

thua nao, Indigenous Thai Fermented Soybean: A Review

¹E. Chukeatirote, ²K. Dajanta and ³A. Apichartsrangkoon

¹School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

²Faculty of Food and Agricultural Technology,

Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand

³Department of Food Science and Technology, Faculty of Agro-Industry,
 Chiang Mai University, Chiang Mai 50100, Thailand

Abstract: thua nao is an indigenous fermented soybean of Northern Thailand. The product can be consumed directly or often used as major ingredient in several local dishes. Similar products include Japanese natto, Indian kinema and Nigerian dawadawa. Such fermented foods are commonly known as alkali fermented soybeans due to bacterial proteolytic activity during the fermentation. Interestingly, these are all household fermentations caused by mixed starter culture of bacilli (except natto which is commercialised and produced by a pure starter culture). In this present study, we aim to summarise the scientific basis of Thai thua nao including fermentation process, microbes involved and nutritive data.

Key words: thua nao, alkali fermented soybean, bacilli fermentation

INTRODUCTION

Fermented soybean foods have been traditionally produced and widely consumed in many parts of the world especially in Asia and Africa. Among these are Japanese natto, Korean chungkukjang, Indian kinema and Nigerian dawadawa (Steinkraus, 1996). thua nao is a traditional fermented soybean food popularly consumed in Northern Thailand (i.e., Chiang Mai, Chiang Rai, Mae Hong Son, Lam Phun and Lam Pang). Thai thua nao is not only an important food/condiment but also serve as a low cost protein supplement for the local. The production of these fermented soybean foods is very similar and is still generally prepared at household scale. Major microorganisms are *Bacillus* bacteria and, during the fermentation, their proteolytic activity causes protein hydrolysis and ammonia liberation. The release of ammonia makes alkaline condition and thus these products are often known as the alkaline fermented soybeans. Of these products, natto is best-characterised with detailed scientific data (Ohta, 1986). In contrast, there is little scientific information of thua nao and, even worse, such available information is also scattered. This present study is therefore, introduced to document current status of thua nao in terms of manufacture, fermenting microbes and nutritive quality.

thua nao: an alkaline-fermented soybean: Alkaline-fermented foods generally regarded as safe constitute a group of food products popularly consumed in several

Asian and African countries (Steinkraus, 2002). They can be produced from different raw materials. For example, thua nao, natto and kinema are made from soybeans (*Glycine max*), dawadawa from locust beans (*Parkia biglobosa*), ogiri from melon seeds (*Citrullus vulgaris*) and ugba from oil beans (*Pentaclethra macrophylla*). During the fermentation of these alkaline-fermented products, the protein is hydrolysed into amino acids and peptides. Ammonia released during the fermentation raises the pH of the final products (as high as 8) and gives the food a strong ammoniacal smell. In general, most of the alkaline fermentations are practised at household scale depending on mixed bacterial cultures in the environment. No deliberate inoculum is required with an exception of natto which is inoculated with a pure culture of *B. subtilis* var. *natto* (Ohta, 1986).

thua nao fermentation process: Three major raw ingredients for thua nao production are as follows: soybeans, water and naturally occurring bacterial starter culture. After complete fermentation, the mature product with distinct organoleptic properties can be eaten directly or mixed with other spices (i.e., chillies, onions and salt). The so-called thua nao (in Thai: thua, soybean and nao, spoiled) is derived from putrefy smell of ammonia released by bacterial fermentation, principally predominated by *Bacillus* species.

There are four main steps in conventional thua nao manufacturing process namely soaking, boiling, fermenting and incubating (Sundhagul *et al.*, 1972;

Chantawannakul *et al.*, 2002). Such a process is also similar to that of other soy-fermented foods. For detailed descriptions of traditional process of thua nao, it typically starts with the soaking and boiling of soybean seeds for 3-4 h until soft. The cooked soybeans are then packed in bamboo basket lined and covered with banana leaves and allowed to undergo spontaneous fermentation at ambient temperature for 2-3 days. However, there are differences in the procedures and equipment employed in different areas and communities. For instance, in the production of thua nao at Fang District in Chiang Mai, dry soybeans are cooked in boiling water for 7 h after being washed twice with clean water, then fermented in woven bamboo baskets lined with fern leaves, then covered with the plastic bags and placed outside the building exposed to sunlight for 3 days.

The mature thua nao is indicated by grayish brown colour of soybean seeds, the appearance of a slight mucilaginous substance and the presence of a strong ammonia-like odour. thua nao is regarded as spoiled when they liberate putrid or rancid smell or appeared contaminated with mould or yellow-pigmented slimy material on the beans. A major problem of cooked thua nao is a short shelf-life (~2 days); if stored under ambient conditions. Hence, in order to extend the shelf-life of this product, sun-dried processed of thua nao is employed and it has another local name, the so-called thua nao kab. To achieve this, the mature thua nao was finely ground, moulded into a ball, pressed to form a flat disc and then sun-dried. This thua nao kab product can be stored for several months.

Bacillus species as predominant in thua nao fermentation: As mentioned earlier, thua nao is still produced conventionally by natural microflora. It should be noted, however that *Bacillus* species are always present in high numbers. According to Chukeatirote *et al.* (2006), *Bacillus* species especially *B. subtilis* predominated from the beginning to the end of the fermentation. Occurrence of *B. subtilis* as predominant species in thua nao product was also reported by Chantawannakul *et al.* (2002) and Leejeerajumnean (2000). Other *Bacillus* species were also found including *B. cereus*, *B. licheniformis*, *B. megaterium* and *B. pumilus* (Chukeatirote *et al.*, 2006; Leejeerajumnean, 2000). Apart from dominant *Bacillus* species, a few bacterial groups (i.e., lactic acid bacteria and unidentified Gram-positive cocci) were also identified only at the onset and thus their involvement in fermentation is possibly limited (Boontim *et al.*, 2003; Chukeatirote *et al.*, 2006).

Nutritive quality of thua nao

Proximate composition: Proximate composition is basically required for nutritional data of foods. This information can be used to indicate nutritive status, to

represent food labeling and to guideline further formulation. It is expected that there exist some differences of these values between thua nao product and raw soybean due to biochemical changes during fermentation. As described by Chukeatirote and Thakang (2006), the content of protein, fat and reducing sugar of fresh thua nao appeared to be slightly higher than unfermented soybean. These changes are possibly caused by several extracellular enzymes produced during fermentation (Chukeatirote *et al.*, 2006). Overall data of proximate analysis of thua nao products are as follows: 64.91% moisture, 1.65% ash, 7.69% crude fibre, 14.33% crude protein and 1.83% fat; whereas those features of thua nao kab were 11.88, 4.94, 13.46, 36.43 and 17.96%, respectively (Chukeatirote and Thakang, 2006).

Amino acid profiles: Amino acid content and pattern are also important from nutritional viewpoint. Besides, some amino acids are considered to play a key role in product taste. thua nao products appear to be a good protein source based on the amino acid profiles. Available data show that all amino acids are present in thua nao with quantities of total free amino acids ranging from 11.03-61.23 g kg⁻¹, as dry basis. The major amino acids are Trp, followed by Glu, Cys, Lys and Leu. All essential amino acids are also present in considerable amounts. It should be noted interestingly that there exist a large proportion of bitter amino acids accounting for more than 50% of total free amino acids (Dajanta, 2010).

Isoflavones: Isoflavones, a type of phenolic compounds, exhibit estrogen-like activity which has been reported to exhibit several health beneficial effects (Wiseman, 2006). Similar to other fermented soybeans, thua nao products contain high amounts of isoflavone compounds. Besides, the use of pure starter culture to prepare thua nao has showed to increase the content of aglycone isoflavones (Dajanta *et al.*, 2009). Aglycone compounds are of great interest because of their bioavailability, high rate of absorption in animals and humans (Izumi *et al.*, 2000; Kano *et al.*, 2006) and high antiproliferative activity on human cancer cells (Peterson *et al.*, 1998).

Aroma and flavouring agents: Microbial fermentation of soybeans causes biochemical changes including organoleptic properties. Such changes affect texture, odour and flavours of the products. Traditionally produced thua nao is typically illustrated as a fish sauce-like product due to a strong ammoniacal smell. In contrast, natto produced by a pure starter culture has a fruity/nutty aroma. Leejeerajumnean *et al.* (2001) reported that thua nao products consist of aldehydes, aliphatic acids, esters and sulphur compounds, whereas natto condiments are devoid of these chemicals. Besides, several pyrazine compounds found in natto are proposed

to be the main contributors to natto odour characteristics (Sugawara *et al.*, 1985; Owens *et al.*, 1997).

CONCLUSION

The thua nao is the indigenous alkaline-fermented soybean of Thailand. This condiment plays a key role as dietary culture of local Thai people. In terms of nutrition, thua nao is regarded as a good source of protein supplement in which other health benefits must be further explored. At present, scientific data of Thai thua nao are limited and scattered. We hence made an attempt to update its information currently available with an anticipation to attract researcher's attention on this local food product. Several issues are open for intensive experiment in order to develop thua nao product. For instance, the use of pure starter culture is a good start and potential for product improvement. Alternative research relevant to nutritional quality (i.e., enhancement of amino acids and flavouring agents), health benefits (i.e., anticancer, antimicrobial and antioxidant activities), as well as fermentation process, would provide a new image of this indigenous food to Thai and world community.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Royal Golden Jubilee Ph.D. Research Scholarship and the Thailand Research Fund for financial support.

REFERENCES

- Boontim, N., S. Lumyong and M. Moriguchi, 2003. Characterisation of Thai fermented foods by organic acid contents. *ScienceAsia*, 29: 301-306.
- Chantawannakul, P., A. Oncharoen, K. Klanbut, E. Chukeatirote and S. Lumyong, 2002. Characterisation of proteases of *Bacillus subtilis* strain 38 isolated from traditionally fermented soybean in Northern Thailand. *ScienceAsia*, 28: 241-245.
- Chukeatirote, E. and P. Thakang, 2006. Chemical composition of thua nao-a fermented soybean food of Northern Thailand. *Chiang Mai J. Sci.*, 33: 243-245.
- Chukeatirote, E., C. Chairun, A. Siengsubchart, C. Moukamnerd and P. Chantawannakul *et al.*, 2006. Microbiological and biochemical changes in Thua nao fermentation. *Res. J. Microbiol.*, 1: 38-44.
- Dajanta, K., 2010. Production of high nutritional fermented soybean (thua nao) by *Bacillus subtilis*. Ph.D. Thesis, Chiang Mai University, Chiang Mai, Thailand.
- Dajanta, K., E. Chukeatirote, A. Apichartsrangkoon and R.A. Frazier, 2009. Enhanced aglycone production of fermented soybean products by *Bacillus* species. *Acta Biol. Szeged*, 53: 93-98.
- Izumi, T., M.K. Piskula, S. Osawa, A. Obata and K. Tobe *et al.*, 2000. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.*, 130: 1695-1699.
- Kano, M., T. Takayanagi, K. Harada, S. Sawada and F. Ishikawa, 2006. Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. *J. Nutr.*, 136: 2291-2296.
- Leejeerajumnean, A., 2000. *Bacillus* fermentation of soybeans: Characterisation of traditional thua nao manufacture. Ph.D. Thesis, The University of Reading, Reading, UK.
- Leejeerajumnean, A., S.C. Duckham, D.J. Owens and J.M. Ames, 2001. Volatile compounds in *Bacillus*-fermented soybeans. *J. Sci. Food Agric.*, 81: 525-529.
- Ohta, T., 1986. Natto. In: *Legume-Based Fermented Foods*, Reddy, N.R., M.D. Pierson and D.K. Salunkhe (Eds.). CRC Press, Florida, pp: 85-95.
- Owens, J.D., N. Allagheny, G. Kipping and J.M. Ames, 1997. Formation of volatile compounds during *Bacillus subtilis* fermentation of soy beans. *J. Sci. Food Agric.*, 74: 132-140.
- Peterson, T.G., G.P. Ji, M. Kirk, L. Coward, C.N. Falany and S. Barnes, 1998. Metabolism of the isoflavones genistein and dichanin A in human breast cancer cell lines. *Am. J. Clin. Nutr.*, 68: 1505-1511.
- Steinkraus, K.H., 1996. *Handbook of Indigenous Fermented Foods*. 2nd Edn., Marcel Dekker Inc, New York, pp: 148.
- Steinkraus, K.H., 2002. Fermentation in world food processing. *Comp. Rev. Food Sci. Food Saf.*, 1: 23-32.
- Sugawara, E., T. Ito, S. Odagiri, K. Kubota and A. Kobayashi, 1985. Comparison of compositions of odour components of *natto* and cooked soybeans. *Agric. Biol. Chem.*, 49: 311-317.
- Sundhagul, M., P. Smanmathuroj and W. Bhodacharoen, 1972. Thua-Nao: A fermented soybean food of northern Thailand. I. Traditional processing method. *Thai J. Agric. Sci.*, 5: 43-56.
- Wiseman, H., 2006. Isoflavonoids and Human Health. In: *Flavonoids, Chemistry, Biochemistry and Applications* andersen, O.M. and K.R. Markham (Eds.). CRC Press, Florida, pp: 371-396.