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Thua nao: Thai fermented soybean

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ABSTRACT

Thua nao is a traditionally fermented food in Thailand. It is manufactured by fermenting cooked soybeans with naturally occurring microbes. There are also similar products including *natto* in Japan, *kinema* in India, and *chongkukjang* in Korea. In Thailand, *thua nao* is widely consumed, especially by people in the northern part. The product is generally regarded as a protein supplement and widely used as a condiment. Two major types of *thua nao* can be distinguished; fresh and dried forms. To date, scientific information on *thua nao* is scarce and thus this article aims to document the updated knowledge of Thai *thua nao*.

1. Introduction

Soybean [*Glycine max* (L.) Merrill], belonging to the family Leguminosae, can be processed into various products. Fermentation of soybean is one of the techniques resulting in novel foods (i.e., fermented soybeans) with unique features. Other advantages include an extension of the product's shelf-life. Fermented soybean is traditional and popularly consumed in many parts of the world, especially in Asia [1–3]. In general, there are only three major raw materials (soybeans, water, and naturally occurring microbes). The fermentation processes are also very similar. However, the final products are various and, presently exist in different forms and names. One of the best known examples is Japanese *natto* [4]. Other similar products include Korean *chongkukjang* [2], Indian *kinema* [3], and Thai *thua nao* [5]. For these products, *Bacillus* species are predominant and expected to be responsible for the fermentation in which their proteases help accelerate the hydrolysis of protein, thus releasing ammonia [1]. Such a mechanism provides an alkaline condition. These fermented soybean products are therefore often termed “alkaline fermented soybeans” [1].

Thua nao, classified as an alkaline fermented food, is prepared using soybean (Fig. 1). Alkaline fermentation is a technical term used to define the process in fermentation of protein-rich foods leading to an alkaline condition [1]. Alkaline fermented foods are generally regarded as safe and can be found as traditional diets in most parts of the world, especially in Asian and African countries. Considered from plant-based products, different raw materials can be used to produce such products. The most well-known representatives of the alkaline fermented foods are soybean-based (i.e., *chongkukjang*, *kinema*, *natto*, and *thua nao*). It should be noted, however, that other plant seeds (nonsoybean products) can also be used, e.g., *ugba* and *iru* of Western Africa are made from oil bean (*Pentaclethra macrophylla*) and locust bean (*Parkia biglobosa*), respectively [6,7].

Thua nao is a conventional fermented soybean food popularly consumed in northern Thailand (i.e., Chiang Mai, Chiang Rai, Lampang, and Mae Hong Son). It is the pride of culinary cultures for centuries. *Thua nao*, devised by local wisdom from generation to generation, is considered as a low-cost meat substitute and can be applied in various uses. *Thua nao* can be consumed directly as a staple food and/or as a flavor enhancer in many local dishes (Fig. 1). Traditional *thua nao* has an unpleasant odor, grayish or brownish in color, and has a slightly sticky cover. In contrast, *natto* produced by a selective starter culture of *Bacillus subtilis* does not have (or slightly has) an ammoniacal smell and is covered with a white massive mucous substance [8]. Other related fermented soy products include Indian *kinema*, Chinese *douchi*, and Burmese *chine pepoke* (see Table 1). These products do have similar properties (i.e., stickiness and typical flavor). Interestingly, such products are located in a specific region referred to as a theoretical triangle, with an individual angle on Japan (*natto*) and Korea (*chongkukjang*), Sikkim and Nepal (*kinema*), and Thailand (*thua nao*). This hypothesis has been developed and proposed by Tamang [10], and is referred to as the ‘*kinema*–*natto*–*thua nao*’ (KNT) triangle; this

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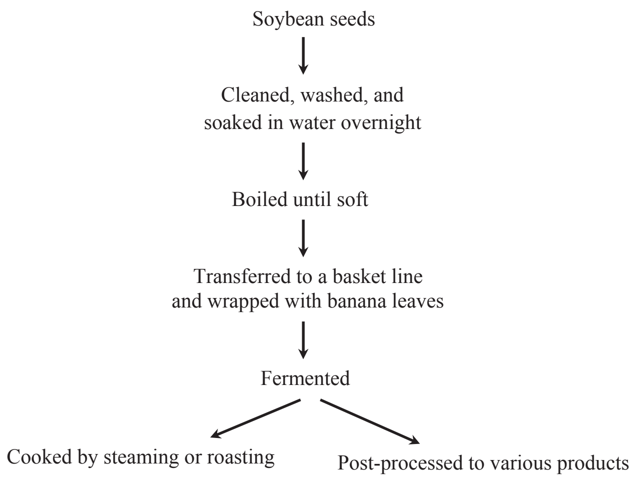

Fig. 1. Overall production process of *thua nao*.

concept also postulates that such fermented soy products may have originated from some places in the KNT area possibly in the Yunnan Province of China. This hypothesis also suggests that *B. subtilis* strains involved in the fermentation might have come from the same origin [11,12]. It should be noted, however, that *tempeh*, an ethnic fermented soy product from Indonesia once being included in the original triangle concept, is prepared by molds and not bacteria [13]. Some features of *thua nao* and other similar soy products are given in Table 1.

Traditional *thua nao* fermentation relies on microbial activity, especially that of the *Bacillus* group [14]. Proteolytic activity is a key step in the fermenting process. During the fermentation, soy proteins are hydrolyzed into peptides, amino acids, and ammonia, resulting in an alkaline condition (pH 8–9) that would help prevent contamination of spoilage microbes [1]. At present, most of the alkaline fermented foods are produced at the household level using mixed microbial cultures. No specific inoculum is thus required and only Japanese *natto* is fermented with a pure starter culture of *B. subtilis* var. *natto* for commercial purposes [4,8].

2. *Thua nao* preparation and fermentation

There are only three major raw ingredients used in *thua nao* production: soybeans, water, and mixed natural microbes. For typical manufacture of *thua nao*, the four major steps are as follows: (1) soaking; (2) boiling; (3) fermenting; and (4) postfermentation process. In brief, the soybeans are soaked overnight and washed thoroughly. These soaked soybeans are then boiled for 3–4 hours until soft. The cooked soybeans are placed in bamboo baskets lined and covered with banana leaves and allowed to undergo spontaneous fermentation at ambient temperature for 2–3 days (Fig. 1). The complete fermented soybean can be observed from the grayish brown color of soybean seeds. The product typically has a strong ammonia-like odor and may be covered by a slight mucilaginous substance (Fig. 2). *Thua nao* with a rancid smell or which appears contaminated with mold or yellow-pigmented slimy material on the beans will be regarded as spoiled. Although freshly prepared *thua nao* can be consumed directly, it is far more common to cook it by steaming (or roasting) before eating. In addition, some spices (i.e., chilies, onion, and salt) can be added to enhance the taste. Fresh *thua nao* has a short shelf-life (~2 days), if stored under ambient conditions. Some postfermentation processes are developed by the locals with the aim of extending the shelf-life. Cooked *thua nao* can be formed into a flat disk and exposed to sunlight, resulting in the dried form of *thua nao*, known as *thua nao kab* (Fig. 2). Alternatively, the dried product is thoroughly grounded into fine powder. These dried forms of *thua nao* product can be kept for several months.

3. Biochemistry and microbiology of *thua nao* fermentation

Soybeans are protein-rich substrates. Enzymatic degradation of soy proteins is hence definitely a key process in *thua nao* fermentation. In general, the soy proteins are hydrolyzed into peptides, amino acids, and ammonia. This proteolysis and other enzymatic activities are expected to contribute to distinct attributes of *thua nao* including texture, appearance, flavor, and aroma. As a result of the ammonia release, the pH of *thua nao* can reach as high as 8. This alkaline nature not only provides a selective condition for certain bacteria (i.e., *Bacillus* species), but also causes unfavorable conditions for other microbes to grow. Ammonia is considered as a major chemical giving a strong smell to the product. An attempt has been made to control ammonia formation using a buffering system in the fermentation which can be achieved by either adding phosphate buffer in the fermenting soybeans or fermenting the soybeans under carbon dioxide atmosphere [15]. The changes in enzymatic profiles of proteases, amylases, phytases, and lipases have also been reported during fermentation of *thua nao* [16].

The essential bacteria of *thua nao* fermentation belong to the *Bacillus* species. The *Bacillus* species, especially *B. subtilis*, are predominant from the start to the end of the fermentation [14–16]. Occurrence of *B. subtilis* as a major species in *thua nao* has also been reported by Inatsu et al [17]. Other *Bacillus* species are also found including *B. licheniformis*, *B. megaterium*, and *B. pumilus* [15,16]. These *Bacillus* species are very proteolytic and are thus a key target for strain screening in research aiming to improve the production of *thua nao* by several researchers. For example, *B. subtilis* strains 38 and TN51 exhibiting strong proteolytic activity are used as a pure starter culture in *thua nao* fermentation [14,18]. Changes in protease activity during the soybean fermentation prepared by *B. subtilis* strain TN51 have been reported [19]. An increased proteolysis of *thua nao* indicating a high fermentation rate is also described when

Table 1
Traditional *Bacillus*-fermented soybeans.

Product	Country	Type	Uses	References
Thua nao	Thailand	Non-salted	Flavor enhancer Main dish	[5]
Natto	Japan	Non-salted	Eaten directly with rice Flavor enhancer Accompaniments to drinks Main dish	[4]
Kinema	India	Nonsalted	Main dish	[3]
Dawadawa	Nigeria and Ghana	Salted	Flavor enhancer	[9]
Chongkukjang	Korea	Nonsalted	Flavor enhancer	[2]

Fig. 2. *Thua nao* products. (A) fresh *thua nao* (Photo courtesy of Dr Katekan Dajanta); (B) *thua nao kab* and cooked *thua nao* in local market of Chiang Rai (Photo courtesy of Mr Pichitchai Nantarat).

using *B. subtilis* BIOTEC 7123 [20]. Apart from the *Bacillus* species, a few bacterial groups (i.e., lactic acid bacteria and Gram-positive cocci) are also present, but their role in fermentation may not be relevant [16,21]. In terms of food safety, some bacteria in the Enterobacteriaceae and fungi are found, but there is no presence of *B. cereus*, *Staphylococcus aureus*, and *Vibrio* species in commercial *thua nao* products studied [22].

4. Nutritional data of *thua nao*

As mentioned, various biochemical changes occur during *thua nao* fermentation. It is hypothesized that protein hydrolysis is possibly a major activity. Microbial proteases help accelerate the protein degradation into various peptides and amino acids. In addition, there is a conversion of isoflavones, which are abundant in soybeans, by these microflora. The biochemical mechanisms of these fermented soybean products are similar, although it should be noted that the difference in nutritional quality can be observed due to: (1) soybean variety; (2) fermentation conditions; and (3) indigenous microflora. This part aims to provide an insight of what we currently know regarding the nutritional aspects of *thua nao*.

4.1. Proximate composition

Data on the composition of foods are significant as they can be referred to diet quality. Such data are also necessary as a guideline for food reference data. Proximate analysis is one system and includes some fundamental information such as water (moisture), ash, crude fat, crude protein, and crude fiber. For *thua nao*, the proximate data can be traced back to the original work of Sundhagul et al [23]. Table 2 shows proximate composition data of *thua nao* obtained from different research work [23–25]. It should be noted that there are variations in data obtained which may result from different soybean cultivars, cultivation, and microbial cultures used.

Table 2
Compilation of proximate data of *thua nao* products.

Chemical composition	FTN [23]	FTN [24]	TNK [24]	FTN [25]
Protein (%)	16.9	14.33	36.43	38.94–42.06
Fat (%)	7.4	1.83	17.96	20.37–25.22
Ash (%)	2.5	1.65	4.94	4.70–5.44
Fiber (%)	5.2	7.69	13.46	12.92–28.06
Reducing sugar (g/L)	ND	0.38	0.77	0.27–0.77
Ammonia (g/100 mL)	ND	0.02	0.03	ND
Moisture (%)	56.4	64.91	11.88	57.22–64.78
pH	8–8.6	6.7	5.9	7.08–8.25

FTN, fresh *thua nao*; ND, data not available; TNK, *thua nao kab*.

4.2. Amino acid profiles

Thua nao products appear to contain a variety of amino acids. Available data show that all amino acids are present in *thua nao* with quantities of total free amino acids ranging from 11.03 g/kg to 61.23 g/kg, as dry basis. The major amino acids are tryptophan, followed by glutamic acid, cysteine, lysine, and leucine. All essential amino acids (i.e., leucine, methionine, phenylalanine, and valine) are present in considerable amounts. It should be noted, interestingly, that a large proportion of bitter amino acids (i.e., arginine, histidine, and isoleucine) account for > 50% of total free amino acids [26]. In addition, when using a pure starter culture of *B. subtilis* TN51, an increase of total free amino acids, including essential amino acids, are found [27]. A significant increase of hydrophobic amino acids and a decrease of charged amino acids are also detected, which is possibly by a mechanism of bacterial biotransformation [27].

4.3. Isoflavones

Isoflavones, a group of phenolic compounds, exhibit estrogen-like activity which has been reported to exhibit several beneficial health effects [28]. It has been shown that soybean and related products are a good source of isoflavones [28,29]. In general, soy isoflavones are a group of heterocyclic phenols comprised of aglycones, β-glucosides, acetylglucosides, and malonylglucosides. Two major groups can be classified simply as aglycones and glucosides based on their distinct chemical structures (i.e., the presence of the glucose subunit in glucoside compounds) [30]. Of these, the glucoside forms are predominant in soybeans [30] whereas numerous studies show that aglycones are more abundant in fermented soybeans [3,31]. *Thua nao* products have also been assessed and the results obtained are in agreement in that a significant increase of aglycone isoflavone is found; in addition, the use of a pure inoculum of *B. subtilis* strain TN51 has proved to help increase the amounts of daidzein and genistein (aglycone group) [32]. Aglycone compounds are of great interest because clinical studies show that they can be absorbed faster and in greater amounts than the glucosylated counterparts [33,34]. It is thus promising to use an appropriate starter culture to develop an aglycone-rich product which would benefit novel functional food development.

4.4. Aroma and flavoring agents

Microbial fermentation causes biochemical changes affecting organoleptic properties. Such changes include texture, odor, and flavors of the products. Traditionally produced *thua nao* is typically described as an unpleasant smelling product, due to a strong ammoniacal smell. In contrast, *natto*, produced by a pure starter culture, has a fruity/nutty aroma. Leejeerajumnean et al [35] reported that *thua nao* products contain aldehydes, aliphatic acids, esters, and sulfur compounds, whereas none of these chemicals was present in *natto*. Several pyrazine compounds found in *natto* are thought to be the main contributors to the odor of *natto* [36]. Similar data are obtained in which the aldehydes and ketones were major volatiles found in *thua nao* [37]. However, it should be noted, interestingly, that the use of the pure culture of *B. subtilis* strain TN51 causes a shift in volatile profiles resulting in an improvement of the organoleptic quality of the product [37].

5. Conclusion

Thua nao is an indigenous alkaline-fermented soybean of Thailand. In terms of nutrition, the product is protein-rich and thus can be regarded as a protein supplement. Other health benefits have also been reported and should be further explored. This article presents scientific data of Thai *thua nao*, with anticipation of attracting researchers' attention to this local food product. There are several issues waiting for detailed study, especially for improvement of the product's quality. A use of pure starter culture is one approach. Alternative research regarding nutritional quality (i.e., amino acids, isoflavones, and flavoring compounds), health benefits (i.e., anticancer, antimicrobial, and antioxidant activities), and the fermentation process, would provide a new image of this indigenous food to Thai and world community. Last, but not least, knowledge transfer (from the scientific to local community) should be considered.

Conflicts of interest

The author has no conflict of interest to declare.

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