis. In addition, buffer curves were established for a rice portion taken at some distance from pieces of fish, with rice for a sample adjacent to pieces of fish, and for some of the fish portions. Samples (10 g) were blended, mixed with water, and then titrated with 0.11 N sodium hydroxide solution.

RESULTS AND DISCUSSION

Within a few hours after packing the jars, sufficient liquid had accumulated so that the entrapped air could be removed by pressing on the surface. Fermentation was rapid as shown by clouding of the free liquid and by changes in the bacterial count. In the first preparation, the *p*H dropped from 6.55 to 3.95 in 24 hr and to 3.90 in 48 hr. Apparent total acidity as measured by titration was 0.26% acid calculated as lactic acid in 24 hr and 0.71% in 72 hr. When a total acidity of 0.71% was attained, two samples of the rice portion and two samples of the rice adjacent to the fish were titrated to *p*H 7.0 and 8.4. The great differences obtained between the two samples demonstrated that the buffer capacity depended upon the relative amounts of fish and rice in the sample.

Well-blended samples yielded a moisture content of 75.0% and a total protein content ($N \times 6.25$) of 32.9, 32.4, and 32.9% (dry basis). Salt content was 3.04%.

The bacteriological counts and identification of isolated cultures (Table 1) yielded results similar to those often observed with fermenting vegetables. The first plating yielded a miscellaneous collection of aerobic bacterial species. Among the 25 isolates from each of the next two platings, cultures of *Leuconostoc mesenteroides* and *Streptococcus faecalis* predominated. Later platings yielded cultures of *Pediococcus cerevisiae* and *Lactobacillus plantarum*. One yeast culture was isolated from the last plating.

The second preparation differed from the first in that no cultures of *L. mesenteroides* were isolated (Table 2), and the first plating yielded a number of strains of *Micrococcus* spp. *P. cerevisiae* and *L. plantarum* played the major roles in acid production.

Differences obtained in buffer capacity among the fish portion, the rice portion adjacent to the fish, and the rice portion further removed from the fish are shown in Fig. 1. Possibly, if this preparation were aged longer, greater equilibrium would have been established. The results (Fig. 1)

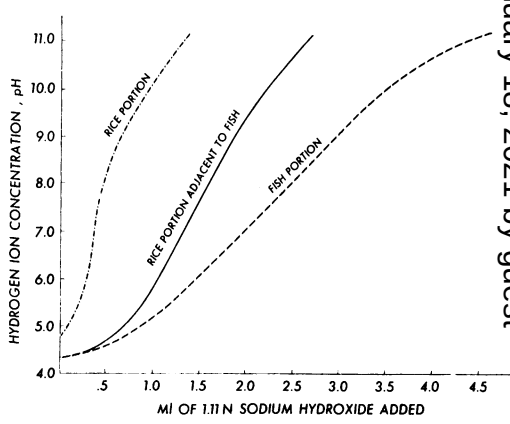

FIG. 1. *Titration of three portions of burong dalag (10-g samples fermented for 8 days). Estimated acidity of rice portion at pH 7.0 is 0.4% (calculated as lactic acid) and at pH 8.4, 0.57% as lactic acid; acidity of portion adjacent to fish at pH 7.0, 1.4% as lactic acid, at pH 8.4, 1.75% as lactic acid; and acidity of fish portion at pH 7.0, 2.0% as lactic acid and at pH 8.4, 2.72% as lactic acid.*

showed a marked difference in buffer capacity, which is reflected by the total acidity.

The two blended portions yielded 40.9 and 40.2% protein, dry basis, and 79.5% moisture.

Moist products of this type are subject to rapid growth of molds and yeasts and, therefore, great care was exercised in sealing the surface of the fermenting mixture with the plastic covers after each sampling. There is little doubt that such growth had occurred with some of the products obtained on the open market. After 7 and 10 days, the fermented products were considered to have attained their optimal flavor and texture characteristics. When cooked with a tomato sauce at this time, the product had a pleasant, slightly acid, fish-rice flavor with no rancid or oxidized flavor.

The product, *burong dalag*, would seem to offer possibilities as a method of preservation of a high-protein food. When properly packed to exclude air, sufficient acid was produced and retained to preserve the product without resorting to high-temperature cooking. Unfortunately, the requirements for a good fermentation and subsequent preservation are poorly understood by those who prepare the product in the Philippines.

Studies in the fields of microbiology and chemistry could do much to clarify the numerous problems and underlying alterations. Such studies should lead to a better understanding and to more standardized methods of preservation of some of the foods of the Far East.

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