

## Assessment of usage and fermentation-related components of *pa daek*, a salt-fermented freshwater fish paste, for household use in a semi-mountainous village in Laos

Junichiro Marui<sup>1</sup>, Katsumi Hasada<sup>1</sup>, Souphachay Phouphasouk<sup>2</sup>, Sayvisene Boulom<sup>2</sup>

<sup>1</sup> Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

<sup>2</sup> Faculty of Agriculture, National University of Laos, Vientiane, Laos

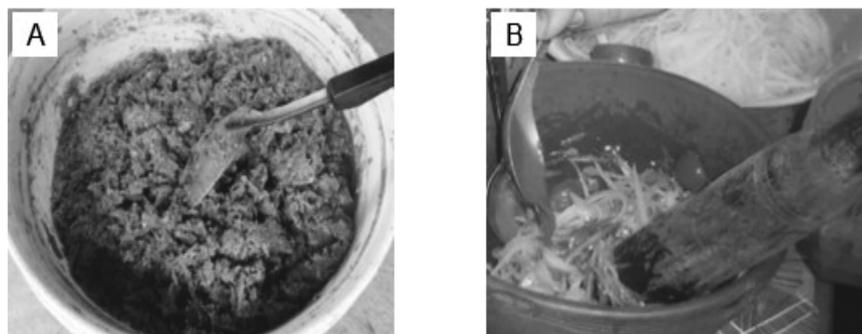
### Abstract

A salt-fermented freshwater fish paste, locally called *pa daek* in Laos, has been popularly used countrywide as an all-purpose seasoning as well as a protein-rich preservative food. Although commercial products are currently available in markets, the in-house production and consumption of *pa daek* utilizing various indigenous freshwater fish species from surrounding water environments such as rivers and paddy ponds continues today in rural households in the country. An interview survey of 137 households in a semi-mountainous rural village revealed frequent use of the *pa daek* in the daily diets. The concentrations of lactic and glutamic acids, which are closely related to the preservability and palatability of *pa daek*, varied widely among the homemade products examined, indicating the need for the widespread dissemination of knowledge and techniques of the *pa daek* production between rural households to manage the long-term fermentation and use of the product.

### Introduction

A salt-fermented freshwater fish paste, locally referred to as *pa daek* in Laos (Fig. 1A), has been popularly used countrywide as an all-purpose seasoning as well as a protein-rich preservative food. Similar types of fermented fish products have traditionally been produced and consumed in Southeast and East Asian countries. Although commercial products are currently available in markets, the in-house production and consumption of *pa daek* utilizing various indigenous freshwater fish species from surrounding water environments such as rivers and paddy ponds continues today in rural households in Laos. In the production of *pa daek*, well-washed fish is mixed with salt and rice bran. The mixed material is stuffed in a covered container followed by fermentation at an ambient temperature. According to producers, although *pa daek* is deemed to be edible after 2–3 months of fermentation, 6–12 months are required to enhance palatability. Fermented products are usually stored without refrigeration while being used and can last for a year or longer. Halophilic lactic acid bacteria species such as *Tetragenococcus halophilus* and *muriaticus* have been detected in *pa daek* products with salt concentrations of 15–20% (Marui et al. 2015). In our experimental *pa daek* production, lactic acid started to increase after 20 days of fermentation, reaching a plateau after 2 months to create acidic environment that should be advantageous for preventing the potential growth spoilage bacteria in the

highly salted products (Marui et al. 2019a, b). A fermentation period-dependent increase of a variety of free amino acids derived from fish protein hydrolysis to enhance the taste and nutritional benefits of the product is another distinct feature of *pa daek* fermentation (Marui et al. 2019a,b). To strengthen and utilize fully such benefits of *pa daek* for sustainable nutrition supply in rural areas, the present study aimed to clarify the current status of *pa daek* use in households, as well as its fermentation-related quality components such as lactic and glutamic acid.



**Fig. 1.** *Pa daek* made in a rural household in Laos (A), and Lao-style papaya salad seasoned with *pa daek* (B).

## Materials and methods

### *Usage survey of pa daek in a village and sample collection*

An interview survey of 137 households on the use of *pa daek* was conducted from August to October 2016 in a semi-mountainous rural village in Feuang District, Vientiane Province, Laos. The homemade *pa daek* products analyzed in the present study were collected from the same village in May 2017. Information about the age (months since the start of the fermentation) of each sample was provided by the producer.

### *Measurement of the fermentation-related quality components of homemade pa daek*

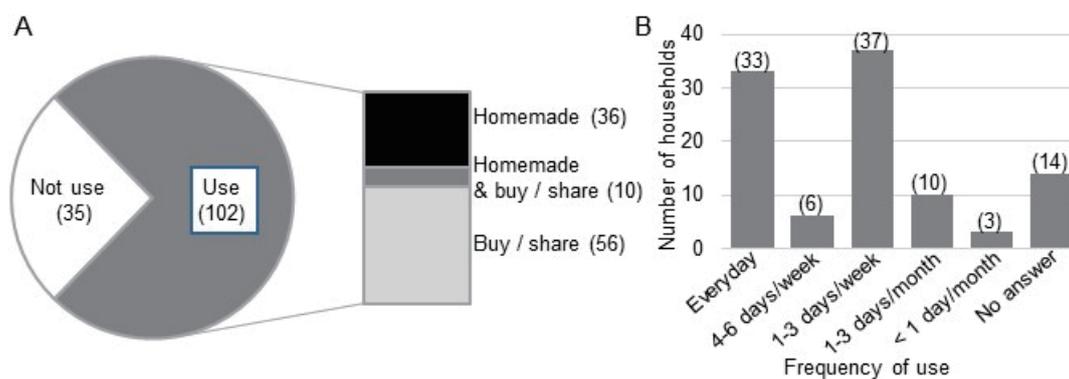
The collected *pa daek* samples were minced by a blender and stored in a freezer at  $-20\text{ }^{\circ}\text{C}$  until analysis. For the salt, pH, and lactic acid measurements, each minced sample was mixed vigorously in 10 mL of sterilized water. The mixture was then centrifuged at  $15,000 \times g$  for 10 min at  $4\text{ }^{\circ}\text{C}$ , followed by the collection of supernatant for the analyses. The salt concentration and pH values were measured using a LAQUA twin compact salt and pH meter (Horiba Ltd., Kyoto, Japan), respectively. Lactic acid content was measured using a D-/L-lactic acid enzymatic test kit (R-Biopharm AG, Darmstadt, Germany).

For the glutamic acid contents, 2 g of each minced sample was diluted to 50 mg/mL with 3% sulfosalicylic acid. The mixture was cooled on ice for 1 h, 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

### *Frequent use of pa daek in rural households*

The interview survey conducted in the present study revealed that *pa daek* was used by more than 70% of the households (Fig. 2A). Among the *pa daek* users, approximately 70% used it at least once a week (Fig. 2B). It is noteworthy that 30% of the households used it every day (Fig. 2B). *Pa daek* is used for seasoning a variety of Lao dishes, such as papaya salad (Fig. 1A), soups, and dipping sauces in daily diets (data not shown). The fish body in *pa daek* is also grilled or deep fried to eat (data not shown). These results suggest the importance of *pa daek* as a palatable seasoning to enhance appetite as well as a source of nutrients such as protein and amino acids. Despite such a high frequency of use, more than half of the users said they obtained the *pa daek* by purchasing or from relatives instead of in-house production. Although *pa daek* production methods have been passed down for generations among families and local communities, it may become increasingly difficult to maintain such important traditional food utilization techniques because of changes in living environments and lifestyles. Meanwhile, the respondents who did not report using *pa daek* mentioned not only a disliked flavor, but also experiences of physiological disorders, presumably caused by eating spoiled products, as unfavorable factors (data not shown). Analytical assessments of the fermentation-related components that determine both the palatability and preservability of *pa daek* are needed to clarify the current status of homemade products for developing a scientific approach to promote the production and use of *pa daek* further for the improvement of rural livelihoods.



**Fig. 2.** Schematic representation of the usage (A) and frequency (B) of *pa daek* in rural households. The quantity of responses to each option is presented in parentheses.

**Table 1.** Age, pH, salt, lactic and glutamic acid concentrations of homemade *pa daek* products.

Sample number	Age* <sup>1</sup> (months)	Salt (%)	pH* <sup>2</sup>	Lactic acid* <sup>3</sup> (%)	Glutamic acid (mg/100g)
1	8	11	6.2	1.56	115
2	8	11	6.2	1.06	Not detected
3	8	11	6.7	1.33	Not detected
4	13	20	5.9	0.48	616
5	2	11	5.0	1.81	609
6	2	18	5.3	0.72	183
7	8	6.6	4.9	1.81	921
8	2	11	6.0	1.60	815
9	2	15	5.0	2.08	623
10	5	25	5.6	0.08	275
11	13	15	5.6	1.33	689
12	12	12	7.5	Not detected	Not detected
13	13	22	5.4	0.40	316
14	6	10	6.8	0.70	1286
15	7	8.4	6.8	0.10	688
16	3	16	6.5	0.43	694
17	7	17	6.8	0.85	465
18	15	12	7.2	0.06	Not detected
19	13	14	6.6	0.30	626
20	14	15	6.0	0.57	515
21	4	15	5.8	0.96	404
22	2	15	6.1	0.11	442
23	13	13	5.5	0.73	673
24	5	4.4	6.6	0.22	Not detected

\*<sup>1</sup> Months since start of fermentation is presented

\*<sup>2</sup> pH values of sample extracts prepared as describe in the section of Materials and Methods are presented

\*<sup>3</sup> Total amount of D- and L-lactic acids is presented

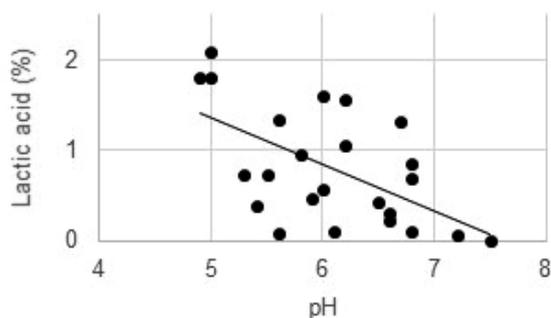
### ***Variability of fermentation-related quality components among homemade pa daek products***

In the present study, 24 *pa daek* products made and used in village households were collected to analyze the fermentation-related components (Table 1). The time since the start of fermentation ranged from 2 to 15 months, indicating the usefulness of the traditional *pa daek* fermentation technique for furnishing a seasonally available indigenous freshwater fish with long shelf life as well as palatability. Salt is thought to be an important factor to control microbial growth in *pa daek* products by lowering the water activity, even though the salt concentration in *pa daek* products varied widely from 4.4% to 25% (Table 1). The minimum inhibitory water activity values for the growth of microorganisms differ between species (Grant 2004). In our previous study, halophilic lactic acid bacteria species such as *Tetragenococci* spp. were commonly

detected in *pa daek* products with a salt concentration of not less than 11%, while lactobacilli were common in those with a salt concentration of less than 10% (Marui et al. 2015). Further studies are now in progress to investigate the preservability and microbial diversity of *pa daek* products with relatively low salt concentrations.

Lactic acid is a characteristic component of *pa daek*. It is made by halophilic lactic acid bacteria in the initial phase of fermentation, accompanied by a pH decrease in the fermenting materials (Marui et al. 2019a, b). The creation of acidic conditions in such products should be advantageous to secure preservability. Among the *pa daek* samples examined in the present study, a wide range of variation in lactic acid concentration was observed (average: 0.80%, standard deviation: 0.61%), as well as a negative correlation with the pH of the product (average: 6.1, standard deviation: 0.70) ( $r=-0.58$ ,  $p<0.01$ ,  $n=24$ ) (Fig. 3), indicating that lactic acid is an important factor to determine the product pH. It is necessary to determine the causes of the low lactic acid concentration and neutral pH observed in some products. Microbial profiling of the halophilic lactic acid bacteria and other species involved in the variation of lactic acid concentration and pH are required to secure the long shelf life of *pa daek*.

During *pa daek* fermentation, varieties of proteogenic amino acids are released in the product (Marui et al. 2019a, b). The free amino acids generated mainly by protein hydrolysis in fish meat during long-term fermentation emphasize the uniquely delicious taste as well as the nutritional benefits of *pa daek* products. Among the free amino acids, the present study focused on glutamic acid, which was found to exist at relatively high levels in *pa daek* products (Marui et al. 2019a, b). Glutamic acid is commonly abundant in fermented Asian fish or soybean seasonings such as fish sauce/paste, soy sauce, and miso, and is well known as a natural taste element that makes a variety of foods palatable (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. The glutamic acid concentration of the 24 *pa daek* samples examined in the present study varied from undetectably low levels to 1% (Table 1), with an average of 0.4% and standard deviation of approximately 0.3%. Although a fermentation period-dependent increase of glutamic acid was observed in our *pa daek* fermentation analysis (Marui et al. 2019a, b), no correlation was observed between the glutamic acid concentration and age (period since the start of fermentation) in the product examined in the present study (data not shown). Endogenous proteolytic enzymes such as lysosomal cathepsins and proteases in fish muscle are considered to be involved in protein hydrolysis, preferably in the acidic environment of the *pa daek* fermentation process, because such enzymes are released from fish muscle lysosomes and function preferably in an acidic environment (Mukundan et al. 1986). On the other hand, the non-enzymatic conversion of glutamic acid to insipid pyroglutamic acid was previously found in soy sauce fermentation and its associated storage period (Hori et al. 1956); this could also happen in long-term *pa daek* fermentation and daily household use. It is also interesting to consider the possible involvement of microbial metabolism in the increase and decrease of glutamic acid concentrations in *pa daek* products.



**Fig. 3.** Scatter diagram of the lactic acid concentration and pH of homemade *pa daek* products. The linear approximation is indicated.

## Conclusion

The present study revealed the frequent use of *pa daek* in the daily diets of rural households in Laos. The results emphasize the continued importance of traditional fermented freshwater fish products for fully utilizing indigenous fishery resources for sustainable nutrition supply in rural areas. The concentrations of lactic and glutamic acids, which are closely related to the palatability and preservability of *pa daek*, varied widely among the homemade products examined, indicating the need for the widespread dissemination of knowledge and techniques between rural households to manage the long-term fermentation and use of the product. Microbiological and biochemical research and education should facilitate further efforts to gain a better understanding of traditional fermentation techniques to achieve the stable production of *pa daek* with good quality.

## References

- Grant WD (2004) Life at low water activity. *Phil Trans R Soc Lond B* 359(1448):1249–1267
- Hori S, Ogawa T, Aoki R, Kondo Y, Ota Y (1956) Studies on the behavior of L-glutamic acid in shoyu. Part. I. Studies on the various types of L-glutamic acid and their formation mechanism in shoyu. *Nippon Nogeikagaku Kaishi* 30:519–523 (in Japanese)
- Marui J, Boulom S, Panthavee W, Momma M, Kusumoto K, Nakahara K, Saito M (2015) Culture-independent bacterial community analysis of the salty-fermented fish paste products of Thailand and Laos. *Biosci Microbiota Food Health* 34:45–52
- Marui J (2019a) Science of *pa daek*, a traditional fermented seasoning of Laos. *Kagaku* 89:824–829 (in Japanese)
- Marui J, Giavang Y, Phouphasouk S, Yiale Y, Boulom S (2019b) Fermentation period-dependent changes of lactic and amino acid concentrations in *pa daek*, a salt fermented freshwater fish paste in Laos. *JIRCAS Working Report 90*, Japan International Research Center for Agricultural Sciences, Tsukuba. pp. 95–100
- Mukundan MK, Antony PD, Nair MR (1986) A review on autolysis in Fish. *Fish Res* 4:259–269
- Yamaguchi S, Ninomiya K (2000) The use and utility of glutamates as flavoring agents in food. *J Nutr* 130:921S–926S