Chapter 3

Nutritional and Physicochemical Qualities of Commercial *Thua Nao*

3.1 Introduction

Thua Nao is a fermented soybean product locally produced and consumed by people in Northern Thailand. Similar fermented soybean products have been described in several countries (i.e. *Natto* in Japan, *Kinema* in India, *Chungkukjang* in Korea and *Dawadawa* in Nigeria). *Bacillus* species, especially *Bacillus subtilis*, are predominant and have been shown to be responsible in the fermentation process (Sundhagul *et al*., 1972; Chantawannakul *et al.*, 2002; Chukeatirote *et al*., 2006; Inatsu *et al.*, 2006). The most important activity that occurs during the fermentation process is proteolysis leading to unique flavour and taste (Leejeerajumnean *et al.*, 2001; Parkouda *et al*., 2009). It is generally accepted that food composition data are useful and needed to specify the association between food and nutritional status, to design regulatory standards, and to help improve product formulation.

Although these fermented soybean products are valued for their high protein content, there are only a few in which their food composition data are available. Indeed, despite the significant role as a protein source, the complete data of amino acid profiles are scarce and restricted to *Natto* (Zarkadas *et al*., 1997), *Kinema* (Sarkar *et al*., 1997) and *Douchi* (Li *et al*., 2007). This criterion is important and can represent one of the key characteristics of nutritional quality of the food product. About *Thua Nao*, the various information of proximate composition has been recorded by Sundhagul *et al*. (1972) and Chukeatirote and Thakang (2006), including changes in biochemical and microbiological profiles during traditionally fermentation of *Thua Nao* (Chukeatirote *et al.*, 2006). Besides, health-promoting compounds such as subtilisin and gamma-polyglutamic acid of *Thua Nao* has recently been reported by Inatsu *et al*. (2006). However, such detailed information on other aspects is not available for *Thua Nao* product. For example, the antioxidant potential and

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antimicrobial capacity including the phytochemical distribution of traditional *Thua Nao* samples have not been investigated. Apart from proximate composition and microbiological quality, this present study was therefore conducted to analyse free amino acid components, phytochemicals and some biological activities of commercial *Thua Nao* collected from local markets in Chiang Mai, Thailand.

3.2 Materials and methods

3.2.1 *Thua Nao* samples

Commercial fresh *Thua Nao* products used in this study were collected from six local markets: Mae Wang (MW), Mae Hia (MH), Mae Taeng (MT), Jom Thong (JT), San Patong (SP) and San Sai (SS) in Chiang Mai, Thailand. The samples once collected were transported to the laboratory in portable coolers and stored at -20°C until used.

3.2.2 Microbiological analysis

Fermented soybeans (5 g) were homogenised with 45 ml of sterile 0.1% peptone water (Merck, Germany) by stomaching for 2 min. Serial dilutions were prepared in 0.1% peptone water and 1 ml of appropriate dilutions were poured in duplicate plates of plate count agar (Merck, Germany) for viable counts of aerobic mesophilic bacteria, and yeast malt extract agar (HiMedia M424, India), pH 3.5 for yeasts and moulds. Spore counts were also determined with plate count agar with suspensions heated at 85°C for 20 min. Cultures were incubated in 37°C for 2 days (plate count agar for bacteria), 25°C for 3-5 days (yeast malt extract agar for yeasts and fungi). The colonies were then counted and expressed as logarithmic colony forming units per gram (log CFU/g) of sample.

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3.2.3 Physicochemical analysis

Proximate analysis of commercial *Thua Nao* products was determined using the standard AOAC methods (AOAC, 2000), No. 955.04 for protein content, No. 905.02 for fat content, No. 945.46 for ash measurement, and No. 990.19 for moisture content. Reducing and total sugars were determined using the dinitrosalicylic reagent

method (James, 1995). For pH, approximately 5 g of fermenting soybeans (wet weight) were homogenised in a blender with 50 ml of distilled water for 15 sec and the pH value of the suspension was measured with a pH meter (Consort C830, CE, Belgium). The colour of soybean surface was determined in L a* b* system by colourimeter Minolta Data Processor DP-301 (Chroma Meter CR-300 Series, Japan).

3.2.4 Free amino acids analysis

Preparation of samples

Two grams of freeze-dried *Thua Nao* (LABCONCO, FREEZONE PLUS, USA) were ground to a fine powder, defatted with distilled petroleum ether (Labscan, Dublin, Ireland) in Soxhlet apparatus and stored in screw-capped plastic tubes at -20°C until required. Preparation of each sample was carried out in triplicate.

Analysis of free amino acid contents

Defatted 100 mg samples were extracted with 25 ml of 70% (v/v) ethanol (Merck, Darmstadt, Germany) in an Ultra-Turax (T25 basic IKA-WERKE, Staufen, Germany) at approximately 6500 rpm for 10 sec at room temperature, and then centrifuged (KUBOTA 6930, Japan) at 2000*g* for 20 min at 4°C. The solvent was removed from the supernatants at 45°C by a vacuum evaporator (Büchi Rotavapor R-200, Switzerland). The collected suspension was finally dissolved in 10 ml borate buffer and filtered through a 0.45 µm filter (Sartorius GmbH, Gottingen, Germany) prior to HPLC analysis.

Free amino acids (FAA) were determined using pre-column derivatisation with 9-fluorenylmethyl chloroformate (Fmoc-Cl) (Sigma-Aldrich Co. St. Louis, MO., USA) followed by reversed-phase high performance liquid chromatography (RP-HPLC) in accordance with the protocol of Sarkar *et al.* (1997). A Shimadzu HPLC system was used with the following conditions: 5µm Restek C_{18} column (Restek, Belfast, UK), 250 x 4.6 mm Restek C_{18} guard column, a column heater, an Fl000 fluorescence detector (263 nm excitation and 313 nm emission), and a C-R6A Chromatopac (Shimadzu, Tokyo, Japan) integrator. Derivatives of FAA were separated using a binary gradient of Eluent A (20 mM ammonium dihydrogen orthophosphate (Ajax Finechem, New Zealand) and 15% methanol (Fisher Scientific,

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UK) and Eluent B (90% acetonitrile (Labscan) in water) as follows: 0 - 3 min, 18% B; 3 - 10 min, 23% B; l0 - 36 min, 48% B; 36 - 37 min, 55% B; 37 - 50 min, 100% B; 50 - 55 min, 18% B. The flow rate was 1 ml/min and the temperature was controlled at 37°C. Each amino acid was identified and quantified on the basis of each external standard curve (linearity of $R^2 > 0.995$).

Amino acid groupings

Amino acids were grouped as basic (Lys + His + Arg), acidic (Asp + Glu + Asn), total charged (basic + acidic), hydrophilic (total charged + Thr + Ser), hydrophobic (Val + Leu + Ile + Phe + Tyr + Trp + Met) and apolar (hydrophobic – Tyr) (Sarkar *et al*., 1997). In addition, taste attributes as described by Tseng *et al*. (2005) were also considered and used to categorise amino acids as monosodium glutamate-like (MSG-like) (Asp + Glu), sweet tasting (Ala + Gly + Ser + Thr), bitter tasting (Arg + His + Ile + Leu + Met + Phe + Trp + Val) and tasteless (Cys + Lys + Pro).

3.2.5 Isoflavone compounds analysis

Extraction of isoflavone compounds

Soybean isoflavones were extracted using the method of Achouri *et al.* (2005). Two grams of fine freeze-dried sample was dissolved with 10 ml of 80% (v/v) methanol (Merck) in a 50 ml screw-cap tube, vortexed for 1 min, sonicated in a FB 15046 sonicator (Fisher Scientific FB 15046, Germany) at 50 - 60 Hz of ultrasonic frequency for 15 min, and centrifuged at 3200 rpm for 30 min. The sample residue from the first extract was subjected to repeated extractions with 10 ml of 80% (v/v) methanol. Supernatants from two extractions were then combined, concentrated with an evaporator (Büchi Rotavapor R-200) and dried by nitrogen gas flow at room temperature. Dried isoflavones extract was subsequently dissolved with 3 ml of 80% (v/v) methanol and filtered through a 0.2 μm membrane filter (Sartorius) prior to HPLC injection. The sample was separately extracted in triplicate. The reliability of the extraction method was assessed by addition of known concentration of glycitein standard (Plantech, Reading, UK) into cooked non-fermented soybean before

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extraction of isoflavones. Recovery percentages for glycitein were calculated as 98% (n = 5).

Analysis of isoflavone compounds

The isoflavones were analysed by HPLC according to the method of Kim and Chung (2007). The Dionex HPLC system used was equipped with a P680 HPLC pump, ASI-100 automated sample injector, thermostatted column compartment TCC-100, Agilent Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), and PDA-100 photodiode array detector. The mobile phase was composed of 0.1% (v/v) acetic acid in filtered MilliQ water (solvent A) and 0.1% (v/v) acetic acid in acetonitrile (solvent B). The injection volume was 20 µl and the components were eluted using the following solvent gradient: from 0 to 50 min 15 – 35% solvent B; then held at 35% solvent B for another 10 min; and from 60 to 65 min re-equilibrated back to 15% solvent B with the flow rate of 1 ml/min. The spectra were collected between 240 and 400 nm and eluted isoflavones were monitored with UV at 254 nm. Stock solutions of six isoflavone standards (daidzin, glycitin, genistin, daidzein, glycitein, and genistein) (Plantech, Reading, UK) and internal standard of flavone (Sigma) were prepared in 80% (v/v) methanol. Calibration curves were plotted by using peak area and corresponding isoflavone concentration. The identity and purity of isoflavones in the samples were analysed by comparing the retention times and UV spectra of the standards.

3.2.6 Total phenolic compounds analysis

Methanol extraction

Based on the procedure of Lee *et al.* (2007a), the ground powder of the lyophilised sample (30 g) was extracted with 300 ml of 80% (v/v) methanol (Merck) for 24 h at room temperature with continuous shaking. The extracts were filtered through Whatman No.1 paper, concentrated under vacuum (Büchi Rotavapor R-200) at 40°C and freeze-dried (LABCONCO, FREEZONE 4.5). The lyophilised extracts were stored at -20°C, and before measuring the content of total phenolic compounds, antioxidant and antimicrobial activities, the extracts were dissolved in methanol.

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Assay of total phenolic compounds

Total phenols were analysed using the protocol of Lin *et al.* (2006). The methanolic extract solution (0.1 ml) was added to a mixture of 1.9 ml of deionised water and 1 ml of Folin-Ciocalteu phenol reagent (Sigma). After 8 min incubation, 5 ml of 20% (w/v) sodium carbonate (Ajax Finechem, Australia) were added and this mixture was then heated for 1 min. The absorbance was measured at 750 nm by a spectrophotometer (Perkin Elmer UV WINLAB, USA). Quantification of the total phenolics was performed using the linear regression equation of the gallic acid (Sigma) standard curve, and expressed as gallic acid equivalents (GAE).

3.2.7 Antioxidant activity assay

Determination of DPPH radical-scavenging activity

Free radical scavenging activity of the extracts was determined using the stable free radical 2,2-Diphenyl-picrylhydrazyl (DPPH) (Fluka Biochemica, Buchs Switzerland) method (Yun, 2005). One milliliter of methanolic lyophilised extracts (various concentrations from 1 – 20 mg/ml) was added to 2 ml of 75 μM methanolic solution of DPPH. The mixture was shaken and allowed to stand in the dark at room temperature for 5 min. The decrease in absorbance at 517 nm (Perkin Elmer UV WINLAB version 2.85.04) was then measured against methanol. The inhibitory percentage of DPPH was calculated according to the equation as follows: % scavenging activity = [1 – (As/Ac)] x 100; where As and Ac were the absorbance values at 517 nm of DPPH with sample and DPPH without sample (control), respectively. The percentage of scavenging activity obtained was subsequently plotted against the sample concentration. The half maximal inhibitory concentration (IC_{50}) was then calculated from the equation analysed from the logarithmic regression curve between soybean extract concentration (mg/ml) and scavenging activity (Parejo *et al.*, 2003). In addition, the efficient concentration representing amount of antioxidant required to decrease the initial DPPH concentration by 50% (EC_{50}) was calculated from the following formula: $EC_{50} = IC_{50}/$ [DPPH] in mg/ml. The antiradical power (ARP) describing the effectiveness of antioxidant and radical scavenging capacity was also determined as follows: ARP = $1/(EC_{50} \times 100)$.

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β-carotene-linoleate model assay

The total antioxidant activity of *Thua Nao* extracts was determined by using the β-carotene linoleic acid model system (Shon *et al*., 2007). A solution of β-carotene was prepared by dissolving 2 mg of β-carotene (Fluka, Spain) in 10 ml of chloroform (Labscan). Two milliliters of this solution were transferred into a 100 ml round-bottom flask. After the chloroform was removed under vacuum, 40 mg of purified linoleic acid (Sigma), 400 mg of Tween 40 emulsifier (Fluka, Spain), and 100 ml of aerated distilled water were added to the flask with vigorous shaking. This emulsion (4.8 ml) was added into 0.2 ml of 10 mg/ml sample extract. For control, 80% methanol was used in the reaction instead of the sample extracts. The mixture was then shaken and stored at 50°C for 2 h. The absorbance of the samples was measured at 470 nm (Perkin Elmer UV WINLAB) against emulsion without β-carotene (blank) at the beginning (0 min) and at the end of the experiment (120 min). Antioxidant activity was then calculated using the following equation: % Total antioxidant activity = $100 \times [1 - \{(A_{so} - A_{se})/(A_{co} - A_{ce})\}]$ where A_{so} and A_{se} were absorbance of the sample at 0 and 120 min, and A_{co} and A_{ce} were absorbance of the control at 0 and 120 min.

3.2.8 Antimicrobial activity assay

Testing microorganisms

The methanol extracted powders were tested against fifteen microbial pathogens. The testing microbes obtained from Thailand Institute of Scientific and Technological Research were *Staphylococcus aureus* TISTR118, *S. epidermidis* TISTR518, *Micrococcus luteus* TISTR884, *Bacillus cereus* TISTR687, *Escherichia coli* TISTR780, *Pseudomonas aeruginosa* TISTR781, *Salmonella typhimurium* TISTR292, *Enterobacter aerogenes* TISTR1468, *Candida albicans* TISTR5779 (ATCC10231), *C. famata* TISTR5098, *C. glabrata* TISTR5006, *Saccharomyces cerevisiae* TISTR5049 (ATCC4105), and *S. ellipsoideus* TISTR5194. *Listeria monocytogenes* DMST17303 and *Salmonella enteritidis* DMST15676 were obtained from the Department of Medical Science Thailand.

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Antimicrobial assay

The freeze-dried *Thua Nao* extracts were dissolved in the 80% methanol (v/v) to a final concentration of 500 mg/ml, centrifuged at 2000*g* for 30 min and filtered supernatant of sample by 0.2 μm Millipore filters (Minisart, Sartorious, Germany). Antimicrobial tests were then carried out by the paper disc diffusion method (Kim *et al*., 2004). The tested microorganisms were adjusted their turbidity to 0.5 McFarland standards from overnight agar cultures and swabbed over the dried surface of trypticase soy agar (Merck, Darmstadt, Germany) plate for bacteria and yeast malt agar (Merck) for yeasts. The antimicrobial activity was determined with the paper disc. Discs (6 mm diameter, Macherey-Nagel GmbH, Düren, Germany) were placed onto an agar plate inoculated with a test organism and loaded immediately of *Thua Nao* extracts fluid (20 μl). The diameter (mm) of inhibition was measured after incubation for 16 to 18 h at 35°C in incubator. Ofloxacin (5 μg/6 mm disc) and Netilmicin (30 μg/6 mm disc) purchased from Oxoid (Oxoid, UK) were used as standard antibiotics to compare the sensitivity of *Thua Nao* extracts against test microorganisms and the negative control was 80% (v/v) methanol. All determinations were made in separated triplicate. The antimicrobial potential of *Thua Nao* extracts were expressed in relative magnitude of inhibition (RMI) calculated as the ratio of area defined between zone of inhibition including disc of sample and the zone of negative control.

3.2.9 Statistical analysis

Data were expressed as means ± standard deviation of triplicate or duplicate observations. The data were also subjected to analysis of variance (ANOVA), *t*-test, and Duncan's multiple range tests. The significant differences between means were defined at $P \leq 0.05$.

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3.3 Results and discussion

3.3.1 Microbiological quality

Total viable count (TVC), spore count (SP) and total yeasts and moulds of commercial *Thua Nao* collected from six local markets in Chiang Mai are shown in

Table 3.1. The TVC examined were in the range of 9.57 - 10.59 log CFU/g. Similar number of viable counts has been reported in traditional *Thua Nao* collected from Lam Phun and Lam Pang as 8.43 to 10.64 log CFU/g (Sundhagul *et al*., 1972). Spore bacterial count was over 91% of TVC number in all samples studied indicating that spore-forming bacteria are the most important group responsible for *Thua Nao* fermentation. Previous studies have also shown the predominance of spore-forming bacteria in the fermentation of *Daddawa*, *Kinema*, *Dawadawa*, and *Thua Nao* (Sundhagul *et al*., 1972; Ogbadu and Okagbue, 1988; Tamang and Nikkuni, 1996; Dakwa *et al*., 2005; Chukeatirote *et al*., 2006). In traditional *Thua Nao*, Chukeatirote *et al*. (2006) identified the Genus and species of spore-forming bacteria involved in the product based on cell morphology and biochemical test showing that *Bacillus subtilis* is the most predominant species in this product. Moreover, *B. pumilus* and Gram-positive cocci were present in *Thua Nao* at 72 h-fermentation, while *Lactobacillus* spp. were identified at the beginning of the fermentation (12 - 24 h).

Table 3.1 Microbiological qualities of commercial *Thua Nao* products collected from six markets in Chiang Mai.

Source of *Thua Nao*	**Total viable count**	**Spore count**	**Yeast and mould**
Mae Wang	10.23 ± 0.02^{c}	9.66 ± 0.02^{ab}	<1
Mae Hia	10.59 ± 0.01^{a}	9.64 ± 0.02^{ab}	<1
Mae Taeng	10.33 ± 0.02^{b}	9.84 ± 0.07^{b}	<1
Jom Thong	9.60 ± 0.06^{d}	9.61 ± 0.06^{ab}	<1
San Patong	9.57 ± 0.03^{d}	9.76 ± 0.08^{b}	<1
San Sai	10.18 ± 0.04^{c}	9.42 ± 0.31^{b}	<1

Data are mean ± standard deviation (n = 3) and expressed in the unit of log CFU/g. Means within same column with different superscripts are significantly different ($P \leq 0.05$).

The problem of spoilage and pathogenic microorganisms contaminant in traditional fermented soybean which usually prepared by home-made based method were reported in previous literatures. Nout *et al*. (1998) reported the presence of several foodborne pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, Enterobacteriaceae, coliform and *Escherichia coli* in commercial *Kinema*.

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Furthermore, Omafuvbe *et al*. (2000) also described the occurrence of *Staphylococcus epidermidis* and *Micrococcus luteus* in soy-*Daddawa*. For traditional *Thua Nao* product, *Bacillus cereus* and Gram-positive cocci have also been reported by Leejeerajumnean (2003) and Chukeatirote *et al.* (2006). However, the strain identification of spoilage and foodborne organisms was not involved in this study. In order to solve the problem of hygiene and upgrade the quality of soy-fermented product, several investigators employed pure starter culture(s) in association with controlled production process to produce *Kinema* (Sarkar and Tamang, 1995; Tamang and Nikkuni, 1996), soy-*Daddawa* (Omafuvbe *et al*., 2002; Terlabie *et al*., 2006), *Chungkukjang* (Lee *et al*., 2005a) and *Natto* (Wei *et al*., 2001).

3.3.2 Physicochemical quality

The characteristics of commercial *Thua Nao* are shown in Figure 3.1. It should be noted that only slightly sticky substance could be detected and the visible dark brown colour of *Thua Nao* collected from six markets appeared widely different. This may be due to the effects of discrepancy between production processes including variety of soybean, boiling time of cooked soybean, fermentation conditions and period of soy incubation. Moreover, a typical unpleasant ammoniacal and fishy smells were liberated from all these products.

The physicochemical quality of commercial *Thua Nao* was investigated and the results are shown in Table 3.2. It was found that in general *Thua Nao* products showed the significant difference in chemical composition including ash, protein, fat, pH, fibre, reducing sugar and total sugar. However, there was no significant difference of moisture in these products with level ranging 62.94 to 64.78% dry basis ($P \leq 0.05$) except the product from SS (57.22%) which contained the lowest water. The lightness (L) of commercial *Thua Nao* products were between 38.63 and 47.18, redness (a*) ranged from 6.91 – 9.10; and yellowness (b*) were between 14.79 and 23.12.

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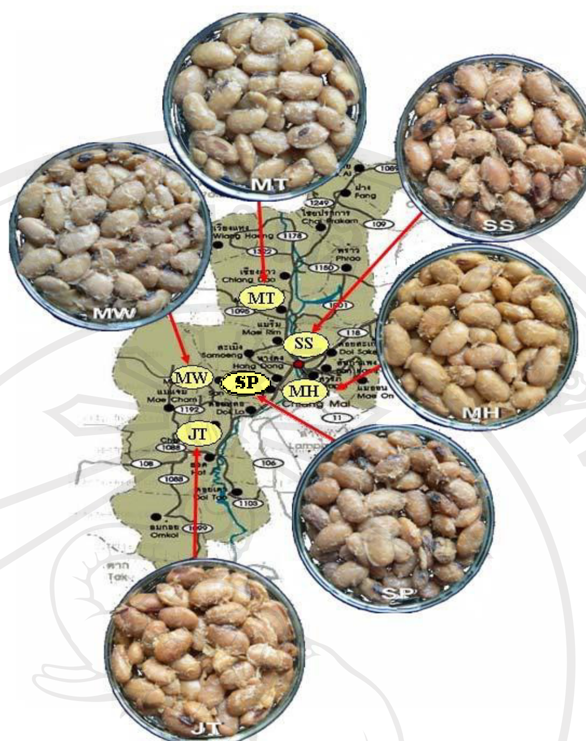

Figure 3.1 Geographic location of *Thua Nao* sampling sites (Chiang Mai, Thailand). The appearance of the *Thua Nao* products collected was also showed. MW, Mae Wang; MH, Mae Hia; MT, Mae Taeng; JT, Jom Thong; SP, San Patong; SS, San Sai.

Table 3.2 Physicochemical quality of commercial *Thua Nao* collected from six markets in Chiang Mai

Quality	**MW**	**MH**	**MT**	**JT**	**SP**	**SS**
Moisture	64.78 ± 0.54^{a}	62.94 ± 0.97^{a}	63.45 ± 2.86^{a}	63.10 ± 0.90^{a}	63.66 ± 1.18^{a}	57.22 ± 0.10^{b}
Protein	40.15 ± 0.65^{bc}	40.52 ± 0.60^{b}	39.44 ± 0.93^{bc}	39.95± 0.27^{bc}	42.06 ± 0.32^{a}	38.94 ± 0.21^{c}
Fat	20.37 ± 0.21^{d}	25.22 ± 0.08^{a}	20.53 ± 0.25^{d}	20.24 ± 0.13^{d}	22.22 ± 0.43^{c}	22.81 ± 0.13^{b}
Ash	5.33 ± 0.01^{a}	4.70 ± 0.04^{c}	5.37 ± 0.09^{a}	5.34 ± 0.02^{a}	5.44 ± 0.06^{a}	5.00 ± 0.14^{b}
Fiber	28.06 ± 0.24^{a}	27.87 ± 0.02^{ab}	26.71 ± 0.05^{c}	27.51 ± 0.26^{b}	27.96 ± 0.11^{ab}	12.92 ± 0.29^{d}
Reducing sugar	3.89 ± 0.06^{c}	3.12 ± 0.07^{e}	4.98 ± 0.01^{b}	3.47 ± 0.06^{d}	7.74 ± 0.11^{a}	2.70 ± 0.07^{f}
Total sugar	5.16 ± 0.13^{d}	5.70 ± 0.09^{c}	5.79 ± 0.05^{c}	7.38 ± 0.10^{b}	10.91 ± 0.06^{a}	4.79 ± 0.01^{e}
pH value	7.47 ± 0.01^{d}	7.08 ± 0.01^{f}	7.09 ± 0.01^{e}	8.25 ± 0.00^{a}	8.17 ± 0.00^{b}	7.89 ± 0.01^{c}
Colour L	43.61 ± 1.55^{b}	44.80 ± 1.96^{b}	47.18 ± 0.67^{a}	39.29 ± 0.29^{c}	39.53 ± 0.29^{c}	38.63 ± 1.40^{c}
Colour a*	7.77 ± 0.27b^{c}	9.10 ± 0.41^{a}	8.01 ± 1.10^{abc}	8.81 ± 0.40^{ab}	6.91 ± 0.56^{c}	7.98± 0.76^{abc}
Colour b*	18.43 ± 0.52^{b}	23.12 ± 1.36^{a}	18.30 ± 1.62^{b}	17.02 ± 0.72^{bc}	15.86 ± 0.14^{c}	14.79 ± 2.15^{c}

Data are mean ± standard deviation (n = 3) and expressed in the unit of % dry matter. Means within same row with different superscripts are significantly different ($P \leq 0.05$). MW, Mae Wang; MH, Mae Hia; MT, Mae Taeng; JT, Jom Thong; SP, San Patong; SS, San Sai.

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Previous reports including this study have verified the chemical composition of commercial fresh *Thua Nao* collected from local markets in Lam Phun, Lam Pang (Sundhagul *et al*., 1972), Chiang Rai (Chukeatirote and Thakang, 2006), and Chiang Mai (this study), as illustrated in Table 3.3. In general, the results obtained were similar to previous investigations except the high content of fat, possibly due to difference of soybean cultivar and production process. Essential unsaturated fatty acids including linoleic (an omega-6 fatty acid) and linolenic (an omega-3 fatty acid) acids are major components in soybean and thus soybean fat could be considered as healthy diet due to abundance in essential unsaturated fatty acids, low level of saturated fatty acids and absence of cholesterol (Liu, 2004). This study showed the pH value of the *Thua Nao* variable between 7.08 and 8.25, slightly different from the values of collected *Thua Nao* reported by Sundhagul *et al*. (1972) and Chukeatirote and Thakang (2006). The alkaline pH of *Thua Nao* is a typical characteristic of the product resulting from the basis end components especially ammonia via proteolysis of fermented organisms in soybean (Hesseltine, 1965; Omafuvbe, 2006).

Table 3.3 Chemical composition of commercial fresh *Thua Nao*[1]

Quality	**This study**	**Sundhagul *et al*. (1972)**	**Chukeatirote and Thakang (2006)**
pH	7.08 – 8.25	8.00 – 8.60	6.70
Moisture (%)	57.22 – 64.78	56.40 – 64.60	64.91
Protein (%)	38.94 – 42.06	38.76	40.84
Fat (%)	20.37 – 25.22	16.97	5.22
Fiber (%)	12.92 – 28.06	11.93	21.91
Ash (%)	4.70 – 5.44	5.73	4.70
Reducing sugar (%)	2.70 – 7.74	-	1.08

The values expressed in unit of dry matter. [1]Commercial fresh *Thua Nao* collected from different local markets in Chiang Mai (this study), Lam Phun, Lam Pang (Sundhagul *et al*., 1997) and Chaing Rai (Chukeatirote and Thakang, 2006). (-), data not available.

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3.3.3 Free amino acid profiles

The typical HPLC chromatograms of amino acid standards are presented in Figure 3.2a. The chromatogram of free amino acids extract from soybean products presented similar chromatograms to the pure amino acid standard (Figure 3.2b, c).

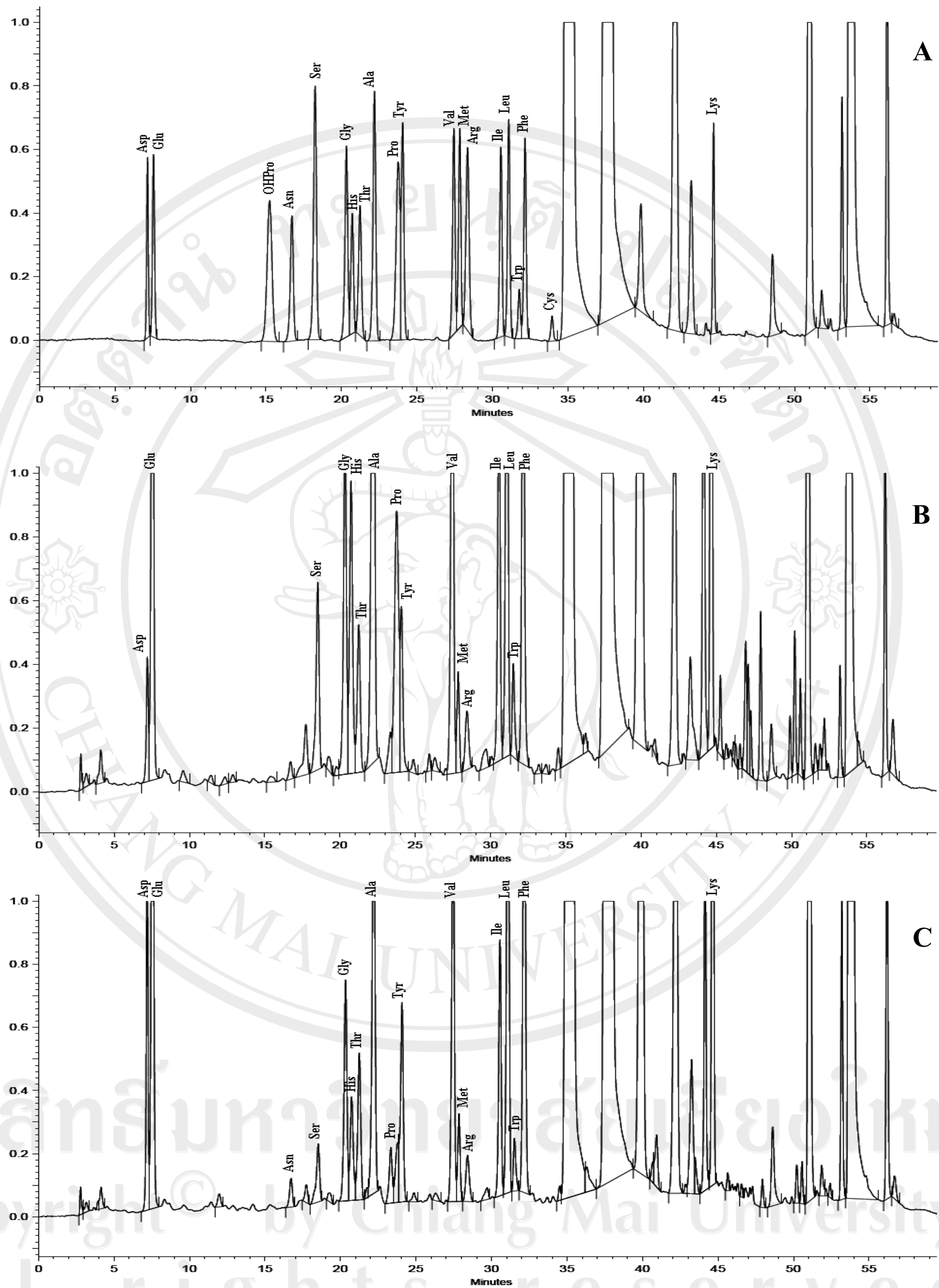

Figure 3.2 Reverse-phase HPLC patterns of authentic amino acids (A) and those extracted from *Thua Nao* products of Mae Taeng (B) and Mae Wang (C). Peaks are labeled with three letter abbreviations for amino acids.

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Table 3.4 shows profiles of free amino acids (FAA) in *Thua Nao* samples collected from six local markets in Chiang Mai, Thailand. Differences in amino acid contents were observed among these samples. The total amounts of FAA ranged from 11.03 (SS sample) to 61.23 g/kg (MT sample). The key factors causing such variations are probably due to different types of soybeans, fermenting microbes and process (Sarkar *et al*., 1997; Wei and Chang, 2004; Lee *et al*., 2005a). Previous studies also reported the contents of free amino acid in traditional fermented soybeans such as *Kinema* having total FAA of 21.21 - 106.11 g/kg (Sarkar *et al*., 1997) and *Chungkukjang* of 34.88 - 90.17 g/kg (Lee *et al*., 2005a). In general, Trp appears to be the most abundant amino acid, followed by Glu and Cys, respectively. The content of these three amino acids was very high representing at least 39% of total residues (MW, 55%; MH, 39%; MT, 48%; JT, 64%; SP, 59%; SS, 48%). In contrast, previous studies have showed that Glu and Asp were the most abundant amino acids in *Kinema* (Sarkar *et al*., 1997) and *Sufu* (Han *et al*., 2004). While the highest content of Leu, Phe and Glu were present in *Chungkukjang* (Lee *et al*., 2005a). In addition, OHPro displayed the lowest amount in commercial *Thua Nao* with proportion of 0.16 to 0.26%, except MT and JT samples that Asn (0.15%) or Pro (0.02%) was the lowest component respectively. Trp, Cys and Met were described as the major limiting amino acids in *Kinema* (Sarkar *et al*., 1997). The contents of sulphur-containing amino acids (Cys and Met) in soybeans are usually low (Imsande, 2003). Thus, high content of Cys in these *Thua Nao* samples (except the SS sample) is unexpected; this finding also indicates nutritive value of the *Thua Nao* products due to high content of sulphur-containing amino acids.

The profiles of essential amino acids (EAA) are also presented in Table 3.4, interestingly, all *Thua Nao* samples contained sufficient amounts of all EAA. Based on Lee *et al*. (1978), EAA can be classified in groups of either seven or ten amino acids. The EAA profiles of these products were in the range of 20.23 - 35.39% for EAA_7 and 59.10 - 65.97% for EAA_{10}, with the most abundant of Trp, Leu, and Lys. While in *Chungkukjang*, 47 - 53% of EAA were reported with the highest content of Leu and Phe (Lee *et al*., 2005a). MT sample contained the significant larger amount of EAA_7 than other samples ($P \leq 0.05$), with higher content around 6 times than the lowest content sample (JT). While SP and MT samples presented the highest contents

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of EAA_{10} with 5 times of the lowest contents sample (SS). These data (high content of Cys and EAA) suggest that *Thua Nao* products could be considered as a good source of protein.

Table 3.4 Compositions and quantities of free amino acid in *Thua Nao* products

FAA	***Thua Nao* sample**[1]					
	MW	**MH**	**MT**	**JT**	**SP**	**SS**
Ala	2.03 ± 0.03^{b}	1.77 ± 0.09^{b}	4.44 ± 0.30^{a}	0.12 ± 0.01^{d}	1.89 ± 0.09^{b}	0.79 ± 0.04^{c}
Arg	0.34 ± 0.02^{b}	0.24 ± 0.01^{c}	0.52 ± 0.00^{a}	0.10 ± 0.01^{d}	0.52 ± 0.01^{a}	0.12 ± 0.00^{c}
Asn	0.24 ± 0.02^{b}	0.50 ± 0.00^{a}	0.09 ± 0.01^{d}	0.04 ± 0.01^{e}	0.21 ± 0.00^{c}	0.10 ± 0.00^{d}
Asp	1.23 ± 0.04^{b}	1.35 ± 0.08^{ab}	0.27 ± 0.01^{c}	0.22 ± 0.01^{C}	1.53 ± 0.22^{a}	0.26 ± 0.00^{c}
Cys	5.09 ± 0.25^{c}	2.79 ± 0.09^{d}	8.62 ± 0.07^{a}	1.71 ± 0.10^{e}	5.63 ± 0.21^{b}	0.10 ± 0.00^{f}
Glu	4.75 ± 0.04^{b}	4.26 ± 0.39^{b}	4.87 ± 0.18^{b}	1.13 ± 0.07^{c}	6.11 ± 0.47^{a}	1.29 ± 0.02^{c}
Gly	0.66 ± 0.02^{c}	1.07 ± 0.09^{a}	1.06 ± 0.03^{a}	0.15 ± 0.00^{d}	0.93 ± 0.05^{b}	0.25 ± 0.02^{d}
His	1.06 ± 0.02^{c}	1.76 ± 0.18^{b}	3.19 ± 0.19^{a}	0.29 ± 0.00^{d}	1.29 ± 0.13^{c}	0.45 ± 0.03^{d}
OHPro	0.08 ± 0.01^{bc}	0.08 ± 0.02^{bc}	0.10 ± 0.01^{a}	0.06 ± 0.01^{c}	0.09 ± 0.00^{ab}	0.03 ± 0.00^{c}
Ile	1.09 ± 0.04^{c}	1.51 ± 0.03^{b}	1.69 ± 0.09^{a}	0.12 ± 0.00^{e}	1.48 ± 0.07^{b}	0.24 ± 0.02^{d}
Leu	3.50 ± 0.17^{ab}	3.57 ± 0.14^{ab}	3.70 ± 0.19^{a}	0.27 ± 0.00^{c}	3.26 ± 0.10^{b}	0.52 ± 0.02^{c}
Lys	3.43 ± 0.06^{b}	2.46 ± 0.09^{c}	4.40 ± 0.14^{a}	0.35 ± 0.01^{d}	3.29 ± 0.19^{b}	0.50 ± 0.04^{d}
Met	0.46 ± 0.08^{b}	0.34 ± 0.02^{c}	0.44 ± 0.01^{b}	0.13 ± 0.00^{d}	0.63 ± 0.01^{a}	0.13 ± 0.03^{d}
Phe	3.43 ± 0.06^{a}	2.33 ± 0.25^{c}	3.56 ± 0.10^{a}	1.43 ± 0.01^{d}	2.69 ± 0.03^{b}	1.01 ± 0.05^{e}
Pro	0.56 ± 0.00^{d}	1.05 ± 0.01^{b}	3.24 ± 0.12^{a}	0.00 ± 0.00^{f}	0.83 ± 0.00^{c}	0.21 ± 0.00^{e}
Ser	0.23 ± 0.01^{b}	0.70 ± 0.00^{a}	0.68 ± 0.04^{a}	0.05 ± 0.00^{c}	0.21 ± 0.01^{b}	0.05 ± 0.00^{c}
Thr	0.55 ± 0.01^{a}	0.24 ± 0.01^{c}	0.54 ± 0.05^{a}	0.20 ± 0.01^{c}	0.33 ± 0.03^{b}	0.08 ± 0.00^{d}
Trp	17.67 ± 1.08^{b}	6.77 ± 0.39^{c}	15.67 ± 1.95^{b}	5.64 ± 0.10^{c}	21.30 ± 2.04^{a}	3.92 ± 0.43^{c}
Tyr	2.19 ± 0.01^{a}	0.42 ± 0.00^{f}	1.67 ± 0.06^{c}	1.01 ± 0.05^{d}	1.95 ± 0.01^{b}	0.71 ± 0.01^{e}
Val	1.53 ± 0.05^{d}	2.00 ± 0.04^{b}	2.47 ± 0.12^{a}	0.17 ± 0.00^{E}	1.75 ± 0.09^{c}	0.26 ± 0.00^{e}
EAA_7[2]	13.99 ± 0.02^{b}	12.47 ± 0.57^{c}	16.80 ± 0.69^{a}	2.67 ± 0.01^{d}	13.43 ± 0.46^{bc}	2.75 ± 0.12^{d}
EAA_{10}[2]	33.05 ± 1.10^{b}	21.23 ± 0.00^{c}	36.19 ± 1.07^{a}	8.71 ± 0.11^{d}	36.54 ± 2.64^{a}	7.24 ± 0.28^{d}
Total FAA	$\mathbf{50.11\pm1.45^{c}}$	$\mathbf{35.22\pm0.60^{d}}$	$\mathbf{61.23\pm0.29^{a}}$	$\mathbf{13.20\pm0.00^{e}}$	$\mathbf{55.92\pm3.24^{b}}$	$\mathbf{11.03\pm0.33^{e}}$

Data are mean ± standard deviation (n = 2) and contents of free amino acid (FAA) expressed in g/kg dry sample. Means within a row varying letter are significantly different ($P \leq 0.05$).

[1]MW = Mae Wang; MH = Mae Hia; MT = Mae Taeng; JT = Jom Thong; SP = San Patong; SS = San Sai.

[2]EAA, essential amino acids were calculated according to the method of Lee *et al*. (1978); EAA_7: Val+Leu+ Ile+Thr+Lys+Phe+Met; EAA_{10}: EAA_7+His+Arg+ Trp.

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Based on amino acid structure and charge (Barrantes, 1975), amino acids can be grouped into hydrophilic, hydrophobic, and apolar classes as shown in Table 3.5. There appeared to be a preferential accumulation of some certain amino acids and thus representing their abundance in a particular group. The results clearly indicated the high content of amino acids with hydrophobic rather than hydrophilic property. In addition, the apolar amino acids were also a major group in these *Thua Nao* products. This result is in agreement with earlier report (Sarkar *et al*., 1997). Moreover, when considered from taste characteristics, FAA can also be classified into four major groups: MSG-like, sweet tasting, bitter tasting and tasteless (Tseng *et al*., 2005). As shown in Table 3.6, the most abundant tasty FAA class of the *Thua Nao* samples was the bitter FAA representing 53.75 - 69.39% of total FAA. It has been proposed that hydrophobic and apolar amino acid contents may cause a bitter flavour of the food product (Cho *et al*., 2004). Such a correlation (high content of hydrophobic and bitter FAA) was also observed in this study.

Table 3.5 Contents of FAA group based on charge criteria[1]

FAA	***Thua Nao* sample[2]**					
	MW	**MH**	**MT**	**JT**	**SP**	**SS**
Basic	4.82 ± 0.03^{bc}	4.46 ± 0.08^{c}	8.11 ± 0.33^{a}	0.74 ± 0.00^{d}	5.10 ± 0.32^{b}	1.07 ± 0.07^{d}
Acidic	6.22 ± 0.07^{b}	6.11 ± 0.48^{b}	5.23 ± 0.15^{c}	1.40 ± 0.07^{d}	7.84 ± 0.69^{a}	1.66 ± 0.02^{d}
Total charged	11.04 ± 0.05^{b}	10.56 ± 0.39^{b}	13.35 ± 0.48^{a}	2.14 ± 0.07^{c}	12.95 ± 1.01^{a}	2.72 ± 0.05^{c}
Hydrophilic	11.82 ± 0.04^{b}	11.50 ± 0.40^{b}	14.56 ± 0.57^{a}	2.40 ± 0.07^{c}	13.48 ± 1.03^{a}	2.86 ± 0.05^{c}
Hydrophobic	29.88 ± 1.10^{b}	16.95 ± 0.08^{c}	29.20 ± 1.39^{b}	8.76 ± 0.04^{d}	33.06 ± 2.29^{a}	6.80 ± 0.36^{d}
Apolar	27.68 ± 1.11^{b}	16.53 ± 0.08^{c}	27.54 ± 1.45^{b}	7.76 ± 0.10^{d}	31.11 ± 2.29^{a}	6.09 ± 0.35^{d}

Data are mean ± standard deviation (n = 2) and expressed in the unit of g/kg dry basis.. Means within a row varying letter are significantly different ($P \leq 0.05$).

[1]Calculated according to the method of Sarkar *et al*. (1997). Basic: Lys+His+Arg; Acidic: Asp+Glu+Asn; Total charged: basic+acidic; Hydrophilic: total charged+Thr+Ser; Hydrophobic: Val+Leu+Ile+Phe+Tyr+Trp+Met and Apolar: hydrophobic-Tyr.

[2]MW = Mae Wang; MH = Mae Hia; MT = Mae Taeng; JT = Jom Thong; SP = San Patong; SS = San Sai.

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Table 3.6 Composition of FAA class based on taste characteristics[1]

FAA	*Thua Nao* sample[2]					
	MW	MH	MT	JT	SP	SS
MSG-like	5.98±0.09[b]	5.61±0.48[b]	5.14±0.17[b]	1.36±0.07[c]	7.63±0.69[a]	1.55±0.02[c]
Sweet FAA	3.46±0.05[b]	3.79±0.00[b]	6.71±0.42[a]	0.52±0.00[c]	3.36±0.15[b]	1.17±0.03[c]
Bitter FAA	31.27±1.14[b]	18.94±0.09[c]	32.91±1.20[ab]	9.16±0.04[d]	34.87±2.42[a]	7.36±0.33[d]
Tasteless FAA	9.07±0.18[c]	6.31±0.19[d]	16.27±0.32[a]	2.06±0.10[e]	9.75±0.02[b]	0.81±0.05[f]

Data are mean ± standard deviation (n = 2) and expressed in the unit of g/kg dry basis. Means within a row varying letter are significantly different ($P \leq 0.05$).

[1]Calculated according to Tseng *et al*. (2005): MSG-like = Asp + Glu; sweet tasting= Ala + Gly + Ser + Thr; bitter tasting = Arg + His + Ile + Leu + Met + Phe + Trp + Tyr + Val; tasteless = Cys + Lys + Pro.

[2]MW = Mae Wang; MH = Mae Hia; MT = Mae Taeng; JT = Jom Thong; SP = San Patong; SS = San Sai.

3.3.4 Isoflavone compounds

The variation of isoflavone glucosides (daidzin, glycitin and genistin) and aglycones (daidzein, glycitein and genistein) in commercial *Thua Nao* collected from Mae Taeng is shown in Figure 3.3. The *Thua Nao* extract showed the largest proportion of agycone isoflavone (89% of total isoflavones) with the most predominant daidzein (45%). This result is in agreement with Wei *et al*. (2008) for the mainly proportion of daidzein in *Natto* produced from four strain of *B. subtilis.* Conversion of β-glucoside, malonylglucoside, and acetylglucoside isoflavones into aglycone form during fermentation via deglycosylation of β-glucosidase which produced from *Bacillus* has been reported previously (Kuo *et al*., 2006). In addition faster rate of daidzein conversion from daidzin than genistein from genistin was also reported by Kuo *et al*. (2006). Numerous studies reported the conversion of isoflavone aglycone in other soy-fermented foods by means of deglycosylation of β-glucosidase which produced from fermenting microorganisms during soybean fermentation process such as fermented soymilk (Chien *et al*., 2006; Chun *et al*., 2007; 2008), *Sufu* (Yin *et al*., 2004; 2005), *Tempeh* (Miura *et al*., 2002), and *Miso* (Yamabe *et al*., 2007). Aglycone soy isoflavones exhibit higher bioavailability and are absorbed faster with higher amounts in humans than are other forms (Izumi *et al*., 2000). Various health benefits such as prevention of mammary cancer (Gotoh *et al*., 1998; Peterson *et al*., 1998, Jung *et al*., 2006), reduced risk of cardiovascular diseases, improvement of bone health and menopause symptoms (Potter *et al*., 1998; Ishimi *et al*., 2002),

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antimutagenic effects (Park *et al*., 2003), and antidiabetic effects (Liu *et al*., 2006) related to isoflavone aglycone in soy foods.

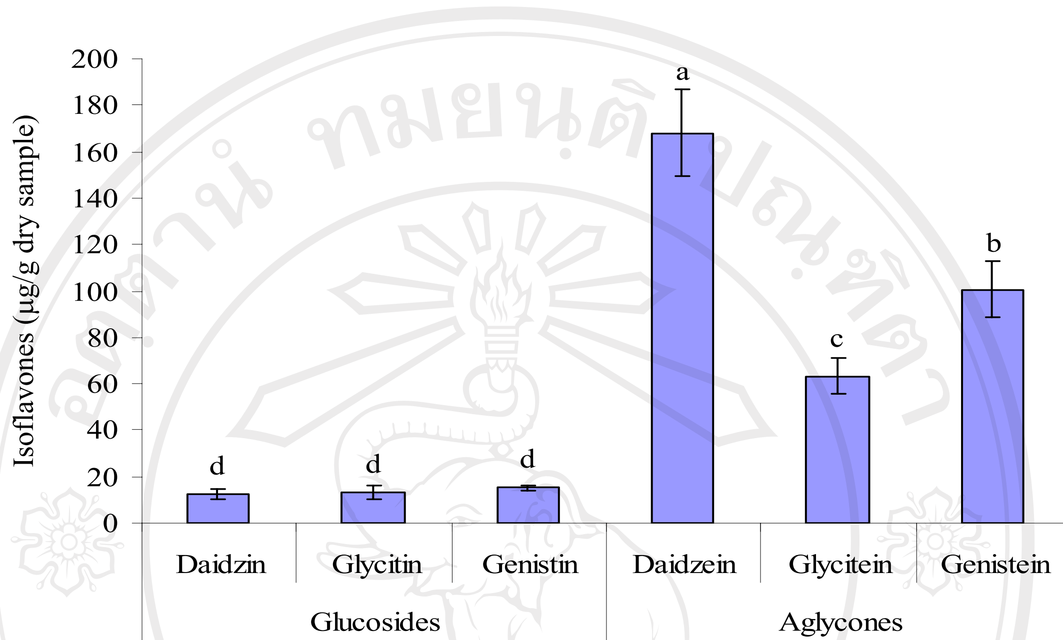

Figure 3.3 Composition and content of isoflavone compounds in *Thua Nao* collected from Mae Taeng. Each value represents mean ± SD (n = 3). Means (bar value) with different letters are significantly different ($P \leq 0.05$).

3.3.5 Phenolic compounds and antioxidant activity

In soybeans, phenolic compounds are one of the major groups of compounds acting as a primary antioxidants or free radical scavenger (Shahidi *et al*., 1992; McCue and Shetty, 2003). This study attempted to verify their contents and antioxidant activities of *Thua Nao* extracts. The contents of total phenolic compounds of *Thua Nao* extracts were measured according to the Folin-Ciocalteau method and their antioxidant activities were determined using two different methods: DPPH-free radical scavenging and inhibition of β-carotene co-oxidation in a linoleic acid model. The antiradical scavenger effect is the process to measure hydrogen donating ability of antioxidant component in sample to eliminate DPPH free radicals changing to form the stable DPPH-H (Yen and Duh, 1994) and the β-carotene linoleic model, antioxidants compounds can prevent the β-carotene-bleaching by neutralising the linoleic acid-free radical and other free radicals formed (Jayaprakasha *et al*., 2001). As shown in Table

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3.7, the total phenolic contents of methanol extracts of the various *Thua Nao*, varying on the source of sampling, range between 30.46 and 44.58 mg GAE/g extract. These values are relatively consistent to the findings of Lin *et al*. (2006), with ranged 23.70 to 45.72 mg GAE/g extract in soybean *Kojis*.

Table 3.7 Antioxidant activities and total phenolic compounds of *Thua Nao* extracts

Thua Nao	**TPC (mg GAE/g ext.)**	**DPPH-radical scavenging**			**AOX (%)**
		IC_{50} (mg/ml)	**EC_{50} (mg/mg DPPH)**	**ARP**	
Mae Wang	35.44 ± 2.09^{b}	2.64 ± 0.13^{bc}	89.15 ± 3.19^{c}	1.12 ± 0.04^{b}	59.45 ± 2.13^{a}
Mae Hia	35.25 ± 0.81^{b}	2.43 ± 0.09^{d}	82.11 ± 2.05^{d}	1.22 ± 0.03^{a}	56.27 ± 0.03^{b}
Mae Tang	33.11 ± 0.54^{b}	2.81 ± 0.02^{b}	94.90 ± 0.54^{b}	1.05 ± 0.01^{c}	51.94 ± 0.43^{c}
Jom Thong	43.33 ± 0.26^{a}	2.47 ± 0.08^{cd}	83.40 ± 1.96^{d}	1.20 ± 0.03^{a}	56.14 ± 0.31^{b}
San Patong	30.46 ± 0.85^{c}	3.19 ± 0.02^{a}	107.73 ± 0.36^{a}	0.93 ± 0.00^{d}	47.21 ± 2.61^{d}
San Sai	44.58 ± 2.27^{a}	2.51 ± 0.02^{cd}	84.94 ± 0.37^{d}	1.18 ± 0.01^{a}	58.72 ± 2.06^{ab}

Data are mean ± standard deviation (n = 3). Means within same column with different superscripts are significantly different ($P \leq 0.05$). TPC, total phenolic compounds; IC_{50}, half maximal inhibitory concentration; EC_{50}, efficiency concentration = IC_{50}/concentration of DPPH in mg/ml; ARP, anti-radical power = 100/EC_{50} (Prakash *et al*., 2007); AOX, total antioxidant determined by using the β-carotene linoleic acid system at 10 mg/ml of dried sample extracts.

Table 3.7 shows the scavenging activities of *Thua Nao* extracts in terms of half-inhibition concentration (IC_{50}), efficiency concentration (EC_{50}), and anti-radical power (ARP) and total antioxidant activity (AOX) measured by auto-oxidation of β-carotene and linoleic acid coupled reaction is also illustrated in Table 3.7. The wide variations of inhibiting free radical and total antioxidant were found in different commercial *Thua Nao*. The power of free radical terminators in IC_{50}, EC_{50} and ARP values of traditional *Thua Nao* extracts ranged from 2.43 to 3.19 mg/ml of sample extract including total antioxidant effect ranged from 47.21 to 59.45% at 10 mg/ml of sample extract. Differences of these properties may be derived from soybean variety (Troszynska and Ciska, 2002; Prakash *et al*., 2007), fermentation process e.g. fermentation temperature and period (Lee *et al*., 2007a) and starter organisms (Lin *et al*., 2006). SS, JT and MH samples did not show significant difference ($P > 0.05$) in the effect of free radical scavenger, but they presented noticeably higher activity than other samples together with evidence of lower IC_{50} and EC_{50} but higher ARP values (Table 3.7). Concerning total antioxidant effect, MW *Thua Nao* extract displayed

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significantly the highest inhibition amongst the *Thua Nao* collected. Interestingly, the San Sai sample exhibited a high antioxidant potential, with the highest antioxidant effects in all tests examined, in contrast the lowest antioxidant potential was identified in the extract of San Patong product.

The antiradical power and total antioxidant effect of collected *Thua Nao* in this study weaker than the inhibition effects that were reported in black soybean *Chungkukjang* (Shon *et al*., 2007) and *Koji* (Lee *et al*., 2007a). This discrepancy may be resulted from the influence of other polyphenolics involved in black soybean, anthocyanin pigment which has been reported to possess antiradical effect (Wang *et al*., 1999; Kathkonen and Heinonen, 2003) including difference in soybean cultivars and starter organisms. However, the effect in this study was relatively higher as compared to that of methanol extract of pure starter *Kinema* (Moktan *et al*., 2008). The weaker antioxidant effects of pure starter fermented black soybean *Chungkukjang* have been demonstrated than that found in natural fermentation products (Shon *et al.*, 2007). This phenomenon was affected by variety and selectivity of enzymes to degrade glycosidic linkages of the original glycoside phenolics into aglycone derivatives which indicated a greater biological effect (Georgetti *et al*., 2009).

Positive correlations between antioxidant activities and total phenolic contents of soybean and *Kinema* have been reported (Prakash *et al*., 2007; Moktan *et al*., 2008). Also, the finding of Kwak *et al.* (2007) suggested that higher level of phenolic and isoflavone compounds correlated with higher antioxidant activity significantly in *Chungkukjang*. In the present study, the high contents of isoflavones and phenolic components (Figure 3.3 and Table 3.7) were expected to be responsible for the higher free radical scavenging effects and total antioxidant activities of *Thua Nao* extracts. Besides antioxidant phytochemicals isoflavones and phenolics, other components including oligoproteins, free amino acids and melanoidins involved in fermented soybeans have been reported to support the antioxidant effect (Saito *et al*., 2003; Prakash *et al*., 2007; Rufian-Henares and Morales, 2007a; Wang *et al*., 2008).

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3.3.6 Antimicrobial activity

The inhibition activity of *Thua Nao* methanol extracts against foodborne pathogenic bacteria and some strains of yeasts were investigated by disc diffusion method. As shown in Figure 3.4 and Table 3.8, it was found that the methanol extracts of *Thua Nao* could inhibit only *B. cereus*. The extract of collected product from San Sai showed the highest antibacterial effect with the significant greater relative magnitude of inhibition ($P \leq 0.05$). The result in this study is contrary to that of Kim *et al*. (2004b) who reported the antibacterial effect of methanol extracts of *Chungkukjang*, traditional Korean fermented soybean, against *S. aureus* and *E. coli*. The study indicated that the phenylacetic acid produced by *B. licheniformis* during fermentation of soybean is one of the main compounds of antimicrobial activity of *Chungkukjang*. Yun (2005) demonstrated that *E. coli*, *S. aureus*, and *S. epidermidis* are the most resistant strains to antibiotic effect of *Doenjang* extracts. Also, the study reported the strongest antimicrobial effects against the facultative and obligate bacteria of ethanol and ethyl acetate *Doenjang* extracts. However, in this study these testing bacterial strains and the suitable solvents of antimicrobial substance extracts were not studied.

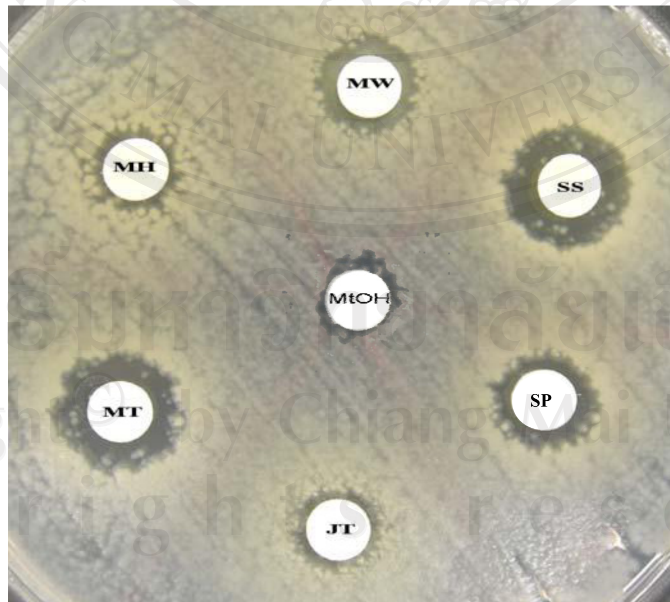

Figure 3.4 Inhibition zone against *B. cereus* TISTR687 of commercial *Thua Nao* collected from six local markets in Chiang Mai: MW, Mae Wang; MH, Mae Hia; MT, Mae Taeng; JT, Jom Thong; SP, San Patong; SS, San Sai and MtOH, negative control of methanol.

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Table 3.8 Antimicrobial activities of methanol extracts of commercial *Thua Nao* based on relative magnitude of inhibition[1]

Microorganisms	MW	MH	MT	JT	SP	SS
Staphylococcus aureus TISTR118	-	-	-	-	-	-
Staphylococcus epidermidis TISTR518	-	-	-	-	-	-
Micrococcus luteus TISTR884	-	-	-	-	-	-
Bacillus cereus TISTR687	1.2 ±0.2[cd]	1.1 ± 0.0[d]	1.7 ±0.2[ab]	1.3 ± 0.0[cd]	1.5 ± 0.1[bc]	1.9 ±0.0[a]
Escherichia coli TISTR780	-	-	-	-	-	-
Pseudomonas aeruginosa TISTR781	-	-	-	-	-	-
Salmonella typhimurium TISTR292	-	-	-	-	-	-
Salmonella enteritidis DMST15676	-	-	-	-	-	-
Enterobacter aerogenes TISTR1468	-	-	-	-	-	-
Listeria monocytogenes DMST17303	-	-	-	-	-	-
Candida albicans TISTR5779	-	-	-	-	-	-
Candida famata TISTR5098	-	-	-	-	-	-
Candida glabrata TISTR5006	-	-	-	-	-	-
Saccharomyces cerevisiae TISTR5049	-	-	-	-	-	-
Saccharomyces ellipsoideus TISTR5194	-	-	-	-	-	-

Data are mean ± standard deviation (n = 3). Means within same row with different superscripts are significantly different ($P \leq 0.05$);
- = no inhibition zone; MW, Mae Wang; MH, Mae Hia; MT, Mae Taeng; JT, Jom Thong; SP, San Patong; SS, San Sai.
[1]Relative magnitude of inhibition = area of inhibition zone of sample/area of inhibition zone of 80%methanol.

3.4 Conclusion

Food composition data are necessary to be considered from a nutritionist's viewpoint. It provides valuable information of nutritive value of the food products. In addition, these data can be used as nutritional standard or as the basis recommendation for Government's health policy. Previous reports including this study have verified the chemical composition of commercial *Thua Nao* as concluded in Table 3.3. This is required for the benefit of the nation and its own people. The development of the product can also be improved in an expectation that the nutritional quality would be better. *Thua Nao* products in this study appear to be a good protein source based on the amino acid profiles and potent to antioxidant diet food with the great contents of antioxidant phytochemicals and their strong antioxidant activities. Further work on development of *Thua Nao* nutritive quality using pure starter culture is being undertaken and the availability of these data is thus important as standard values.

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