



Metadata and the Fatty Acids Content in Thai Shrimp Paste

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Received June 30th, 2022

Accepted July 7th, 2022

Published July 21st, 2022

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ABSTRACT

Introduction: The nutritional value of shrimp paste depends on the manufacturing process, such as the concentration of salt in the raw materials and the species of krill or small shrimp. In theory, different krill sources should have different bacterial species in communities. These are a major cause of the different chemical and physical properties of shrimp paste, especially the nutritional properties. This study focuses on microbial communities using the metagenome technique and the fatty acids content in the shrimp paste.

Objective: The metagenomic profiles of the bacteria and analysis of fat content in Thai shrimp paste.

Methods: Seven samples of fermented shrimp paste were obtained from various local markets and fishing villages and were analyzed for the fat content using an in-house method based on AOAC (2016). For analysis of the 16S rDNA metagenomic bacterial profile using Illumina sequencing.

Results: KK3 sample has the highest level of Saturated fatty acid and unsaturated fatty acid (Omega 3, Omega 6, Omega 9). A total of seven metagenomic samples were sequenced that report the dominant bacteria were *Bacillus*, *Lactobacillus*, *Staphylococcus*, and *Vibrio*.

Conclusion: This study can be the baseline information applied to develop the product of local or small enterprises.

Keywords: metagenomic profiles; shrimp paste; fatty acids

Introduction

Thai shrimp paste (kapi) is a fermented cuisine made primarily from salt, shrimp, or krill. Fermentation is a preservation method that uses a chemical transformation to turn raw materials into products via activities of an endogenous enzyme found in the raw material. The nutritional value of shrimp paste depends on the manufacturing process, for example, the concentration of salt and the species of krill or shrimp in the raw materials [1]. Proteins and lipids are hydrolyzed by natural microbial enzymes in krill. Microbes in krill survive in halophilic conditions, directly affecting total characteristics of shrimp paste [2].

This study is interested in three regions known for shrimp paste production in Thailand including the West coast of the upper Thai gulf, the East coast of the upper Thai gulf, and the lower Thai gulf. The raw materials for making shrimp paste on the West coast of the upper Thai gulf region using estuarine push net catching shrimp or krill from the deep-sea during December and used to preserve with salt in a ratio of about 10:1. For the East coast of the upper Thai gulf region using estuarine push net catching shrimp or krill in the estuary area and then put in the sun for drying raw material, which operates for preservation with salt in ratio about 8:2. Moreover, what is different is in the West coast of the upper Thai

gulf, shrimp or krill are caught using estuarine push net in the deep sea but on the East Coast of the upper Thai will use a estuarine push net to catch shrimp or krill around the estuary area. In addition, in the southern region, krill or shrimp are washed before being crushed. [3-5]

The shrimp paste contained a variety of fatty acids, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and Polyunsaturated fatty acids (PUFA) which are important sources for health benefits, especially PUFA fatty acid group (omega-3 and omega-6) that contribute significantly for human health associated with obesity, metabolic disease, insulin action, cardiovascular and immune functions [6]. Omega3 fatty offers beneficial effects on depression, rheumatoid arthritis, and asthma [7]. Additionally, alpha-linoleic acid (ALA) which is one of the omega-3 appears to benefit the heart, immune system, and nervous system [8]

According to Ali and colleagues (2020), shrimp paste contains essential amino acids more than non-essential amino acids and the fermentation period affects the concentration of amino acids and PUFA fatty acid group. The study found longer fermentation of shrimp paste resulted in a 6-week increase in essential oil production, almost equivalent to raw material (*Acetes* sp.), and an increase in PUFA production more than raw material [9]. Therefore, the food preservation process by fermenting shrimp paste has the potential to develop a healthy seasoning.

In theory, different krill sources should have bacterial communities containing varied species. These differences are a major cause of the different chemical and physical properties of shrimp paste, especially the nutritional properties. The growth of microorganisms depends on the raw materials used in the fermenting process [10]. The main raw material in shrimp paste was divided into two genera: *Acetes* and *Mesopodopsis* [11]. *Acetes* sp. is a genus of small shrimp with red tail, length 10-40 mm. Habitat in brackish and sometimes shallow coastal waters. Usually moved through the middle of the water or near the water surface, which was found in mangrove forests and seagrass, can be found from the surface of the water to a depth of about 20 meters. [12] *Mesopodopsis* sp. has general form long and slender and back eyes, length 10-30 mm. Habitat in coastal and brackish water areas. During the day, it will be in seabed and will float to the surface during the night [13]

The diverse types of shrimp paste will provide different nutrients because it may be the effect of the quantities of krill and the process of making shrimp paste differentially in each locality. However, there are enormous local shrimp pastes, for which the nutritional values and quality control processes have not been reported.

The metagenomic data from shrimp paste in Southeast Asia still lack data to support details of

spontaneous fermentation. Therefore, this study is interested in identifying the bacterial communities from local fisherman's villages in Thailand, using metagenomic next-generation sequencing, which is any of several high-throughput sequencing methods that can be used to directly identify the bacterial community from the sample without culturing [14].

Furthermore, to support the idea that "the different types of shrimp paste will provide different nutrients," the fatty acids content (omega-3, omega-6, and omega -9) of the samples where the types of shrimp used were identified in the previous study were measured.

Methodology

Fermented shrimp paste sampling

In three regions of the Thai gulf, Thailand (Figure1), seven samples of fermented shrimp paste were obtained from various local markets and fishing villages. The sampling areas were located and marked by GPS coordinates (Table 1) for further reference. Fermented shrimp paste samples were kept at 4°C in a refrigerator and metagenomic DNA was extracted within one month after sample collection. Totally seven fermented shrimp pastes were successfully collected and marked as KK1, KK2, KK3, SR1, SR2, SR3, and TP1.



Figure 1 The sampling areas in three regions of the Thai gulf, Thailand

Analysis of PUFAs and nutritional values in Thai fermented shrimp paste

Fermented shrimp paste samples were analyzed for the fat content including saturated fatty acids (SFAs): Polyunsaturated fatty acids (PUFAs) were measured using an in-house method based on AOAC (2016) 2003.05.

Metagenomic DNA extraction

DNA was extracted from shrimp paste using NucleoSpin® Soil kits (Macherey-Nagel, Düren, Germany). In the lysis process, lysis SL2 buffer and enhancer SX were used to increase DNA extraction efficiency. The concentration and purity of metagenomics DNA were measured by a 1.0% agarose gel electrophoresis and 260/280 nm absorbance using an Epoch microplate spectrophotometer (Bio-Tek). DNA samples with an A260/280 not less than 1.8 were collected at –20°C.

Table 1 Fermented shrimp paste sampling area

Shrimp paste samples	krill tissue (Previous study)	sampling area	GPS location
KK1	<i>Mesopodopsis orientalis</i>	Samut Songkhram	13°25'33.1"N, 99°58'41.7"E
KK2	<i>Acetes japonicus</i>	Samut Songkhram	13°25'28.1"N, 99°58'40.6"E
KK3	<i>A.japonicus</i>	Samut Songkhram	13°25'28.1"N, 99°58'40.6"E
SR1	<i>A.japonicus</i>	Chonburi	13°09'53.6"N, 100°55'50.2"E
SR2	<i>A.adonics</i>	Chonburi	13°09'53.6"N, 100°55'50.2"E
SR3	<i>A.japonicus</i>	Chonburi	13°09'53.6"N, 100°55'50.2"E
TP1	<i>A.japonicas</i>	Songkhla	6°52'12.4"N, 100°58'08.5"E

DNA analysis

Total metagenome DNA extracted from shrimp paste was sent to be analyzed for metagenome bacterial profile for the 16S rDNA V3-V4 region amplicon analysis [15] using Illumina sequencing (OMICS center, Chulalongkorn University)

Results

In addition, our previous research has identified krill species in Thai shrimp pastes (Table1), it was found that KK1 was reported as *M. orientalis* whereas KK2, KK3, SR1, SR2, SR3, and TP1 were reported as *A. japonicas*. The sample was collected from different areas of the Thai gulf but most of the shrimp found were identified as *A. japonicas*, which was inferred to be the habitat of this krill species in the Thai gulf.

Analysis of fatty acids in Thai fermented shrimp paste

The highest fat level was found in the KK3 sample from the province of Samut Songkhram, with a range of 1.35 to 3.17 g of fat per 100 g of sample. The percentage of saturated fatty acids ranged from 67.17 to 86.43 percent, with the highest concentration found in the KK1 sample and the percentage of unsaturated fatty acids ranged from 13.57 to 32.83 percent, with the highest concentration found in the KK2 sample from Samut Songkhram province (data not shown). On the other hand, when calculating the amount per 100 grams of shrimp paste, it was found that the KK3 sample has the highest level of Saturated fatty acid and unsaturated fatty acid (Omega 3, Omega 6, Omega 9) shown in Table 2.

Metagenomic analysis

A total of seven metagenomic samples were sequenced using next-generation sequencing (MiSeq; Illumina, San Diego, CA, USA). The dominant bacteria were *Bacillus*, *Lactobacillus*, *Staphylococcus*, and *Vibrio*. Furthermore, *Exiguobacterium* is also a dominant bacterium but was reported only in SR1 and SR2. The diversity of the bacterial community is shown in figure 2.

From the previous study, it was known that only KK1 from Samut Songkhram province used *M. orientalis* as the main raw material but the diversity of the bacterial community is shown that has the same pattern with KK2, KK3, SR1, SR2, SR3, and TP1.

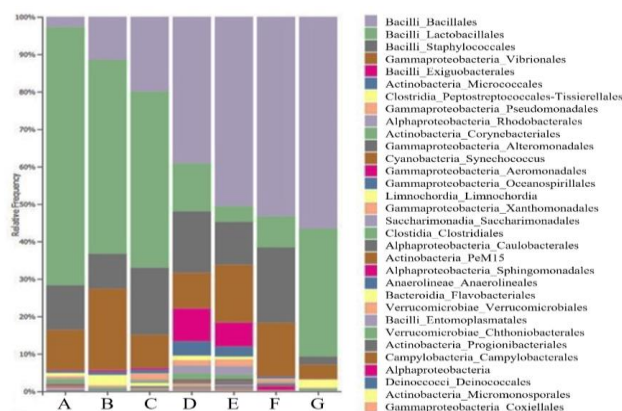


Figure 2 Metagenomic profile of Fermented shrimp paste from shrimp paste samples (A)KK1, (B)SR3, (C)KK3, (D)SR2, (E)SR1, (F)TP1, and (G)KK2.

Table 2 Fatty acids in Thai shrimp paste samples

Shrimp paste samples	Fat content (g/100g)	Saturated fatty acid (g/100g)	Unsaturated fatty acid (g/100g)	Omega 3			Omega 6		Omega 9
				ALA (g/100g)	EPA (g/100g)	DHA (g/100g)	LA (g/100g)	AA (g/100g)	OA (g/100g)
KK1	1.74	0.24	1.50	0.00	0.06	0.03	0.04	0.00	0.06
KK2	1.35	0.44	0.91	0.00	0.10	0.10	0.06	0.00	0.10
KK3	3.17	0.65	2.52	0.00	0.25	0.16	0.06	0.00	0.25
SR1	1.76	0.46	1.30	0.00	0.12	0.13	0.04	0.00	0.12
SR2	1.60	0.46	1.14	0.00	0.12	0.13	0.03	0.00	0.12
SR3	1.40	0.30	1.10	0.00	0.08	0.05	0.03	0.00	0.08
TP1	1.88	0.29	1.59	0.01	0.02	0.01	0.03	0.01	0.02

Discussion

Although the KK1, KK2, and KK3 were collected close to each other in the Samut Songkhram province, KK1 is used with different krill species from KK2 and KK3, but the diversity of the bacterial community showed the same pattern of microbial compositions. SR1, SR2, and SR3 samples are used *A. japonicus* that has the same raw material, only SR1 and SR2 have bacteria in phylum *Exiguobacterium*. These results suggest that the distinct species of krill will not directly provide to different microorganisms in shrimp paste, which may depend on the salt concentration used, length of fermentation, source of raw materials (shrimp habitat), and contamination in the process.

Omega 3 includes DHA and EPA fatty acids, an essential fatty acid that is recommended daily intake is 250-500 mg [16], The recommended daily intake of DHA in the human brain is 2.4–3.8 mg per day [17]. For Thai recommended daily intakes-Thai RDI suggests a daily intake of total fat is 65 g and recommended intakes for polyunsaturated fatty acids including the omega-3 and omega-6 range from 6-11 percent of your total daily energy intake (EPA and DHA combined 250-2000 mg per day), percent daily values are based on a 2000 calorie diet [18]. The shrimp paste is a useful source of DHA and essential amino acids. According to studies, a healthy ratio of omega-6 to omega-3 fatty acids appears to be between 1:1 to 4:1 [19].

Conclusion

This study is a novel investigation of the amount of omega-3, omega-6, and omega-9 in local Thai shrimp paste in comparative analysis. Furthermore, an analysis of the fatty acids in shrimp pastes also revealed that Thai shrimp paste is nutritious and healthy for healthy adults. Thai recommended daily intake of EPA and DHA is 250-2000 mg per day. From the analysis, it was found that most of the shrimp paste samples with 100 g of

essential fatty acids were sufficient to the recommended daily requirements. But in shrimp paste, there is also salt and calories that should be careful so this study provides fundamental data that can be used for the nutritional fact label and the precaution label. Moreover, reduction of salt in fermentation should be applied to produce healthier products. Therefore, this study reveals baseline information that can be applied to develop the product of local or small enterprises for further analysis to produce low-salt shrimp paste.

Acknowledgments

The authors appreciate Ratchadapisek Somphot Research Funding CU_GR_62_45_23_18 from Chulalongkorn University. Special thanks to Mr. Moohummad Roze Imamhem from Itisam Wittaya foundation school for local fermented shrimp paste samples and processes from Songkhla province, Thailand. We acknowledge Assistant Professor Kornrawee Aiemsomboon, Ph. D. from the Marine Science Department of Chulalongkorn University for kindly krill species investigation. And I appreciate the program in biotechnology, faculty of science, Chulalongkorn University.

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