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# Effect of Amino Acids and Taste Components on Fermented Fish Sauce (Budu) from Thailand

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## Abstract

Budu is one of the most popular fermented fish products in Thailand's southern area due to its distinctive flavor. It is being manufactured in large quantities for usage in cuisine as seasonings and sauces. The objective of this study was to determine the effect of amino acids on the distinctive taste components of Budu in southern Thailand. The amino acids in Budu were determined using GC-MS after fish was fermented for 6–12 months as recommended by the manufacturer. Lysine, glutamic acid, and aspartic acid are the three most abundant amino acids, with 1600, 1,540, and 1,260 mg/100g, respectively. Additionally, it was revealed that the umami taste was formed by a group of amino acids (glutamic acid and aspartic acid) followed by sweetness and bitterness. Sensory analysis discovered salty tastes, followed by umami, sour, sweet, and bitter. Four Budu samples generate a salty and umami flavor. Salt is mixed with cleaned fresh fish and fermented to enable native enzymes to auto-digest the protein and produce amino acid-rich products. Fish enzymatic fermentation produces short chain peptides and amino acids that contribute to the umami flavor and taste. Additionally, the fermentation process creates a high glutamic acid concentration, as well as other amino acids and nucleotides that add to the umami flavor of the products. The study findings will be information that is particularly benefit to consumer and manufacturers to promote Budu products in the country's region.

**Keywords**: *Budu, Taste, Amino acid, Southern, Thailand*



## INTRODUCTION

In Thailand's southern border cuisine, Budu is a fish sauce that is often used as a flavoring component and has a high salt content due to the fermentation process (Sukjuntra, 2018). The production of Budu contains small saltwater fish, mainly anchovies, which are combined with salt at 25–30% (w/w) and then spontaneously fermented under anaerobic conditions for 6–12 months. This food has a salty and unique flavor and is brown or dark brown in color (Mohamed et al., 2012). The manufacturing procedures utilized to create these items vary by nation, since each culture has its own traditional processes. (Mohamed & Mustafa, 2021). As a result of the fermenting process, Budu develops a distinct flavor. Hydrolysis of fish proteins is initiated by enzymes found in fish and

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certain halotolerant and halophile bacteria, which subsequently create amino acids, peptides, and ammonia, making it an excellent source of protein (Lee et al., 2016; Wu et al., 2017). Moreover, the high salt content of Budu sauces is able to inhibit the spread of harmful microorganisms, resulting in their signature salty umami and aroma (Mohamed et al., 2012). Free fatty acids are released during the process, which is called lipolysis, and play a critical role in determining taste qualities (Chen et al., 2017). In Thailand, the fermentation of Budu was considered an indigenous method because it required minimal practical guidance on a small industrial scale, but it required a lengthy fermentation time to guarantee that the fish combination was completely solubilized. Furthermore, no control measures are used during this spontaneous process, which is often linked with inconsistent product quality and poor production yield. Moreover, the unique taste of Budu depends on the type of fish, the ratio of fish to salt, including fermentation time and temperature, as well as specific techniques in each production site (Sukjuntra, 2018; Tsai et al., 2006). Relationships between the amino acid composition and taste analysis for determining basic flavors in each location of the Budu process are important for the quality improvement of Budu production. Therefore, the aim of this study is to identify the amino acid compounds that contribute to the special tastes in four locations of the Budu factory by using gas chromatography-mass spectrometry (GC-MS). These findings may be used to make predictions on the taste that will increase the quality of Budu in Thailand.

## LITERATURE REVIEW

Salting is an easy and economical technique of food preservation that avoids spoiling and allows for fermentation of food products. In Southeast Asia, the process of salting fish is often employed to create fermented fish seasonings. Among a variety of fermented products, fish sauces are the most popular seasonings containing umami components and are normally required in this region's cuisine. Nuoc mam in Vietnam, Tuk trey in Cambodia, Patis in the Philippines, Bakasang in Indonesia, Ngan-pya-ye in Burma, Budu in Malaysia and Nam pla in Thailand are some of the names for the sauce used in the region (Yoshida, 1998). In Thailand, Nam pla is the fish sauce and is used in practically all Thai cuisine. Pla ra and Budu is a traditional fermented fish food common in northeastern and southern regions, respectively (Saisithi et al., 1966). Budu is a colloidal substance that stands between fish sauce and fermented fish paste, while prala is fermented fish paste that contains rice bran as a carbohydrate source. The main ingredients of Nam pla and Budu are the same as seawater fish (*Stolephorus* sp.), whereas Pla ra is freshwater fish (*Trichogaster* sp*., Crossocheilus* sp.). The traditional process of producing fish sauce varies by location. The ratio of salt to fish, the fermentation temperature, the kind of fish, and minor components all have a significant impact on the composition and nutritional quality of fish sauce (Lopetcharat et al., 2001). In fermentation stage, hydrolysis breaks down fish proteins into peptides and amino acids. Small peptides, amino acids, ammonia, and trimethylamine contribute to fish sauce's unique scent and taste (Dougan & Howard, 1975). Budu is a high-protein food that provides a variety of necessary amino acids (Mohamed et al., 2012).



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## RESEARCH METHODOLOGY

### Sample collection

Four Budu samples were purchased and collected from prevalent sites in three southern provinces, namely Yala, Pattani, and Narathiwat. All samples were kept in a chiller (6 °C) until they were needed for analysis. Information about the production process was collected by interviewing the manufacturer.

### Sample preparation

Budu puree (cloudy) and Budu sauce (clear) were mixed thoroughly and 200 ml of methanol was added. Mixed samples were stirred by a magnetic stirrer for 45 min and then centrifuged at 8,000 rpm for 15 min (Refrigerated Centrifuge 5920R, Eppendorf, Germany). The supernatant was filtered, and the sediment was re-extracted until the color of the Budu water became lighter. Extracted samples were then filtered to make it concentrated by using rotary evaporator (Rota vapor R-3, Buchi, Flawil, Switzerland). The extracts were weighed and freeze dried (LABCONCO/FreeZone 2.5, Missouri, U.S.A.) to give the extracts of Budu powder.

### Analysis of amino acid

From Budu powder samples (0.4 g) obtained from freeze drying, 2 g of liquid sample were put into a 100 ml extraction bottle. Then, the sample was added to 10 ml of 6 M HCl and mixed, and the bottle was tightly closed (for Typtophan, add 10 mL of 4.2M NaOH). The samples in the bottle were autoclaved at 121–123 $^{O}$C for 3 hrs and then diluted with 2M NaOH in a 20 ml volumetric flask. The sample was passed through filter paper no. 42 by a syringe filter with a 0.45 µm and performed derivatization with BSTFA with 1%TMCS. The sample was performed on a GC/MS (7000D Triple Quad, Agilent, Santa Clara, CA, USA) using helium as the carrier gas. The column used was a HP-5MS 5% phenyl, 95% dimethyl-polysiloxane (30 m, 0.25 mm, 0.25 m). The injector with a split ratio of 3:1, a split flow of 3 ml/min, and a 2 µl. The initial temperature for the column temperature program was set at 15 ºC/min from 100 ºC to 300 ºC, and the MS Temp was set at 230 °C (Source) and 150 °C.

### Sensory analysis

The panelists were trained and followed the process (Meyer et al., 2016; Scharbert & Hofmann, 2005). There are four males and eight females, aged 25–40 years, who have been trained as tasters to be proficient in taste. The scoring test is one method for training gourmets. The sensory test allows the tester to taste the freeze-dried Budu sample by dissolving the sample in water (Sprinkle, Thailand). The panelists were asked to rate the taste profile on a five-point scale for sweetness, saltiness, sourness, bitterness, umami. Four Budu samples (A, B, C and D) were presented to each panelist in random order.



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## FINDINGS AND DISCUSSION

Production survey

The Budu production process was found in the households and small industries of southern Thailand (Jariya Sukjuntra, 2009). The research team selected and collected samples from four different sources (3 districts of 2 provinces): Narathiwat Province (sample A), Pattani Province (sample B, C and D) as shown in Fig. 1. Budu is made from anchovies, which may be combined with tiny quantities of roundbelly sardine. The fish were washed, marinated with salt in different ratios (fish and salt; 3:1 and 4:1) and fermented in a closed container for a fermentation period of 6–12 months. After Budu fermentation is complete, it is separated and packaged into three kinds of products: Budu sauce (clear sauce), Budu puree (cloudy sauce), and mixed Budu (clear-cloudy; 50:50). This product is turbid because to the presence of heavy sediments and appears darker than Nam pla (Chotechuang, 2013). The production process was shown in Fig.2. Numerous ingredients contribute to the particular taste of Budu, the bulk of which are small fish based on anchovies. Other species, such as roundbelly sardines, are sometimes fermented concurrently (Beddows, 1998). Other factors affecting production are the ratio of fish to salt. with a ratio of 3:1 (B and C) and 4:1 (A and D) while the containers used for fermentation are both glazed water jars and a large plastic bucket. Table 1 contains information about Budu's four manufacturing locations and manufacturing processes in Southern Thailand. Due to the cultural and dietary differences amongst Thai consumers, Budu processing is significantly distinct from Nam pla preparation. Nam pla manufacture begins with washing of fresh fish to eliminate contaminants and limit of microorganisms in the raw materials (Setyahadi, 2014). Generally, cleaned fish is combined with salt in a 2:1 or 3:1 ratio (w/w). The salt-mixed fish is then transferred to a fermentation tank covered with a bamboo mat for fermentation period 12-18 months (Lopetcharat et al., 2001). Nam pla has a distinct rate of fermentation and end product synthesis than Budu (Saisithi et al., 1966).

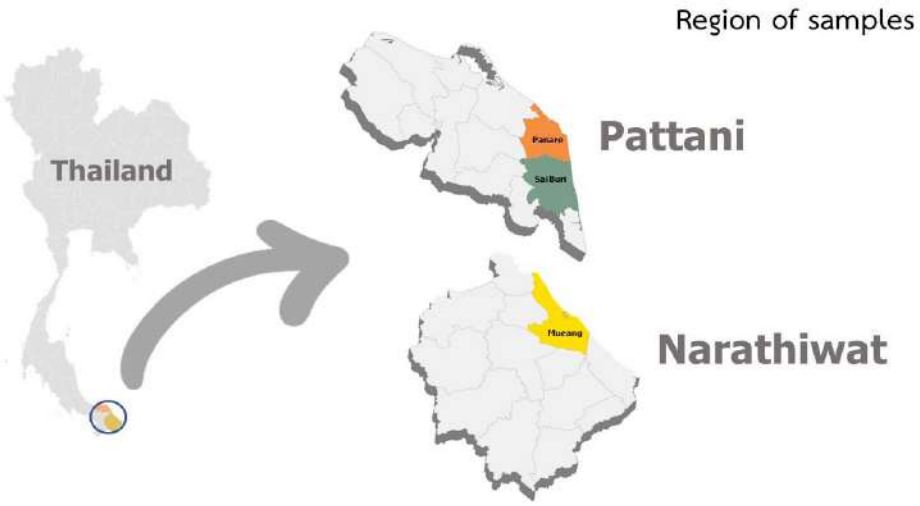


**Fig. 1** A survey of data sources and collecting Budu samples in 4 different sources (3 districts of 2 provinces)



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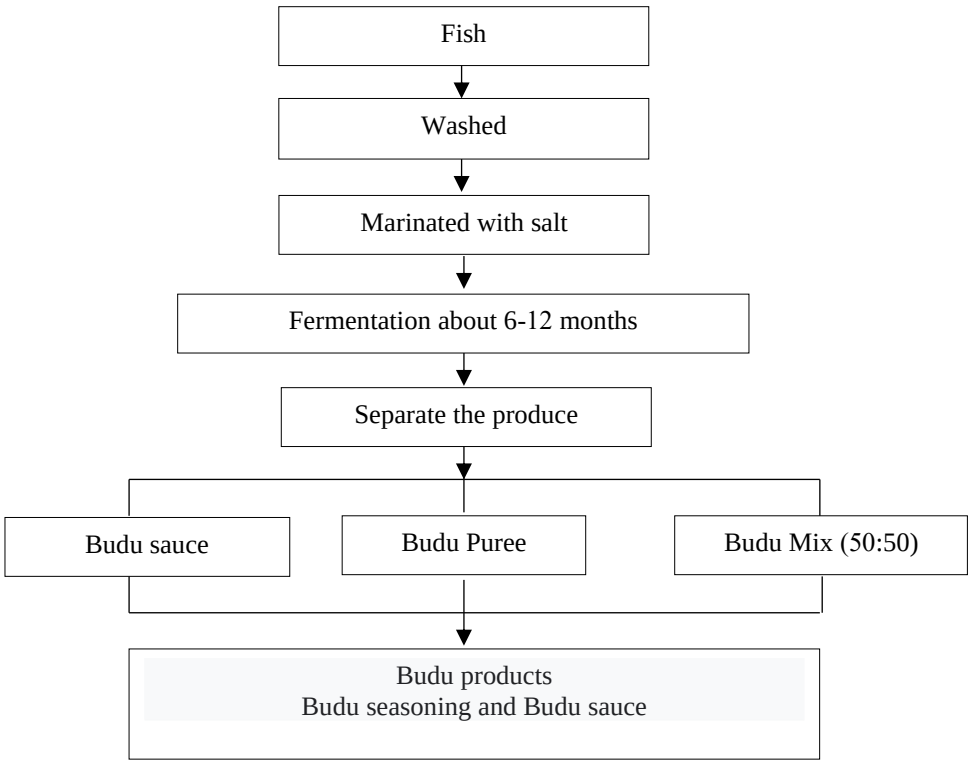


**Fig. 2** Budu production process from the southern border provinces of Thailand



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**Table 1** The information of 4 production sites and production processes of Budu in Southern Thailand

| **Brand code** | **A** | **B** | **C** | **D** |
|---|---|---|---|---|
| **District** | Muang | Saiburi | Panare | Saiburi |
| **Province** | Narathiwat Province | Pattani Province | Pattani Province | Pattani Province |
| **Type of establishment** | SMEs | Factory | SMEs | Factory |
| **Kind of fish** | Anchovies/ Roundbelly sardine | Anchovies/ Roundbelly sardine | Anchovies/ Roundbelly sardine | Anchovies/ Roundbelly sardine |
| **Kind of salt** | Table salt (Pattani) | Table salt (Bangkok) | Table salt (Phetchaburi) | Table salt (Phetchaburi) |
| **Ratio (Fish: Salt)** | 4:1 | 3:1 | 3:1 | 4:1 |
| **Fermentation duration** | 6 months | 12 months | 12 months | 12 months |
| **Fermentation container** | Glazed water jar with dragon patterns (1 meter deep) | Large plastic bucket (500 liters) | Glazed water jar with dragon patterns (1 meter deep) | Glazed water jar with dragon patterns (1 meter deep) or Cement Pond (3 meters deep) |

**Relationship between amino acid and taste**

The results of quantitative testing of amino acids in four Budu samples were found to indicate that the samples with the highest amino acid content were sample B (12,796.9 mg/100g) in Saiburi district, Pattani province. Lysine, glutamic acid, and aspartic acid are the three most abundant amino acids in Budu, with levels of 1,600, 1,540, and 1,260 mg/100g, respectively (Table 2). They account for about 35% of the amino acid content. These findings supported the amino acid profiles of fish sauce previously published (Park et al., 2001). Typically, Budu is used in cuisine as a seasoning. Importantly, Budu is strong in lysine and contains all necessary amino acids. By means of grouping the flavored amino acids and calculating the values of Dose-over-Threshold (DoT), it was found that the group of amino acids that give umami flavor is L-glutamic acid in the range of 77.25–95.17. The most common sweet and bitter amino acids are L-alanine and L-leucine (range of 6.15-7.66 and 4.29-6.36, respectively) (Table 3). Similar to Nam pla, glutamic acid is the most prevalent amino acid in Budu. These findings imply that the flavor active components, like in Nam pla, are related to the umami taste compound. In Southeast Asia, the most popular umami-containing condiments are fish sauces. Fish and salt are the two main components in fish sauce, although the proportions vary by country (Lopetcharat & Park, 2002). The degraded products, amino acids, nucleotides, and salt give a distinctive umami flavor and aroma (Otsuka, 1998).



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**Table 2** The amount of amino acid in the Budu samples A, B, C and D, respectively

| Analyte | Result(mg/100g) | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Aspartic Acid | 970.0 | 1,260.0 | 972.0 | 1,060.0 |
| Glutamic Acid | 1,350.0 | 1,540.0 | 1,250.0 | 1,310.0 |
| Glycine | 614.0 | 704.0 | 612.0 | 661.0 |
| Histidine | 270.0 | 362.0 | 219.0 | 370.0 |
| Isoleucine | 423.0 | 555.0 | 414.0 | 384.0 |
| L-Alanine | 688.0 | 819.0 | 658.0 | 663.0 |
| L-Arginine | 650.0 | 960.0 | 593.0 | 588.0 |
| Leucine | 733.0 | 918.0 | 714.0 | 619.0 |
| Lysine | 1,450.0 | 1,600.0 | 1,320.0 | 1,490.0 |
| Methionine | 293.0 | 381.0 | 288.0 | 264.0 |
| Phenylalanine | 406.0 | 533.0 | 256.0 | 377.0 |
| Proline | 401.0 | 650.0 | 358.0 | 440.0 |
| Serine | 312.0 | 584.0 | 376.0 | 447.0 |
| Threonine | 619.0 | 765.0 | 533.0 | 600.0 |
| Tryptophan | 79.7 | 86.9 | 89.5 | 86.3 |
| Tyrosine | 192.0 | 323.0 | 204.0 | 210.0 |
| Valine | 599.0 | 756.0 | 576.0 | 586.0 |
| Total amino acid | 10,049.7 | 12,796.9 | 9,432.5 | 10,155.3 |



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**Table 3** Taste Qualities, Taste Thresholds (TC), Concentrations in Budu, and Dose-over-Threshold (DoT) Factors of Taste-Active Compounds in Budu sample A, B, C and D, respectively

| Taste compound | TC (µmol/kg) | Dose-over-threshold (DoT) in | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| **Group I: Sweet-Tasting Compounds** | | | | | |
| Glycine | 25000 | 3.27 | 3.75 | 3.26 | 3.52 |
| L-Alanine | 12000 | 6.43 | 7.66 | 6.15 | 6.20 |
| L-Methionine | 5000 | 3.93 | 5.11 | 3.86 | 3.54 |
| L-Proline | 25000 | 1.39 | 2.26 | 1.24 | 1.53 |
| L-Serine | 25000 | 1.19 | 2.22 | 1.43 | 1.70 |
| L-Threonine | 35000 | 1.48 | 1.84 | 1.28 | 1.44 |
| **Group II: Umami-Tasting Compounds** | | | | | |
| L-Aspartic Acid | 4000 | 18.22 | 23.66 | 18.26 | 19.91 |
| L-Glutamic Acid | 1100 | 83.43 | 95.17 | 77.25 | 80.96 |
| **Group III: Bitter-Tasting Compounds** | | | | | |
| L-Histidine | 45000 | 0.39 | 0.52 | 0.31 | 0.53 |
| L-Isoleucine | 10000 | 3.22 | 4.23 | 3.16 | 2.93 |
| L-Arginine | 75000 | 0.50 | 0.73 | 0.45 | 0.45 |
| L-Leucine | 11000 | 5.08 | 6.36 | 4.95 | 4.29 |
| L-Lysine | 80000 | 1.24 | 1.37 | 1.13 | 1.27 |
| L-Phenylalanine | 45000 | 0.55 | 0.72 | 0.34 | 0.51 |
| Tryptophan | 4000 | 0.98 | 1.06 | 1.10 | 1.06 |
| Tyrosine | 4000 | 2.65 | 4.46 | 2.81 | 2.90 |
| L-Valine | 30000 | 1.71 | 2.15 | 1.64 | 1.67 |
| **Total** | | **135.66** | **163.27** | **128.62** | **134.41** |



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Note: If the value is greater than 1, it means that it has contributed or attributed that flavor.

Sensory analysis

The sensory profiles of materials determined by twelve panelists using the quantitative descriptive analysis (QDA) test have five characteristics presented in Fig. 3. The fermentation raised the salty, umami, sour, sweet, and bitter scores. The salty score was the highest of all samples, followed by umami, sour, sweet, and bitter, respectively. Sample D presented the saltiest taste (2.56 score) and sample B presented the most umami taste (2.37 score). The sweet, sour, and bitter tastes showed the highest scores in sample C. Each Budu presented a different taste and number of amino acids depending on the production factors.

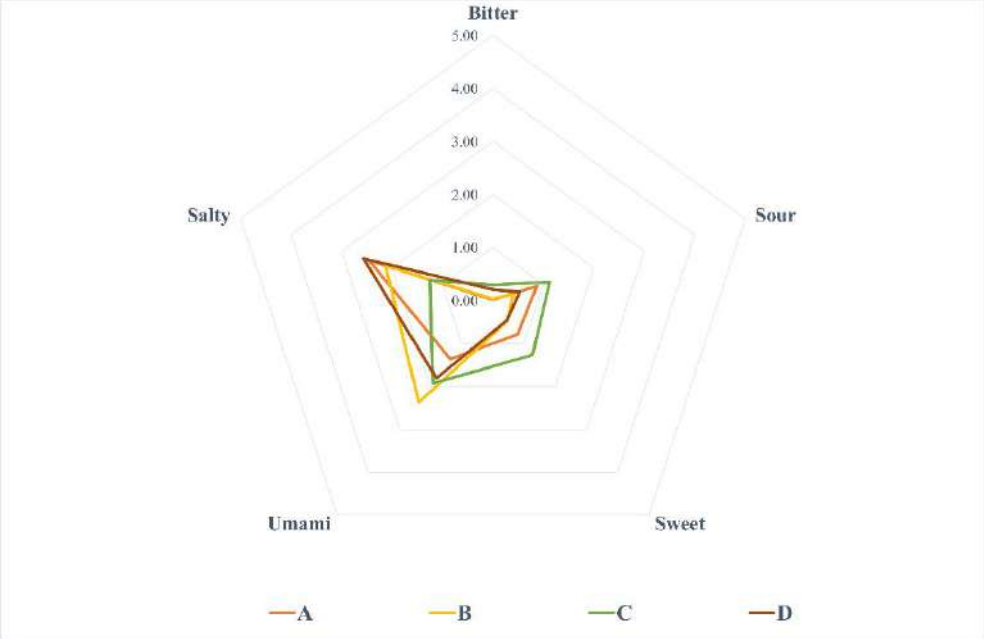


**Fig. 3** Sensory analysis

## CONCLUSION

This study, Budu is made in the southern part of Thailand produced high amount of glutamic acid depending on the treatment of raw materials prior to fermentationGlutamic acid and its salts are very important in flavor of fish sauce. Budu has a high concentration of essential amino acids, including lysine. This data suggests that Budu may be a beneficial source of protein. There are also other factors that affect the taste, such as microorganisms in the fermentation process and other compounds. This research needs more study to contribute to the development of the business by increasing the value of Budu products so they can be exported to the global market.



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