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Nutritional and Physicochemical Qualities of *Thua Nao* (Thai Traditional Fermented Soybean)

Katekan Dajanta [a,b], Ekachai Chukeatirote*[c] and Arunee Apichartsrangkoon [b]

[a] Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand.

[b] Department of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand.

[c] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand.

*Author for correspondence; e-mail: ekachai@mfu.ac.th

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ABSTRACT

The content of total phenolics, DPPH-free radical scavenging effect, total antioxidant and antimicrobial activities of *Thua Nao*, a Thai traditional fermented soybean, collected from six local markets in Chiang Mai, Thailand were investigated. In addition, their physicochemical and microbiological qualities were also evaluated. Based on the proximate analysis results, some variations were found among these *Thua Nao* products. In general, the samples contained 57.22 - 64.78% moisture, 4.70 - 5.44% ash, 12.92 - 28.06% crude fibre, 38.94 - 42.06% crude protein and 20.37 - 25.22% fat. For microbiological quality, total viable counts were in the range of 9.57 - 10.59 log CFU/g. Bacterial spore count was over 91% in all samples indicating that spore-forming bacteria are predominant for *Thua Nao* fermentation. Total phenolic contents of *Thua Nao* extracts were also varied depending on the source; these were between 30.46 - 44.58 mg GAE/g extract. For antioxidant activity, the IC_{50} values as determined by the DPPH method ranged from 2.43 to 3.19 mg/mL of sample extract whereas total antioxidant activity as analysed by the carotene linoleate system were between 47.21 and 59.45% at 10 mg/mL of sample extract. Differences of these properties may be due to soybean variety, fermentation process, and starter organisms. In terms of antimicrobial activity, it was showed that the methanolic extracts of *Thua Nao* could inhibit only *Bacillus cereus*. Our study provides detailed information regarding nutritive quality of *Thua Nao*. The product appears to be a good protein source based on protein content and potent to antioxidant diet food with the great contents of antioxidant phytochemicals and their strong antioxidant activities.

Keywords: fermented soybean, *Thua Nao*, antioxidant activity, total phenolics, physicochemical quality, nutritive value

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 [1-4]. The most important activity that occurs during the fermentation process is proteolysis leading to unique flavour and taste [5, 6]. 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* [7], *Kinema* [8] and *Douchi* [9]. 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*. [1] and Chukeatirote and Thakang [10], including changes in biochemical and microbiological profiles during traditionally fermentation of *Thua Nao* [3]. Besides, health-promoting compounds such as subtilisin and gamma-polyglutamic acid of *Thua Nao* has recently been reported by Inatsu *et al*. [4]. However, such detailed information on other aspects is not available for *Thua Nao* product. For example, the antioxidant potential and 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.

2. MATERIALS AND METHODS

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.

2.2 Microbiological Analysis

Fermented soybeans (5 g) were homo genised with 45 ml of sterile 0.1% peptone water (Merck, Darmstadt, 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, Darmstadt, 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 then incubated at 37°C for 2 days (plate count agar for bacteria), at 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.

2.3 Physicochemical Analysis

Proximate analysis of commercial *Thua Nao* products was determined using the standard AOAC methods [11], 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 [12]. For pH, approximately 5 g of fermented 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).

2.4 Samples Extraction

Based on the procedure of Lee *et al.* [13], the ground powder of the lyophilised sample (30 g) was extracted with 300 mL of 80% (v/v) methanol (Merck, Darmstadt, Germany) 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, Switzerland) at 40°C and freeze-dried (LABCONCO, FREEZONE 4.5, USA). 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.

2.5 Total Phenolics Content

Total phenolics were analysed using the protocol of Lin *et al.* [14]. 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-Aldrich Co., St. Louis, MO, USA). 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 version 2.85.04, USA). Quantification of the total phenolics was performed using the linear regression equation of the gallic acid (Sigma-Aldrich Co., St. Louis, MO, USA) standard curve, and expressed as milligrams of gallic acid equivalents/g of sample extract (mg GAE/g).

2.6 DPPH Radical-scavenging Assay

Free radical scavenging activity of the extracts was determined using the stable free radical 2,2-Diphenyl-picrylhydrazyl (DPPH) (Fluka Biochemica, Buchs, Switzerland) method [15]. One milliliter of methanolic lyophilised extracts (various concentrations from 1 - 20 mg/ml) was added to 2 ml of 75 mM 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, USA) was then measured against methanol. The inhibitory percentage of DPPH was calculated according to the equation as follows: % scavenging activity = $[1 - (As/Ac)] \times 100$; 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 [16]. 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: $ARP = 1/(EC_{50} \times 100)$.

2.7 β-carotene-linoleate Model Assay

The total antioxidant activity of *Thua Nao* extracts was determined by using the β-carotene linoleic acid model system [17]. A solution of b-carotene was prepared by dissolving 2 mg of β-carotene (Fluka, Spain) in 10 ml of chloroform (Labscan, Dublin, Ireland). 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-Aldrich Co., St. Louis, MO, USA), 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 version 2.85.04, USA) 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.

2.8 Antimicrobial Activity Assay

The methanol extracted powders were tested against fifteen microbial pathogens. The testing microbes obtained from Thailand Institute of Scientific and Techno logical 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, *Saccha romyces cerevisiae* TISTR5049 (ATCC4105), and *S. ellipsoideus* TISTR5194. *Listeria monocytogenes* DMST17303 and *Salmonella enteritidis* DMST15676 were obtained from the Department of Medical Science Thailand.

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 supernatant of sample was filtered by 0.2 mm Millipore filters (Minisart, Sartorious, Germany). Antimicrobial tests were then carried out by the paper disc diffusion method [18]. The tested microorganisms were adjusted their turbidity to 0.5 McFarland standards from overnight cultures and swabbed over the dried surface of trypticase soy agar for bacteria and yeast malt agar 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 ml). The diameter (mm) of inhibition was measured after incubation for 16 to 18 h at 35°C. Ofloxacin (5 mg/6 mm disc) and Netilmicin (30 mg/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% 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.

2.9 Statistical Analysis

Data were expressed as means ± Data were expressed as means ± standard deviation of triplicate or duplicate observations. The data were also subjected to analysis of variance (ANOVA) and Duncan's multiple range tests. The significant differences between means were defined at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

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 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 [1]. 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 that the spore-forming bacteria are the major group in the fermentation of *Daddawa* [19], *Kinema* [20], *Dawadawa* [21], and *Thua Nao* [1, 3]. In traditional *Thua Nao*, Chukeatirote *et al*. [3] 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).

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*. [22] reported the presence of several foodborne pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, Enterobacteriaceae, coliform and *Escherichia coli* in commercial *Kinema*. Furthermore, Omafuvbe *et al*. [23] 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 [24] and Chukeatirote *et al*. [3]. However, the

Table 1. Microbiological quality of commercial *Thua Nao* products collected from six markets in Chiang Mai, Thailand.

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 ($P \leq 0.05$).

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* [20, 25], soy-*Daddawa* [26, 27], *Chungkukjang* [28] and *Natto* [29].

3.2 Physicochemical Quality

The characteristics of commercial *Thua Nao* 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 [6]. The physicochemical quality of commercial *Thua Nao* was investigated and the results are shown in Table 2. It was found that in general *Thua Nao* products showed the significant difference in chemical compositions 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 from 62.94 to 64.78% dry basis ($P \leq 0.05$) except the product from SS (57.22%) which contained the lowest water content. 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.

Previous reports including this study have verified the chemical compositions of commercial fresh *Thua Nao* collected from

Table 2. Physicochemical quality of commercial *Thua Nao* collected from six markets in Chiang Mai, Thailand.

	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}
Fibre (%)	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±0.27^{bc}	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.

local markets in several provinces of northern Thailand such as Lam Phun, Lam Pang [1], Chiang Rai [10], and Chiang Mai (this study), as illustrated in Table 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 [30]. 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*. [1] and Chukeatirote and Thakang [10]. 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 [31, 32].

Table 3. Chemical compositions of commercial fresh *Thua Nao*.

	Source of fresh *Thua Nao*		
	Chiang Mai[1]	Lam Phun and Lam Pang [1]	Chiang Rai [10]
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
Fibre (%)	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). (-), data not available.

3.3 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 [33, 34]. 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-Ciocalteu 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 [35] 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 [36]. As shown in Table 4, 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. [14], which were ranged from 23.70 to 45.72 mg GAE/g extract in soybean *Kojis*.

Table 4 also 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) including total antioxidant activity (AOX) measured by auto-oxidation of β-carotene and linoleic acid coupled reaction. 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, 82.11 to 107.73 mg/mg DPPH and 0.93 to 1.22, respectively 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 [37, 38], fermentation process e.g. fermentation temperature and period [13] and starter organisms [14]. Although the SS, JT and MH samples did not show significant difference ($P > 0.05$) in the effect of free radical scavenger, they presented noticeably higher activity than other samples; this is evident by the values of IC_{50}, EC_{50} (lower values), and ARP (higher values). Concerning total antioxidant effect, MW *Thua Nao* extract displayed significantly the highest inhibition among the *Thua Nao* collected. Interestingly, the SS 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 SP product.

The antiradical power and total antioxidant effect of collected *Thua Nao* in this study are weaker than the inhibition effects that were reported in black soybean *Chungkukjang* [17] and *Koji* [13]. 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 [39, 40] including difference in soybean cultivars and starter

Table 4. Total phenolics and antioxidant activities of *Thua Nao* extracts.

Source	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 = $100/EC_{50}$[38]; AOX, total antioxidant determined by using the b-carotene linoleic acid system at 10 mg/ml of dried sample extracts.

organisms. However, the antioxidant effect in this study was relatively higher as compared to that of methanol extract of pure starter *Kinema* [41]. The weaker antioxidant effects of pure starter fermented black soybean *Chungkukjang* have been demonstrated than that found in natural fermentation products [17]. 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 [42].

Positive correlations between antioxidant activities and total phenolic contents of soybean and *Kinema* have been reported [38, 41]. Also, the finding of Kwak *et al.* [43] 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 phenolics (Table 4) 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 [38, 44-46].

3.4 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. Of the tested microbes, it was found that the methanol extracts of *Thua Nao* could inhibit only *B. cereus*. In this study, we used the value of the relative magnitude of inhibition (RMI) which was calculated from the ratio between the area of the inhibition zone and the area of the negative control (80% methanol) to describe the antimicrobial activity. The RMI values of the *Thua Nao* extracts were varied ranging from 1.1 to 1.9 in which the extracts derived from San Sai showed the highest anti-*B. cereus* effect with the significant greater RMI ($P \leq 0.05$). The result in this study is contrary to that of Kim *et al*. [18] 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 [15] 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. It is therefore interesting to further explore whether the suitable solvents were used to extract the antimicrobial substance(s) in the future.

4. CONCLUSIONS

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. 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 protein

content 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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