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# A Study on Production Processes and Quality of Fermented Soybean (Thua – Nao) in the Upper North of Thailand

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## Abstract

The objective of this research was to study the fermented soybean product called Thua – Nao in terms of its production processes and its problems, including quality testing, in the Upper North of Thailand. The experiment was a purposive survey and was conducted from May to July, 2004. Simple random sampling was used to get the representative locations : Chiang Mai, Mae Hong Son and Chiang Rai provinces.

The results showed that the Thua – Nao production processes differed in the different production locations. Factors that caused differences in the production process were soybean variety, boiling time, fermentation time, grinding method, product quantity, marketplace and marketing, and product types. Thua – Nao production starts by boiling soybeans for 5 – 8 hours before putting them in a basket until they are drained of water. After that, they are fermented in a plastic bag for about 2 – 4 days and then are ground in a grinder or mortar, molded into a ball shape and pressed into a thin disc. Lastly, they are dried by sunlight for about 2 – 3 days and stored in a plastic bag or other containers.

Thua - Nao quality was described in terms of nutritional value and type and quantity of microorganisms. It was found that the nutritional value was higher than that of other soybean products. It contained 38.94 – 42.81 % protein, 3.15 – 9.33 % oil, 33.62 – 40.43 % carbohydrate, 5.31 – 7.86 % fiber, 0.01 – 0.09 % ash and 7.30 – 12.02 % moisture content. Thua – Nao had inconsistent quality because of the effect of the season. Not only that, it also contained beneficial bacteria (*Bacillus* spp.) and fungi (*Rhizopus* spp.). *Aspergillus flavus* and *Aspergillus niger* were found to be possible to lower the quality of the products. The total number of microorganisms ranged from $1.56 \times 10^7$ to $4.93 \times 10^7$ CFU/g.

**Keywords:** fermented soybean, quality, nutritional value, microorganism

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# การศึกษากระบวนการผลิตและคุณภาพของถั่วเหลืองหมัก (ถั่วเน่า) ในพื้นที่ภาคเหนือตอนบนของประเทศไทย

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## บทคัดย่อ

วัตถุประสงค์ของการวิจัย คือ เพื่อศึกษากระบวนการผลิตถั่วเหลืองหมักหรือที่เรียกว่า ถั่วเน่า รวมทั้งปัญหาและผลการทดสอบคุณภาพ ในเขตพื้นที่ภาคเหนือตอนบนของประเทศไทย ทำการวิจัยแบบเจาะจง ช่วงระหว่างเดือนพฤษภาคม ถึง กรกฎาคม พ.ศ. 2547 ทำการสุ่มตัวอย่างแบบง่ายเพื่อใช้เป็นตัวแทนการสำรวจในพื้นที่ ผลการสุ่มจังหวัดที่ได้ คือ เชียงใหม่ แม่ฮ่องสอน และเชียงราย

ผลการวิจัย พบว่า กระบวนการผลิตถั่วเน่าแตกต่างกันไปในแต่ละพื้นที่ ปัจจัยที่มีความแตกต่าง คือ สายพันธุ์ ระยะเวลาการต้ม ระยะเวลาการหมัก วิธีการบด ปริมาณผลิตภัณฑ์ ชนิดผลิตภัณฑ์ สถานที่จำหน่าย และการตลาด วิธีการผลิตเริ่มต้นจากการต้มเมล็ดถั่วเหลืองประมาณ 5 – 8 ชั่วโมง จากนั้นนำไปใส่ในภาชนะที่มีช่องเพื่อให้สะเด็ดน้ำ และหมักในถุงพลาสติกประมาณ 2 – 4 วัน พอครบกำหนดจึงทำการบดด้วยครกหรือเครื่องบดจนละเอียด นำไปปั้นเป็นก้อนกลมแล้วกดให้เป็นแผ่นวงกลม ตากแดดให้แห้งโดยใช้เวลาประมาณ 2 – 3 วัน แล้วเก็บในภาชนะหรือถุงพลาสติกเพื่อรอการจำหน่าย

การตรวจวัดคุณภาพของถั่วเน่าในด้านคุณค่าทางโภชนาการ ชนิดและปริมาณของจุลินทรีย์ พบว่า ถั่วเน่ามีคุณค่าทางโภชนาการสูงกว่าผลิตภัณฑ์จากถั่วเหลืองชนิดอื่น โดยมีองค์ประกอบของโปรตีนอยู่ในช่วง 38.94 – 42.81% ไขมัน 3.15 – 9.33% คาร์โบไฮเดรต 33.62 – 40.43% ใยอาหาร 5.31 – 7.86% เถ้า 0.01 – 0.09% และความชื้น 7.30 – 12.02% โดยมีคุณภาพที่ไม่ค่อยสม่ำเสมอ ทั้งนี้ขึ้นอยู่กับฤดูกาล ภายหลังการตรวจวัดเชื้อจุลินทรีย์ พบเชื้อแบคทีเรียและเชื้อราที่เป็นประโยชน์ต่อกระบวนการหมัก คือ แบคทีเรีย *Bacillus* spp. และรา *Rhizopus* spp. และพบเชื้อราที่มีผลกระทบต่อคุณภาพของถั่วเน่า คือ *Aspergillus flavus* และ *Aspergillus niger* แต่ในปริมาณเพียงเล็กน้อย โดยตรวจพบปริมาณจุลินทรีย์ทั้งหมดในถั่วเน่าอยู่ในช่วง $1.56 \times 10^7$ ถึง $4.93 \times 10^7$ CFU/กรัม

## Introduction

The scientific name of soybean is *Glycine Max* L. Merrill, family Leguminosae, subfamily Papilonoideae. It is an important economic crop in Thailand because of its numerous advantages (Lumlertgul and Boonraeng, 2004). It has been called a marvelous plant (Suppadit and Sangla, 2003). The demand for processed soybean consumption in Thailand and on the world market has increased rapidly (Sangla and Suppadit, 2005). There have been many studies that have looked at soybean utilization including soybean processing. Samples of soybean products are cooked soybean, tofu, miso, soy milk, soy sauce, white soy sauce, bean cake, soft bean cake, bean cake scum, Thua – Nao and utility compound extraction such as isoflavone, jenistine, lecithin and phytoestrogen (Siriboriruk, 1999). Fukutake *et al.* (1996) reported that isoflavone and jenistine have a protective effect against cancer. Moreover, the Chiang Mai Field Crops Research Center (1999) reported that lecithin was known as a controller of the nervous system and its phytoestrogens serve a hormonal function in menopausal women.

Thua – Nao is produced by people in many provinces in the Upper North of Thailand (Chiang Mai, Chiang Rai, Lampang, Lamphun, Mae Hong Son, Phrae, Nan, and Phayao provinces). They produce local soybean food products including Thua – Nao for long – term storage. These products include dried Thua – Nao, spicy Thua – Nao, and grilled Thua – Nao (Sangla and Suppadit, 2005). Pintasean *et al*. (2002) reported that the various Thua – Nao products are known as popular foods and cheap protein sources. The people of the Upper North use Thua – Nao as a seasoning for food or for direct consumption (Sundhagul *et al*., 1972). However, the processing methods are limited to the local scale and use old traditional-style technology or indigenous knowledge. The production and quality of Thua – Nao varies depending on the location where it is produced. Production and quality have always been inconsistent (Sangla and Suppadit, 2005).

Thus, the objective of this study was to survey the preliminary data on the production, processing, and quality of Thua – Nao for improving the production technology, quality, and safety of the product.

## Materials and Methods

This study was a purposive research survey (Chanthalukana, 1980). It was conducted at Thua – Nao producing locations in the upper North of Thailand from May to July, 2004. The Thua – Nao producing regions are Chiang Mai, Chiang Rai, Lampang, Lamphun, Mae Hong Son, Phrae, Nan, and Phayao provinces. The experiment used simple random sampling for the representative locations (Chanthalukana, 1980). They were composed of 3 provinces and 7 districts, which were as follows:

1. Chiang Mai was composed of Mae Wang and Fang districts.
2. Mae Hong Son was composed of Khun Yuam, Muang, and Pang Ma Pa districts.
3. Chiang Rai was composed of Mae Jan and Mae Sai districts.

Primary data were collected by interviewing 30 villagers per location. Data on Thua – Nao production methods, its problems, fermentation time, marketplace and marketing, and types of product were recorded. Thua – Nao were sampled for quality testing. Each sample weight was 300 grams and was kept in a vacuum-sealed plastic bag to protect it from microorganism contamination. Nutritional values were composed of carbohydrate, protein, oil, fiber, ash, and moisture content. They were measured according to the Association of Analytical Chemists (AOAC) Manual (AOAC, 1992). The quantity of microorganisms was determined by Total Plate Counts Methods in Nutrient Agar (Sundhagul *et al*., 1972; Suwanpinij, 2001). Microorganism types were classified by Instant Bacteria Classification of API50 CHB (bioMerieux, France) (Sundhagul *et al*., 1972).

## Results and Discussion

### Thua – Nao Production

Thua – Nao was produced using indigenous knowledge. First, soybeans were boiled in water for about 5 – 8 hours. Then, they were put in a basket until they were drained. Next, they were fermented in a plastic bag for about 2 – 4 days. After that, they were ground in a grinder or mortar, molded in a ball shape and pressed into a thin disc. Finally, they were dried using sunlight for about 2 – 3 days and were kept in a plastic bag or some other type of container.

**Differences in Production Methods** (Table 1)

1. Variety

This depended on the consumer. Villagers used mainly varieties Tadeang Muangpai and SJ. 5, as those are the most popular local varieties.

2. Boiling time

This depended on production location and ranged from 5 – 8 hours.

3. Fermentation time

Fermentation time differed depending on the production locations and the season (varying from 2 – 4 days). For example, it takes a longer time to ferment Thua – Nao in the cool season than in dry season.

4. Grinding method

Both grinders and mortar and pestle were used.

5. Product types of Thua – Nao

Villagers produced dried Thua – Nao, spicy Thua – Nao, and grilled Thua – Nao.

6. Quantity and time of production including marketplace and marketing.

This depended on the production location. Chiang Rai province was capable of producing the highest quantity. Mae Hong Son had more marketplaces than Chiang Mai and Chiang Rai, respectively. Marketplaces were in villages, district centers, provincial centers, and nearby provinces.

**Production Problems**

1. Drying

Sunlight was used to dry Thua – Nao. In the rainy season, fungi contaminated the Thua – Nao because of the limited sunlight available for drying. This led to yield losses. Therefore, production quantities were less than in the dry season. In Pang Ma Pa District, Mae Hong Son, a drying warehouse was constructed for solving this problem. Sometimes, Thua – Nao had a bad odor because fermenting microorganisms could not grow. It was understood that a longer drying time is necessary in the rainy and cool seasons than in dry season.

Long – term storage sometimes caused fungal contamination, especially in moist conditions. Lumlertgul and Boonreang (2004) reported that spicy Thua – Nao could be kept for 2 – 3 days, while fried Thua – Nao could be kept for 4 – 5 days. Dried Thua – Nao has a longer storage period (2 weeks) than fried Thua – Nao and spicy Thua – Nao, respectively. For optimal storage conditions, moisture content must not exceed 14 %.

2. Low quality

Product quality was low in terms of color, flavor, smell, and texture. The quality standard differed based on the time and location of production.

3. Limitations of marketing

Production was limited to the local scale and Thua – Nao products are only accepted as a popular food in some districts and provinces in the upper North of Thailand.

**Thua – Nao Quality**

**Nutrition Values**

Table 2 shows the protein, oil, carbohydrate, fiber, ash, and moisture content, which ranged from 38.94 to 42.81 %, 3.15 to 9.33 %, 33.62 to 40.43 %, 5.31 to 7.86 %, 0.01 to 0.09 %, and 7.30 to 12.02 %, respectively. The total nutritional value of Thua – Nao was found to be higher than other soybean products when compared by weight. Samples of other soybean products were cooked soybean, soy milk, soy sauce, white soy sauce (miso), bean cake, soft bean cake and bean cake scum. There are many types of Thua – Nao products, for example, dried Thua – Nao, spicy Thua – Nao, and grilled Thua – Nao. Yoksan (2005) reported that protein from meat (9 %) was less than that from soybean. Clinical results obtained by Kungsadalaumpai (2005) reported that protein from soybean reduced cholesterol and LD – cholesterol level and the body received sufficient protein if consumed in high quantities. Besides this, the lipid content of meat is higher than the lipid content from soybeans, and saturated fatty acids were almost 13 % (Yoksan, 2005). Meanwhile soybean has a low saturated fatty acid content and is a good source of essential fatty acids, including linoleic and linolenic acid. Both of these fatty acids must come from food and cannot be synthesized in the body; therefore, consumers of soybean products do not gain weight (Kungsadalaumpai, 2005). Soybean products are also high in carbohydrates and fiber. Juntawankul (1995) reported that the carbohydrates in soybean are both water soluble carbohydrate and water insoluble carbohydrates. Water soluble carbohydrates are composed of many sugars, such as disaccharide (sucrose : $C_{12}H_{22}O_{11}$), trisaccharide (raffinose : $C_{18}H_{32}O_{16}$), and tetrasaccharide (stachyose : $C_{24}H_{42}O_{21}$). The high fiber content promotes good intestinal function and reduces constipation (Suppadit, 2003). Furthermore, the ash in soybeans contains minerals, including potassium, phosphorus, magnesium, calcium, sodium and sulfur (Yoksan, 2005).

**Microorganism Values**

The type and quantity of microorganisms in Thua – Nao differed depending on the production location (Table 3). This research found *Bacillus* spp. (*Bacillus subtilis*, *Bacillus licheniformi*, *Bacillus thermocatenulatus*), the same species that are found in Nutto (Krusong and Pongsawadmanit, 1985). Nutto is a soybean product from Japan. *Rhizopus* spp., *Aspergillus flavus* and *Aspergillus niger* were also found in Thua – Nao from Muang district, Mae Hong Son and Mae Sai district, Chiang Rai. Long term storage resulted in fungal contamination, especially in moist conditions.

*Bacillus* spp. is an aerobic opportunistic bacteria (Suwanpinij, 2001). Their important role is to release proteolytic enzymes, the so called protease, for breaking down protein into ammonium. Protease digests compounds in soybean, yielding good characteristics such as good taste and smell, a digested complex structure, prolonged storage, increased digestive ability, and dissolubility for simple absorption (Hesseltine and Wang, 1980). *Bacillus* spp. are used as a starter inoculum for Thua – Nao fermentation. Lumlertgul and Boonraeng (2004) stated that using *B. subtilis* as a starter inoculum was better than using *B. licheniformi* and *B. thermocatenulatus*, respectively. A mixture of three bacterial species provided spectacular the contrast results. This might be a result of competition among the three species during the fermentation process. Their toxicity and optimal inoculums should therefore be tested (Juntawankul, 1995). Not only that, Lumtertgul and Boonraeng (2004) and Wood (1998) reported that fermenting Thua – Nao at 35 $^{o}$C was the optimal temperature for the fermentation process as it produced the best soluble protein.

Fungi groups in Thua – Nao were natural fungi. They could cause fungi allergy (Poonwan, 2005). *Rhizopus* spp. are fungi that produce amylase to digest carbohydrates into dextrin and sugar during the fermentation process (Srinakarintarawirot, 2005). They also increase nutritional values. However, *A. flavus* and *A. niger* are fungi that might produce the so-called aflatoxin. They are compounds that can negatively affect health. Consuming high quantities of this aflatoxin can damage liver and blood cells; aflatoxin is also a carcinogen (Suppadit, 2003). However, *A. flavus* and *A. niger* were more beneficial than *Rhizopus* spp. in terms of Thua – Nao fermentation (Lotong and Suwanarit, 2005). They could change sugar to steric acid and decreased the pH until some toxic fungi could no longer grow in the Thua – Nao (Srinakarintarawirot, 2005).

## Conclusions and Suggestions

Currently, the Thua – Nao production process using indigenous knowledge and technology for developing improved soybean nutrition and long term storage conditions has gained popularity. Thua – Nao has higher nutritive values than other soybean products. However, the product quality is inconsistent. In the cool season, an incomplete fermentation process occurs because of cool conditions affecting microorganism growth. Not only that, fungi contamination is the main problem in the rainy season. Thus, optimum fermentation conditions to bring about a temperature of 35 $^{o}$C is a new method for developing fermentation quality. Bacteria found in the Thua – Nao are beneficial for the fermentation process. Although they can cause diarrhea, they release the protease enzyme to digest protein and break down the food compounds yielding good characteristics. Moreover, this disease has not been reported from Thua – Nao consumption. The majority of fungi found in the Thua – Nao can produce aflatoxin, which is a contaminant of this product. Thus, measures should be taken to control and prevent the growth of these fungi in Thua – Nao, involving raw material selection, production, packaging and marketing.

**Table 1** Differences in Thua – Nao production in various locations.

| Location (Province) | Productive Input | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Variety** | **Boiling Time (Hour)** | **Fermenting Time (Day)** | **Grinding Method** | **Quantity (Kilogram/ Villager)/Time of Production (Day)** | **Marketplace/ Marketing** | **Type of Products** |
| Chiang Mai | SJ. 5 or Others | 5 | 3 – 4 | Grinder | 8/1 | District and Provincial Market/ Direct – Sale and Sub – Dealer | Dried Thua – Nao |
| Mae Hong Son | Tadeang Muangpai | 8 | 2 – 4 | Mortar/Grinder | 20/1 | District, Provincial and Other Provincial Market/Direct – Sale and Sub – Dealer | Dried Thua – Nao<br>Spicy Thua – Nao<br>Fried Thua – Nao |
| Chiang Rai | Non – Specific | 6 – 8 | 2 – 3 | Grinder | 32/1 | District and Provincial Market/ Sub – Dealer | Dried Thua – Nao |

**Table 2** Nutritional values of Thua – Nao from various production locations.

| Sampling Location | | Nutritional Values (%) | | | | | |
|---|---|---|---|---|---|---|---|
| **Province** | **District** | **Protein** | **Oil** | **Carbohydrate** | **Fiber** | **Ash** | **Moisture Content** |
| Chiang Mai | Mae Wang | 38.94 | 3.15 | 40.43 | 5.44 | 0.02 | 12.02 |
| | Fang | 42.81 | 3.59 | 38.86 | 6.03 | 0.02 | 8.69 |
| Mae Hong Son | Pang Ma Pa | 42.19 | 8.20 | 33.62 | 7.86 | 0.04 | 8.09 |
| | Muang | 42.38 | 7.23 | 36.08 | 5.89 | 0.06 | 8.36 |
| | Khun Yuam | 42.12 | 4.63 | 38.45 | 5.31 | 0.09 | 9.40 |
| Chiang Rai | Mae Jan | 39.25 | 8.10 | 39.35 | 6.26 | 0.01 | 7.30 |
| | Mae Sai | 41.25 | 9.33 | 34.81 | 6.40 | 0.07 | 8.14 |
| **Mean** | | | | | | | |
| Chiang Mai | | 40.88 | 3.37 | 39.65 | 5.74 | 0.02 | 10.36 |
| Mae Hong Son | | 42.23 | 6.69 | 36.05 | 6.35 | 0.06 | 8.62 |
| Chiang Rai | | 40.25 | 8.72 | 37.08 | 6.33 | 0.04 | 7.72 |
| **3 Provinces** | | **41.12** | **6.26** | **37.59** | **6.14** | **0.04** | **8.90** |
| **Other Soybean Products*** | | | | | | | |
| Cooked Soybean | | 11.00 | 5.70 | 10.80 | 1.60 | - | - |
| Soy Milk | | 2.80 | 1.50 | 3.60 | 0.10 | - | - |
| Soy Sauce | | 5.20 | 0.50 | 8.10 | 0 | - | - |
| White Soy Sauce | | 12.00 | 3.80 | 8.00 | 0 | - | - |
| Bean Cake | | 12.50 | 8.10 | 6.00 | - | - | - |
| Soft Bean Cake | | 7.90 | 4.10 | 0.40 | 0.10 | - | - |
| Bean Cake Scum | | 47.00 | 28.40 | 14.90 | 0.10 | - | - |

**Source:** * Kungsadalaumpai (2005)

**Table 3** Type and quantity of microorganisms of Thua – Nao from various production locations.

| Sampling Location | | Type of Microorganisms | Quantity of Microorganisms (CFU/Gram) |
|---|---|---|---|
| **Province** | **District** | | |
| Chiang Mai | Mae Wang | Bacteria* | $1.56 \times 10^7$ |
| | Fang | Bacteria*, *Rhizopus* spp. | $1.55 \times 10^7$ |
| Mae Hong Son | Pang Ma Pa | Bacteria*, *Rhizopus* spp. | $3.18 \times 10^7$ |
| | Muang | Bacteria*, *A. flavus*, *A. niger* | $5.37 \times 10^6$ |
| | Khun Yuam | Bacteria* | $1.31 \times 10^7$ |
| Chiang Rai | Mae Jan | Bacteria* | $3.29 \times 10^6$ |
| | Mae Sai | Bacteria*, *Rhizopus* spp., *A. flavus.*, *A. niger* | $2.23 \times 10^7$ |
| **Mean** | Chiang Mai | | $1.56 \times 10^7$ |
| | Mae Hong Son | | $4.93 \times 10^7$ |
| | Chiang Rai | | $2.76 \times 10^7$ |
| | **3 Provinces** | | $\mathbf{2.64 \times 10^7}$ |

**Food Note**: * Bacteria = *B. subtilis, B. licheniformis, B. thermocatenulatus*
CFU = Colony Forming Unit

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