Fermentation period-dependent changes of lactic and amino acid concentrations in *pa daek*, a salt-fermented freshwater fish paste in Laos

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Abstract

Pa daek is a salt-fermented freshwater fish paste that can be made and consumed at home and is popularly used as an all-purpose seasoning in Laotian cooking. Nowadays, the products made by small and medium-sized manufacturers are also becoming popular in local markets. The traditional *pa daek* fermentation technique furnishes a seasonally available indigenous freshwater fish with palatability as well as long shelf life. In our *pa daek* fermentation conducted using small clupeid freshwater fish (locally called *pa keo* in Laos), the increase in lactic acid was observed after 20 days of fermentation, reaching approximately 1% after 2 months, and maintaining the same level at after 6 months. The pH decreased from 6.3 to 5.4 as the lactic acid fermentation proceeded, showing the importance of the early phase of the *pa daek* fermentation to secure the preservability. The total amount of 20 free proteogenic amino acids increased in a time-dependent manner throughout the observation period. Significant increases in glutamic acid and lysine in the fermentation could well explain the advantages of the long-term fermentation for making *pa daek* products tasty and nutritious.

Introduction

Pa daek is a salt-fermented freshwater fish paste that can be made and consumed at home and is popularly used as an all-purpose seasoning in Laotian cooking. Nowadays, the products made by small and medium-sized manufacturers are also becoming popular in local markets. The *pa daek* products are usually stored without refrigeration while being used and can last for a year or longer, illustrating the benefits of a traditional fermentation technique that furnishes a seasonally available indigenous freshwater fish with palatability as well as long shelf life. We previously reported the importance of salt concentration to determine the representative lactic acid bacteria in such products (Marui *et al.* 2015). Halophilic lactic acid bacteria species such as *Tetragenococcus halophilus* and *Tetragenococcus muriaticus* have been detected in *pa daek* products with salt concentrations of 15–20% (Marui *et al.* 2015). The long period of *pa daek* fermentation is also of scientific interest for making products that meet consumer preferences. According to the producers, although *pa daek* is deemed to be edible after 2–3 months of

fermentation, 6–12 months are required to enhance palatability. Here, we describe time-dependent changes in lactic and amino acid concentrations in our experimental *pa daek* fermentation procedure, established with small clupeid freshwater fish, locally referred to in Laos as *pa keo*.

Materials and Methods

Pa daek fermentation

In the present study, small clupeid freshwater fish (*pa keo*) were collected from Nam Ngum Reservoir in Laos for use in the *pa daek* fermentation. The length of the fish used in the present study was around 40 mm that was reported to be the standard lengths of the matured *pa keo* in the Nam Ngum Reservoir (Morioka *et al.* 2019). First, the fish were washed well with water, followed by draining in a strainer. The washed fish were mixed with salt and rice bran at a ratio by weight of 3:1:1. The salt concentration in the liquid portion of the mixture was measured using a LAQUA twin compact salt meter (Horiba Ltd., Kyoto, Japan) and adjusted to approximately 15% by adding water. The mixed material was stuffed tightly into plastic containers to remove the air and sealed with screw caps. The fermentation took place at an ambient temperature in the laboratory.

Serial measurements of pH, lactic acid, and amino acids

For the serial measurements of pH and lactic acid, the *pa daek* fermentation was conducted as described above from February to August 2017. The samples were collected after 0, 1, 3, 7, 10, and 20 days, and after 1, 2, 4, and 6 months of fermentation. The collected samples were minced by blender and stored in the freezer at –20 °C until analysis. For the measurements, each minced sample was mixed vigorously in 10 mL of sterilized water. The mixture was centrifuged at 15,000 × g for 10 min at 4 °C, followed by the collection of supernatant for the analyses. The pH was measured using a LAQUA twin compact pH meter (Horiba Ltd.). The lactic acid content was measured using a D-/L-lactic acid enzymatic test kit (R-Biopharm AG, Darmstadt, Germany).

For the free amino acid measurements, fermentation was conducted from December 2015 to June 2016. After 0, 1, 2, 4, and 6 months of fermentation, the samples were collected and stored as described above. Next, 2 g of each minced sample was homogenized in 10% trichloroacetic acid, followed by centrifugation. The glutamic acid content in the supernatant was measured using a fully automated amino acid analyzer (JLC-500/V2; JEOL Ltd., Tokyo Japan).

Results and Discussion

Lactic acid production in the early phase of pa daek *fermentation*

As shown in Fig. 1, an increase in lactic acid was observed after 20 days of fermentation, reaching approximately 1% after 2 months, and maintaining the same level at after 6 months. The pH decreased from 6.3 to 5.4 as the fermentation proceeded. In accordance with the accumulation pattern of lactic acid, the ratio of the pH decrease between 10 days to 2 months of fermentation was more significant compared with an earlier phase of fermentation, suggesting that lactic acid

was a major factor in creating the acidic conditions in *pa daek*. In addition to the high salt concentration, such acidic conditions in *pa daek* products should be effective for preventing the potential growth of spoilage microorganisms that preferably grow in neutral or alkaline pH conditions.

Since the salt concentration in the samples was approximately 15% throughout the fermentation period, the lactic acid in the *pa daek* samples was thought to have been produced by halophilic lactic acid bacteria species such as *T. halophilus* and *T. muriaticus*. Lactic acid

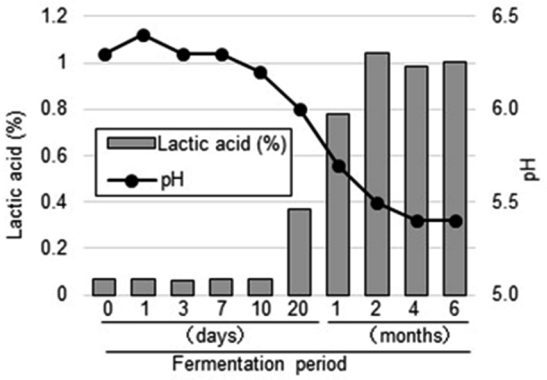

Fig. 1. Lactic acid concentration and pH levels in *pa daek* fermentation (Adapted from Marui, 2019).

concentration reached a plateau after 2 months of fermentation, presumably because of the pH sensitivity of the species. These species were reported to grow and produce lactic acid preferably in the growth media with initial pH values of 6.5 and 7.5, while the media with an initial pH value of 5.8 negatively affected the growth (Kobayashi *et al*. 2004). It is also important to note that the *Tetragenococcus* spp. are facultative anaerobic bacteria. The *T. halophilus* strain achieved a higher growth rate than aerobic cultures in oxygen-free anaerobic cultures (Gürtller *et al*. 1998). Taken together, in practical *pa daek* fermentation, it is deemed desirable to minimize the inclusion of air in the fermenting materials particularly for the first few months of the fermentation process to create and maintain anaerobic conditions favorable for the growth of halophilic lactic acid bacteria species. *Pa daek* producers can estimate the status of lactic acid fermentation in their products by monitoring pH with a measuring device or test paper.

Free amino acids in **pa daek** *fermentation*

The concentration of 20 free proteogenic amino acids in *pa daek* was analyzed until 6 months of fermentation (Table 1). The total amount of free amino acids increased in a time-dependent manner throughout the observation period. The amount in the sample fermented for 6 months (3,194 mg/100 g) reached 16.7 times that of the amount observed at the beginning of *pa daek* fermentation (191 mg/100 g). The free amino acids generated by long-term fermentation should be important factors to furnish *pa daek* products with the characteristic delicious taste and nutritional benefits. For instance, the *pa daek* prepared in the present study contained glutamic acid at the highest level in the amino acids examined (Table 1). Glutamic acid is a well-known natural taste element that makes a variety of foods palatable. It is commonly abundant in fermented Asian fish or soybean seasonings such as fish sauce/paste, soy sauce, and miso

(Yamaguchi *et al*., 2000). Dishes cooked with such kinds of glutamic acid-rich seasonings enhance appetite and go well with rice, which is a staple food in Asian countries. Lysine was detected at a comparable level to glutamic acid, also suggesting the usefulness of *pa daek* in the Laotian rice-based diet. Although lysine is an essential amino acid for humans, it is the first limiting amino acid in rice (STFCJ, 2015). The frequent use of *pa daek* in daily diets might partly complement the possible shortage of dietary lysine.

Table 1. Concentrations of free proteogenic amino acids in *pa daek* fermentation [adapted from Marui et al. (2018), Marui (2019)].

Amino acids	Fermentation period (months)				
(mg/100 g)	0	1	2	4	6
Glutamic acid	16	160	230	320	420
Lysine	24	180	250	330	410
Leucine	14	150	190	270	340
Alanine	18	100	140	200	260
Valine	9	85	120	180	230
Aspartic acid	5	53	80	130	190
Threonine	8	55	80	130	170
Isoleucine	6	55	80	130	170
Serine	9	58	81	120	160
Phenylalanine	7	53	72	120	150
Tyrosine	3	42	55	110	140
Methionine	4	49	65	95	120
Asparagine	4	44	59	81	100
Proline	7	31	43	69	95
Glycine	7	27	39	63	85
Histidine	26	45	56	70	82
Glutamine	7	58	65	47	34
Tryptophan	1	7	10	20	26
Arginine	16	130	21	7	12
Cysteine	ND	ND	ND	ND	ND
Total	191	1,382	1,736	2,492	3,194

ND: Not detected

In our preliminary analysis, glutamic acid and lysine were detected at relatively high levels among the free amino acids examined in homemade *pa daek* samples made from various fish species (data not shown). The free amino acids in *pa daek* are derived mostly from protein hydrolysis occurring in the fermenting fish; thus, the free amino acid profile of *pa daek* products

might vary depending on the fish species used for production. Amino acid profiling of the various freshwater fish species in Laos will be beneficial not only for understanding the nutritional benefits, but also for developing *pa daek* products that are rich in valuable free amino acids.

Further research should explore the detailed mechanisms of the fermentation time-dependent increase in free amino acids that enhances both the taste and nutritional benefits of *pa daek*. In general, endogenous proteolytic enzymes such as lysosomal cathepsins and proteases in fish muscle are considered to be involved in protein hydrolysis in fermented fish production. Such enzymes are released from fish muscle lysosomes and function preferably in an acidic environment (Mukundan *et al*. 1986); thus, the pH decrease attributed to halophilic lactic acid bacteria in *pa daek* fermentation might be one of the necessary factors to increase the free amino acid concentration by promoting fish protein hydrolysis. It would also be interesting to investigate the possible direct involvement of the metabolic systems of halophilic lactic acid bacteria and other species in the generation of beneficial amino acids in *pa daek* products.

Conclusion

The importance of long-term fermentation of *pa daek* for product quality has been empirically recognized by producers for a long time. The present study confirmed such ancient wisdom by revealing a fermentation period-dependent increase in lactic and free amino acid concentrations in *pa daek*. Such a scientific basis is useful for encouraging both homemade and commercial *pa daek* producers to manage the fermentation period carefully to enhance palatability and ensure lactic acid fermentation. Furthermore, information regarding free amino acids should have a positive impact on the promotion of *pa daek* consumption. It is also noteworthy that *pa daek* fermentation conducted using small clupeid freshwater fish (*pa keo*) as the material is useful for a laboratory model of *pa daek* production. *Pa keo* is now popularly used for commercial *pa daek* production in Laos. It is available all year round and easily processed into *pa daek* because of its small size. Further promotion of microbial and biochemical research on *pa daek* with this fermentation model is encouraged for *pa daek* product development, as well as for progress in fermented food science research and education in Laos.

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