Chapter 10
Conclusions and Recommendations

10.1 Conclusions

As noted earlier, *Thua Nao* is an indigenous fermented soybean of Thailand. Due to its pungent odour, inconsistent quality and food safety from conventional process, the product does not gain much attention. *Natto* in contrast is a similar product and presently is one of the best-characterised products among the alkaline fermented soybean foods. *Natto* is also the only example that has been produced industrially due to its distinct flavour and taste. Apart from consumer's acceptability, several healthy benefits have been described (i.e., anti-carcinogenic activity (Jung *et al*., 2006), antidiabetic activity (Liu *et al*., 2006), and antioxidant activity (Georgetti *et al*., 2009)) and thus it is not surprising that *Natto* becomes popular and has been widely consumed by Japanese people. This study is an example of how we can utilise our knowledge (food science and biotechnology) towards improving the *Thua Nao* product.

Our study is also the first to investigate physicochemical and microbiological properties including a data set of nutritive qualities (i.e., total phenolics, free amino acid profiles, and isoflavones) of this indigenous Thai *Thua Nao*. We provide the comprehensive data of commercial *Thua Nao* product with an expectation that these results can be used as nutritional reference and as food regulatory standard. Furthermore, the use of *Bacillus subtilis* strain TN51 as inoculum has showed to be an appropriate means to control the *Thua Nao* product's quality (nutritive and safety issues). In using this starter culture (*B. subtilis* TN51), we strongly believe that the *Thua Nao* product can be improved in terms of taste and smell. Additionally, high nutritional values as shown in this Thesis would be a key factor to promote the product and thus give a new image to *Thua Nao* as a good choice for protein supplement. We would be delighted that our results from this present study could pave a way for *Thua Nao* to gain popularity and finally to scale up for

commercialisation. It should be noted, however that the use of starter culture may affect the production cost.

In this study major conclusions are obtained as shown below:

1. The physicochemical and microbiological qualities including their amino acid profile, total phenolic compounds and some biological activity of commercial *Thua Nao* were varied depending on soybean cultivar and processing conditions i.e., soaking, cooking and fermentation processes as summarised in Table 10.1.

2. Soybean cultivar TG145 appeared to be a suitable raw material for production of high nutritive *Thua Nao* because of its high protein (45.85%), low fat (15.59%), good production yield and small size seeds.

3. The production of *Thua Nao* by fermented autoclaved soybean TG145 cultivar with pure starter culture of *B. subtilis* strain TN51 subsequently incubated in controlled 43°C for 72 h could be improved organoleptical and nutritional qualities (flavour volatiles, free amino acids, isoflavones, phenolics, and antioxidant activity) of the product when compared with natural fermentation (Table 10.1).

4. High temperature (40°C) storage condition did not maintain a desirable quality of the product. Low temperature (4°C) was shown prolong shelf-life storage of *Thua Nao* produced by natural and pure starter fermentations based on visual characteristics at least 40 days for both aerobically and vacuum packages. Also based on the limit of acceptability level of TBA rancidity, only TNB51 packed in vacuum condition could be extended shelf-life for 10 days whilst other cases were not available although storage at 4°C.

Table 10.1 Physiochemical, microbiological, organoleptical and nutritional qualities of various productions *Thua Nao*

Quality	TNB51	TNMX	TNCM
Physicochemical quality			
Moisture (%)	56.74	68.19	57.22 -64.78
pH value	8.00	8.33	7.08 – 8.25
Protein (%)	45.35	44.91	38.94 – 42.06
Fat (%)	16.40	19.06	20.24 – 25.22
Ash (%)	6.43	7.58	4.70 – 5.44
Fiber (%)	16.0	19.06	12.92 – 28.06
Total sugar (%)	6.02	2.64	4.79 – 7.38
Reducing sugar (%)	3.59	2.47	2.70 – 7.74
Ammonia (mg/ml)	0.29	0.42	-
Colour L value	36.42	53.74	38.63 – 47.18
Colour a* value	10.11	6.62	6.91 – 9.10
Colour b* value	14.57	20.06	14.79 – 23.12
Microbiological quality			
Total viable count (log CFU/g)	8.62	9.67	9.57 – 10.59
Spore count (log CFU/g)	8.59	9.62	9.42 – 9.84
Yeast and mould (log CFU/g)	< 1	< 1	< 1
Organoleptical quality[1]			
Overall preference	5.62	4.82	-
Colour	5.58	4.86	-
Odour	5.80	4.32	-
Flavour	5.64	4.68	-
Texture	5.98	4.86	-
Nutritional quality			
Flavour volatile compounds (mg/kg dry sample)	452.68	389.40	-
Free amino acids (g/kg dry sample)	151.12	75.79	11.03 -61.23
Isoflavone compounds (μg/g dry sample)	639.74	320.23	372.70
Phenolic compounds (mg GAE/g extract)	35.18	37.29	30.46 - 44.58
Total antioxidant activity (%, at 10 mg/ml)	62.12	65.65	47.21 – 59.45
DPPH-radical scavenging effect (IC_{50}, mg/ml)	3.27	5.18	2.43 – 3.19
Antimicrobial activity (RMI)[2]	1.60 – 1.90	1.90 -2.60	1.10 – 1.90
Spectrum of antimicrobial inhibition[2]	BC, LM	SA, SE, ML, BC	BC
Shelf-life storage (days) [3]	60	40	40

TNB51, *Thua Nao* produced by fermented autoclaved soybean cultivar TG145 with *B. subtilis* TN51; TNMX, *Thua Nao* prepared by fermentation of boiled soybeans with naturally occurring microbes; TNCM, commercial *Thua Nao* purchased from six local markets in Chiang Mai; Physicochemical quality of samples are presented in dry basis; (-), not determined; RMI, relative magnitude of inhibition; BC, *Bacillus cereus* TISTR687; LM, *Listeria monocytogenes* DMSTI17303; SA, *Staphylococcus aureus* TISTR118; SE, *Staphylococcus epidermidis* TISTR518; ML, *Micrococcus luteus* TISTR884. [1]Data based on 7-hedonic scale. [2] Data based on methanol extraction. [3] Data based on visual quality.

10.2 Recommendation for further investigations

1. Other soybean cultivars including black soybeans should be used in production of *Thua Nao* in order to find the potential of manufacturing high nutritional fermented soybean foods.

2. Further study related to production condition such as steaming times, amounts of *Bacillus* inocula, mixed starter cultures, pre- and post-fermentation process using pure starter culture of *B. subtilis* TN51 should be conducted.

3. Changes in flavour volatiles, free amino acids, isoflavones, antioxidant components and their biological functions should be analysed during *Thua Nao* fermentation.

4. Antimicrobially active components in *Thua Nao* product should be identified in further investigations by purifying before structural identification with Electron Impact Mass Spectrometry (EI-MS) technique (Kim *et al*., 2004).

5. To extend shelf-life of *Thua Nao*, development of preservation technique such as different dehydration process or freeze-drying should be further explored with an expectation for commercial purpose.