

Assessment of Health Risks to People in Northeastern Thailand from the Intake of Sodium and Synthetic Preservatives Contained in Traditional Thai Fermented Fish Sauce (*Nam-Pla-Ra*) and Their Pathogenic Prevalence

Pimnibha Hirunsorn* and Mutiara Nur Isnainia

Department of Food Technology, Faculty of Technology, Khon Kaen University,
Khon Kaen 40000, Thailand

(*Corresponding author's e-mail: juthkh@kku.ac.th)

Received: 21 August 2021, Revised: 6 October 2021, Accepted: 13 October 2021

Abstract

This research aims to assess the health risks in Thailand from sodium and synthetic preservatives in traditional Thai fermented fish sauce (*nam-pla-ra*) and their pathogenic prevalence. Of 54 pasteurized-bottles and 37 bottles of Thai Papaya salad (*somtum*) sold by street vendors, the ingredients containing preservatives were found to be 63 (70 %) comprising 67 % (in bottles) and 53 % (of *somtum* ingredients) of different types, concentrations, for single or combined use. These preservatives analyzed by high performance liquid chromatography (HPLC) method did not comply with Thai Community Product Standards for traditional Thai *nam-pla-ra*. The estimated daily intake of sorbic and benzoic acid is below that of the ADIs (Acceptable Daily Intake) in all cases as the amounts were 0.11 - 3.25 % for benzoic acid and 0.00 - 0.01 % for sorbic acid. The sodium risk was estimated as 46.9 - 81.7 % affecting people aged 35 years old to the elderly (≥ 65 years) in pasteurized-bottles (81.7 %) and *somtum* ingredients (70.3 %). The pathogenic and sanitary indicators of microbes in *nam-pla-ra* obtained from *somtum* vendors need to be improved by educating the *somtum nam-pla-ra* vendors in hygienic standards. The scientific data in this work can provide a proper guide to suitable amounts of intake leading to more effective government regulations for *nam-pla-ra* or related products.

Keywords: Benzoic acid, Pathogenic prevalence, Sodium dietary exposure, Sorbic acid, Traditional Thai fermented fish sauce

Introduction

Ethnic *nam-pla-ra* (fermented fish sauce) is a traditional Thai condiment which is popular in every region of Thailand, especially in the Northern and Northeastern parts of Thailand. It is used in Thai papaya salad, *somtum pla-ra*, as a staple ingredient. *Nam-pla-ra* is made by filtering fermented fish (*pla-ra*) to obtain a raw sauce with some seasoning added (e.g., sugar, tamarind juice, and pickled garlic juice) to the sauce depending on the manufacturers' recipe, followed by pasteurization [1,2]. The unique and tasty *pla-ra* products are very popular with 20,000 - 40,000 tons/year being produced for both domestic consumption and exports overseas. Thailand has achieved the high export value of approximately 900 MB or 27 million USD (5 million tons) for the total of *pla-ra* products [3]. Of all FDA-certified *pla-ra* products, *nam-pla-ra* delivered the highest market share of 48 %, contributing 533 million USD (260 million tons) in export value as reported in 2019. The major destinations for these products are Japan, Cambodia, Australia, The United States, and Spain. The manufacturing zones for *pla-ra* products are scattered over all parts of Thailand but concentrated in the provinces in the Northeastern and Central regions from where they are distributed to large cities such as Khon Kaen in the main provinces.

Despite its appetizing flavor, the high nutrients, and probiotic benefits of *nam-pla-ra*, its high sodium content when consumed over a long period of time can become problematic for health as a result of excess sodium intake. The average daily consumption of *nam-pla-ra* was 10.13 g/person based on the food consumption of the Thai population in 2016 [4]. Therefore, Thais consume around 548 - 572 mg of sodium intake individually from *nam-pla-ra* on a daily basis, which is 1 quarter of the World Health Organization's (WHO) daily recommendation of 2,000 mg or 5 g salt [5]. Meanwhile, the sodium intake of Thais is up to 4,200 - 4,300 mg/day of their total food consumption. An intake of excess sodium is associated with raised

blood pressure and increased cardiovascular risk, chronic kidney disease, osteoporosis, and chronic renal failure (CRF) [6-9]. A high intake of sodium worldwide [10] was the leading dietary risk factor associated with 3 million deaths and 70 million disability-adjusted life-years (DALYs) lost in 2017. Thailand was ranked third in ASEAN for having the most patients with a kidney disease with about 7.6 million people suffering from chronic renal failure (CRF) which requires a medical budget of over 20 billion Baht annually [11].

With regard to diet, Thai people tend to gain sodium mostly from seasonings, condiments, and processed foods which result in an increased intake of sodium. Therefore, Thailand has implemented a salt reduction policy and regulations which will be supported through scientific research and surveys. The Low Salt Thailand Network which collaborates with the Thai Health Promotion Foundation (ThaiHealth) initiated the “Less Salt Project” in 2013, to increase awareness of consumers about salt intake by means of many campaigns, such as “Lod Kem Tham Dai” (Less Salt Action). Sauces and condiments are the leading causes of high sodium intake. In the Northeastern province of Isan, *nam-pla-ra* is in the third rank of the highest salt consumption after salt and fish sauce. Therefore, several research studies have investigated possible alternatives by examining the effects of sodium-reduced or salty sauce and condiments [12-14]. However, they still need at least 12 % salt for preservation purposes and a recommended diet of reduced salt intake is needed to encourage public health awareness. Few chemical risks were found from synthetic preservatives in *nam-pla-ra* products. Chalardsue Magazine funded by the Foundation of Consumers reported in 2018 that the use of preservatives was found in 15 pasteurized bottles of *nam-pla-ra* randomly taken from stores and open markets [15]. All these samples were tested with the Maximum Permitted Levels (MPLs) of each preservative according to the FDA regulations that year which varied from 0 - 831.8 mg/kg for benzoic acid and no sorbic acid was detected. However, the detection results and the labelling information caused controversy because, for example, the sample detected benzoic acid as high as 831.8 mg/kg but the labelling stated there was “no preservative use”.

Thus, it is important that precise amounts of daily intake of *nam-pla-ra* are recommended stating their nutritional value and use of food additives so that future regulations from Thai FDA will include them [16]. Nevertheless, none has reported status of health risks to Thai people from the intake of sodium and synthetic preservatives contained in traditional Thai fermented fish sauce (*nam-pla-ra*). The objective of this study was to determine the sodium content and concentrations of preservatives (benzoic acid and sorbic acid) in fermented fish sauce (*nam-pla-ra*), both pasteurized bottles and *somtum* ingredients obtained from vendors, and their respective dietary exposure (% risk) to people. An analysis of the information found on the labels compared the descriptions with the actual contents. The prevalence of pathogenic microbes in *nam-pla-ra* was also evaluated according to the Thai Community Product Standards. The results of this study will provide useful data to generate a new nutrition labeling policy, safety regulations, and a vigorous campaign for the safe and healthy consumption of *nam-pla-ra*.

Materials and methods

A total of 91 *nam-pla-ra* samples were collected from the central area of Khon Kaen province, Thailand, which included convenience stores, supermarkets, groceries, and open markets (sampling period: January - March 2019). The samples were divided into commercial pasteurized-bottles (54 samples or brands) and open containers from *somtum* vendors (37 samples). The pasteurized bottles were used to collect the samples from vendors with hygienic practice during sampling to prevent cross-contamination. The information about the preservatives (INS number, preservatives, etc.) on the labels of the packaging were also noted and recorded.

Determination of preservative (benzoic acid and sorbic acid) content

Sample preparation and HPLC conditions

Each *nam-pla-ra* sample (10 g) was measured and mixed with 35 mL of 80 % methanol. Then it was centrifuged at 2,000 - 2,500 rpm for 5 min (Hermle LaborTechnik GmbH - Z 200 A Universal Compact Centrifuge). After that, prior to HPLC injection, the supernatant was filtered from each sample with a nylon syringe filter (pore diameter of 0.45 μ m) into a HPLC vial for analysis (United States Department of Agriculture, 2011 with some modifications) [17]. Methanol (Duksan, Korea), Acetic Acid Glacial (QReC, New Zealand) and Ammonium Acetate (Carlo Erba, Italia) were used for the mobile phase preparation.

The HPLC system was carried out by reverse phase liquid chromatography (Pump model 600, injector model 486, and autosampler model 717, Waters, Milford, MA). The UV-Visible detector was used at the wavelength of 235 nm for the detection of benzoic and sorbic acid. The column HPLC was Column Agilent Poroshell 120 EC-C18, 3.0 \times 100 mm, 2.7 μ m and the column temperature was 40 °C. The injection volume

was 1.0 μL while the mobile phase used an ammonium acetate buffer: methanol (33:67), at a flow rate of 0.5 mL/min set for isocratic elution. Total running time was 35 min for each sample.

Standards and standard curve preparation

The standard curve preparation method was followed to carry out an analysis of the preservatives using high performance liquid chromatography (HPLC) with some modifications. Briefly, benzoic (Fluka, Switzerland) and sorbic (Merck, Germany) acid stock solution were prepared at 1,000 mg/L with methanol: Water, 70:30 v/v in a 100 mL-volumetric flask. Then benzoic acid: 20, 15, 10, 5 mL and sorbic acid: 1, 0.75, 0.5, 0.25 mL of the working stock solution were pipetted into a volumetric flask size 50 mL and adjusted to 50 mL with 70 % methanol to achieve 400, 300, 200, 100, ppm and 3.2, 2.4, 1.6, 0.8 ppm of benzoic and sorbic acid solution, respectively. After that, each solution was filtrated by a syringe filter 0.45 μm to obtain clearer solutions which were kept in vials.

Determination of sodium content

Sample preparation and analysis

Sample preparation and instrumental analysis were carried out with some adjustments according to the method stated in Nielsen [18]. A 1 g sample in a glass tube was added with 65 % HNO_3 (QReC, New Zealand) 5 mL then the tube was placed on a block and hot plate at 140 $^\circ\text{C}$ for 2 h for the digestion and cooling process according to the standard preparation above. After the sample was filled with ultrapure grade water, it was then filtrated with Whatman filter paper no. 1 and kept for analysis. Determination of sodium was performed using an atomic absorption spectrometer (AAS; PinAAcle 900 F, USA). An air-acetylene flame was used in an AAS machine with flow rates of 13.5 L/min of air and 2.0 L/min of acetylene. Absorbance signals were measured using a 330.24 nm line at a bandpass of 0.2 nm.

Standards and standard curve preparation

The standard sodium analysis curve was calculated with slight adjustments following the Nielsen [18] procedure. 1 g of NaCl (99.999 %, Ajax Finechem Pty Ltd., NWS, Australia) was diluted in 5 mL % HNO_3 (QReC, New Zealand) and proceeded by inserting the glass tube in block and hot plate followed by 2 h of digestion at 140 $^\circ\text{C}$. After the digestion cycle was complete it was cooled. A few drops of ultrapure grade 1 water (Milli-Q Millipore 18.2 M Ω cm resistivity, Millipore Corp. Billerica, MS, State) were poured through the glass tube wall. The NaCl extracted from samples was then poured into a volumetric flask of 100 mL. In the next step, the glass tube was rinsed with ultrapure water grade 1 for 5 times and then poured into a volumetric flask. When the ultrapure water grade 1 added reached the line mark, it was then shaken until homogeneous. A standard series was performed in 100 mL volumetric flask: 0, 0.25, 0.5, 1, 2, 4 and 6 mL (0, 25, 50, 100, 200, 400 and 600 ppm). They were adjusted to the marking line with ultrapure grade 1 water and then they were ready for analysis.

Dietary exposure of Khon Kaen population to preservatives and sodium from nam-pla-ra consumption

Dietary exposure known as Estimated Daily Intake (EDI) was calculated by multiplying the concentration of preservatives in each food product with the individual food consumption data from a Thai population survey in 2016. This method was performed based on a deterministic method (IPCS, 2009, Chapter 6) as shown in the following Eq. (19).

$$\text{Dietary exposure} = \frac{\Sigma (\text{Concentration of chemical in food} \times \text{Food consumption})}{\text{Body Weight (kg)}}$$

Probabilistic exposure assessment was conducted to estimate the degree of exposure at the mean and high levels (97.5th percentile) in addition to per capita (all population) and the eater only category. Dietary exposure is the daily chemical (preservative) intake (mg/kg b.w./day). The concentration of chemicals (preservatives) in food was analyzed in mg/kg. Food consumption is the average intake of food containing preservatives per day. The body weight of consumers was in kg. The dietary intake of sodium was calculated in mg/day unit and the ADI for sodium consumption was < 2,000 mg sodium/day according to WHO recommendations [5].

Microbiological analysis

To determine the microbiological numbers, each 10 g sample was transferred into a sterile stomacher bag containing 90 mL peptone water (PW) and homogenized for 2 min. One mL aliquots of the samples

were serially diluted in 9 mL of PW, and 0.1 mL of sample or diluent was plated onto each medium. Total plate count (TPC) (by pour plate method) was determined using Plate Count Agar (PCA) incubated at 35 °C for 24 - 48 h. Baird Parker (BP) Agar and Mannitol egg yolk polymyxin (MYP) agar with egg yolk emulsion were used as selective media for enumeration of *Staphylococcus aureus* [20] and *Bacillus cereus* (ISO 7932:2014) [21], respectively, incubated at 37 °C for 24 h. Tryptose Sulphite Cycloserine Agar (TSC, 37 °C for 24 h) was used for enumeration of *Clostridium perfringens* [22]. Chromocult® Coliform Agar and Plate Count Agar (CCA, 35 °C for 24 - 48 h) for *E. coli* and coliforms (ISO 9308-1:2014) [23]. The results were expressed as the logarithm of the colony forming units per gram (log CFU/g). In order to detect *Salmonella*, each sample was tested for its presence or absence according to ISO 6579:2017 [24]. All media for the microbiological analyses were purchased from HiMedia Laboratories, Mumbai, India.

Statistical analysis

The results are given as mean \pm SD and the ranges of preservative/sodium. Comparisons of the concentration of sodium, preservatives, and the microbial counts in the sample groups were carried out using the statistical program of SPSS 25.0 software for Windows (IBM Corp., NY). The mean values were separated using independent sample t-test, and $p \leq 0.05$ was used to determine significant differences.

Table 1 Frequency, range, mean concentration, and number of samples exceeding MPLs of preservatives (sorbic acid/benzoic acid alone and both combinations) in *nam-pla-ra* products collected in Central Khon Kaen, Thailand.

Bottle (commercial brand) (54 samples)				Sontum vendor container (37 samples)					
Positive samples (%)	Range (mg/kg)	Mean* (mg/kg)	> MPL ^a	> MPL ^b	Positive samples (%)	Range (mg/kg)	Mean* (mg/kg)	> MPL ^a	> MPL ^c
Benzoic acid (BA)									
36 (67 %)	9.8 - 967	184.5 \pm 0.9	36	4	27 (73 %)	5.4 - 4,291.6	354.5 \pm 1.7	27	2
Sorbic acid (SA)									
3 (6%)	2.2 - 3.0	2.5 \pm 0.1	3	0	0 (0 %)	0	0	0	0
SA + BA in combination									
Positive samples (%)	Range (mg/kg)	Mean* (mg/kg)	Ratio > 1 ^d		Positive samples (%)	Range (mg/kg)	Mean* (mg/kg)	Ratio > 1 ^d	
7 (13 %)	0.1 - 0.5	0.3 \pm 0.2	0		6 (16 %)	0.1 - 0.7	0.3 \pm 0.2	0	

*Means of analyzed value of preservative types in positive samples.

^aMPL = Not allowed any usage of preservatives (Thai Community Product Standards (NAM PLA RA)) [2].

^bMPL = Maximum Permitted Level of benzoic acid for condiments; at which allowed not exceed 500 mg/kg.

^cMPL = Maximum Permitted Level of sorbic acid for condiments; at which allowed not exceed 1,000 mg/kg

^dMPL = The combined use of 2 or more food preservatives (classified as the same functional class).

^{b, c, d}Based on Announcement of the Food and Drug Administration Re: Notification of Thai FDA Ministry of Public Health Revised (No. 418) B.E. 2563 Issued by virtue of the Food Act B.E. 2522 Re: Prescribing the principles, conditions, methods and proportion of food additives (No.2) [25].

Results and discussion

Occurrence and dietary exposure to preservatives in *nam-pla-ra* products

Table 1 exhibits the number of sorbic acid and benzoic acid-positive samples, the range, the mean concentrations, and the samples which exceed the permitted criteria of both sets of major Thai regulations in tested *nam-pla-ra* products. Of all the 91 *nam-pla-ra* samples, 63 preservative-positive samples (70 %) comprised 36 of 54 brands (67 %) and 27 from 37 vendors (53 %) with different types, concentrations, and single/combined use. The higher benzoic acid-positive samples (63, 69 %) of the total 91 samples were revealed versus much lower sorbic-acid positive in 3 samples (3.3 %) regardless of the sample sources. The range in the levels of benzoic acid was 5.4 - 4,291 mg/kg, and the mean concentration was 269.5 \pm 1.3 mg/kg. Of the different sources, benzoic acid was mostly found in bottled *nam-pla-ra*, 36 samples were in the range 9.8 - 967 mg/kg, on the other hand, only 3 samples of *nam-pla-ra* obtained from vendors revealed

a much higher range (5.4 - 4,291.6 mg/kg) than expected. No sorbic acid was found in any of the *nam-pla-ra* samples obtained from vendors but it was found in 3 bottles only, with a limited range between 2.2 and 3.0 mg/kg and an average mean of 2.5 ± 0.1 mg/kg. *Nam-pla-ra* products usually contain some added benzoic acid, avg. 269.5 ± 1.3 mg/kg, a small amount of sorbic acid, avg. 2.5 ± 0.1 mg/kg, and the lowest use of BA and SA combined, 0.3 ± 0.2 mg/kg.

According to the Thai Community Product Standards for *nam-pla-ra* products specified by the Thai Industrial Standards Institute (TISI), no preservatives and coloring agents are allowed. To these standards, many product samples were compliant, including 36 bottles and 27 samples obtained from vendors (benzoic acid), 3 bottles (sorbic acid), and 7 bottles and 6 samples from vendors (BA + SA) of *nam-pla-ra* products (**Table 1**). In recent years, a revision of these regulations has reduced MPLs significantly and also specifies controls on excessive quantities of similarly functional additives of at least 2 types (especially health-affected additives) to protect health of consumers. With regard to the use of 2 or more food additives, a maximum level of use permitted is prescribed, and also the sum of the proportions is calculated by dividing the concentrations of each food additive by the maximum level of use permitted for a particular additive which must not exceed 1 [26]. Most of the *nam-pla-ra* samples analysed were within the permitted limits, except for 4 bottles and 2 samples from vendors containing benzoic acid as a preservative. None of the samples had a concentration of sorbic acid and benzoic acid over 1.

No research studies have been reported on preservative issues in such traditional *nam-pla-ra* products. However, a survey report of Chalardsue Magazine in 2018 [15] reported the presence of benzoic acid (12 positive from 15 samples) within the lower ranges (5.67 - 831.8 mg/kg) of samples from commercially pasteurized bottles of *nam-pla-ra* from the open market, which are lower than those reported in the present work. Of the positive samples, none complied with the FDA regulations for that period of time, but 1 sample was not within the permissible limit in line with more recent criteria.

The EDI and risk exposure (% ADI) of sorbic acid and benzoic acid through the consumption of bottled *nam-pla-ra* (result not shown) and somtum ingredients (**Table 2**) among Thai population age groups in the range of 3 - 5.9 and > 3 years are shown. The EDIs for commercially pasteurized bottled *nam-pla-ra* samples were estimated at 0.01 - 0.16 mg/kg b.w./day for benzoic acid and 0.00 mg/kg b.w./day for sorbic acid which represents % ADI at a very low value, 0.11 - 3.25 % for benzoic acid and 0.00 - 0.01 % for sorbic acid based on age groups. Children aged 3 - 5.9 years exposed to benzoic acid at a mean intake of *nam-pla-ra* consumption for the eater only category represented 1.37 %. Moreover, a high intake of benzoic acid among the eater only category was only at 97.5th percentile which increased to 3.25 %. A slightly higher EDI and derived % ADI of *nam-pla-ra* ingredients for *somtum* was found. The EDI was in the range of 0.01 - 0.31 g/kg b.w./day for benzoic acid and 0.00 g/kg b.w./day for sorbic acid. These values contributed to the exposure to benzoic acid at the 97.5th percentile, with mean estimations of 6.24 and 2.63 %. Therefore, the consumption of *nam-pla-ra* (bottled and *somtum* ingredients) is not a matter of serious concern regarding the risks of exposure to preservatives.

Table 2 Estimated daily intake (EDI) of sorbic acid and benzoic acid from *pla-ra* sauce consumption from *somtum* vendor containers by Thai population and the respective comparison with acceptable daily intake (ADI) as % ADI (EDI/ADI*100). ADI of benzoic acid was 5 mg/kg b.w./day and ADI of sorbic acid was 25 mg/kg b.w./day.

	Estimation method	Mean				97.5 th Percentile			
		Per capita		Eater only		Per capita		Eater only	
Preservative types	Age (y)	3 - 5.9	> 3	3 - 5.9	> 3	3 - 5.9	> 3	3 - 5.9	> 3
	Food consumption data of Thai population (2016) (g/kg b.w./day)	0.03	0.04	0.37	0.18	0.3	0.3	0.88	0.52
Benzoic acid	EDI (mg/kg b.w./day)	0.01	0.01	0.13	0.06	0.11	0.11	0.31	0.18
	% ADI*	0.21	0.28	2.62	1.28	2.13	2.13	6.24	3.69
Sorbic acid	EDI (mg/kg b.w./day)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	% ADI*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

ADI = EDI/ADI*100.

*Per Capita = Per capita, the food consumption based on overall Thai population.

*Eater Only = Eater only, the food consumption based on only Thai population eating that defined food type.

*ADI of sorbic acid was 25 mg/kg b.w./day [25,27].

*ADI of benzoic acid was 5 mg/kg b.w./day [25,27].

Sodium content and dietary exposure to sodium through the consumption of *nam-pla-ra*

The sodium contents of 91 *nam-pla-ra* samples are shown as mean, standard deviation in **Table 3**. Commercially pasteurized bottled *nam-pla-ra* had a sodium content of 60.5 mg/g (Range: 40.8 - 82.2 mg/g), which is significantly higher than those of the *somtum* ingredients at 52.1 mg/g sample 32.2 - 77.2 mg/g) ($p \leq 0.05$). The Thai Community Product Standards [2] specify that *nam-pla-ra* or *pla-ra* sauce should consist of at least 120 g/L salt (12 %) with the sodium content distributed equally with 48 mg/mL based on safety and fermentation aspects. The salt content from the average of sodium detected in both groups of samples were in accordance with official standards and with a higher concentration of approximately 1.25 times (Salt concentration: 15.1 % for bottles and 13 % for *somtum* ingredients of *nam-pla-ra*). Fermented condiments and/or processed fish products (i.e., fish sauce) contained high amounts of salt yielding a very high sodium content up to 27.82 % (w/v) of the product [28].

Table 3 Sodium content determined and their dietary intake of sodium through bottled- and vendor-received *nam-pla-ra* products in several age groups for eater only.

	Sodium content determined*	Estimation method		Mean				
		Age (y)	3 - 5.9	6 - 12.9	13 - 17.9	18 - 34.9	35 - 64.9	≥ 65
<i>Pla-ra</i> sauce samples		Daily intake of <i>nam-pla-ra</i> (2016) (g/day)	6.02	8.86	8.96	10.46	10.47	9.58
		EDI (mg/day)	364.3	536.2	542.2	633.0	633.6	579.7
Bottled <i>nam-pla-ra</i>	60.5 ± 0.1 ^a	% ADI	18.2	26.8	27.1	31.6	31.7	29.0
<i>Somtum</i> vendor containers	52.1 ± 0.1 ^b	EDI (mg/day)	313.4	461.3	466.5	544.6	545.1	498.8
		% ADI	15.7	23.1	23.3	27.2	27.3	24.9
		Estimation method		97.5 th percentile				
		Age (y)	3 - 5.9	6 - 12.9	13 - 17.9	18 - 34.9	35 - 64.9	≥ 65
Bottled <i>nam-pla-ra</i>	As above	Daily intake of <i>nam-pla-ra</i> (2016) (g/day)	18	18	18	18	27	27
		EDI (mg/day)	1,089.3	1,089.3	1,089.3	1,089.3	1,633.9	1,633.9
<i>Somtum</i> vendor containers	As above	% ADI	54.5	54.5	54.5	54.5	81.7	81.7
		EDI (mg/day)	937.2	937.2	937.2	937.2	1,405.8	1,405.8
		% ADI	46.9	46.9	46.9	46.9	70.3	70.3

*Means of sodium content in different letters were significantly different ($p \leq 0.05$).

*EDI of sodium in the respective comparison with acceptable daily intake (ADI_{Na}) as % ADI, % ADI = EDI/ADI_{Na} .

Daily sodium intake for different ages at were estimated at means from 364.3 - 633.6 mg/day from the pasteurized bottled samples while a lower range of 313.4 - 545.1 mg/day was calculated for the samples of *somtum* ingredients. At 97.5th percentile, a higher intake of values for different ages was shown, with pasteurized bottled samples: 1,089.3 - 1,633.9 mg/day, and *somtum* samples: 937.2 - 1,405.8 mg/day. In spite of an optimum sodium intake/day from all food products as recommended by WHO (not exceeding 2,000 mg/day - or 1 teaspoon), the values were very high and close to the limit for the consumption of *nam-pla-ra* products. While, in 2012, the estimated salt intake in Southeast Asia from all food consumption in 20 - 30 year old adults was at 7.6 g/day and in 60 - 81 years old was 8.8 g/day based on data obtained either from food frequency questionnaires, 24 h dietary recalls, 5 day food records or 48 h urine sodium measurements [29].

To assess the health risks as the mean estimation, it was found that populations were still safe when exposed to sodium as seen from all % ADI values below 100 %, ranging from 15.7 - 31.7 %. The middle age group of 18 - 34 years and the full adult group (35 - 64 years old) were subject to the highest exposure to sodium at 27 - 32 % from consuming both *nam-pla-ra* from commercially pasteurized bottles and the containers sold by *somtum* vendors, followed by the elderly age group (24.9 - 29 %). The exposure to sodium from the *nam-pla-ra* commercial bottles (18.2 - 31.7 % ADI) was significantly greater than that

from the *somtum* ingredients (15.7 - 27.3 % ADI). The estimation at the 97.5th percentile for the eater only group showed that the risks increased from 46.9 - 81.7 % for the older age groups ranging from 35 years old to elderly from commercially pasteurized bottles of *nam-pla-ra* (81.7 %) and *somtum* sauce ingredients (70.3 %). These findings on sodium content in fermented fish sauce and review of labelling will provide useful data for a new nutritional labeling policy (e.g., lower RDIs for sodium per day), for a campaign/project for increasing consumers' awareness on the consumption risks of high sodium intake, should also encourage product reformulation by manufacturers (e.g., healthier choices with lower levels of sodium in products).

Product label verification of preservatives in bottled *nam-pla-ra* products declaration

More than half of the commercially pasteurized bottles of *nam-pla-ra* surveyed were labeled in Thai (65 %, n = 35) by the manufacturers while the rest were in both Thai and English (35 %, n = 15). Of a total of 54 products, 25 products showed statements about preservatives including the following: "No preservative used" (41 %, n=22), "Preservative use" (2 %, n=1), and type or INS "Benzoic acid" or "INS 211" (n = 2, 4 %). The majority of the products (54 %, n = 29) did not display any information about their use of preservatives.

The Food and Drug Administration of Thailand (FDA Thailand) established mandatory regulations with a declaration of preservative use in "Labeling of Pre-packaged Foods" (No.367) B.E. 2557 [30]. This included the functional class titles of food additives (i.e., preservatives) together with specific names (benzoic acid) or with the International Numbering System (INS). It was stated that Food Additives (INS 211) should be declared if used in an amount sufficient to perform a technological function in food. To determine the accuracy of the preservative statement declared on the product label, the detected preservative results in bottled products from section 3.1 were then analyzed to check their correspondence with the legal declaration which classified 4 main groups (**Figure 1**). The Group 1 Label was for the most serious cases where preservatives were detected but there was incorrect declaration. This label was applied to 43 samples (79.6 %). Almost all the problems on the labelling in this group were because the brand did not provide information on the existing preservative content (26 brands, 60.5 %) or they claimed that the brand did not contain preservatives even though they did (17 brands, 39.5 %). Of the 43 bottles, 33 tested brands contained benzoic acid (76.7 %), 3 brands contained sorbic acid (6.9%) and 7 brands contained both (16.3 %). Groups 3 and 4 provided accurate declarations on the presence or absence of preservatives, respectively, with 3 samples (5.6 %) for Group 3 and 5 samples (9.3 %) for Group 4. Notably, Group 2 did not contain any preservatives nor were they correct declarations. The labeling of preservative use in *nam-pla-ra* or other local Thai products did not correspond to that of the SME manufacturers. Moreover, inaccurate information on labels can have serious consequences so it should be carefully regulated. The use of preservatives represented the 3 most serious health risks for consumers in recent decades [31,32]; however, there appear to be very few reports on the verification of preservative labelling. In addition, the effective implementation of accurate labeling is needed to prevent misunderstandings. Similarly, better communication aiming at educating consumers about food hazards and health concerns is required.

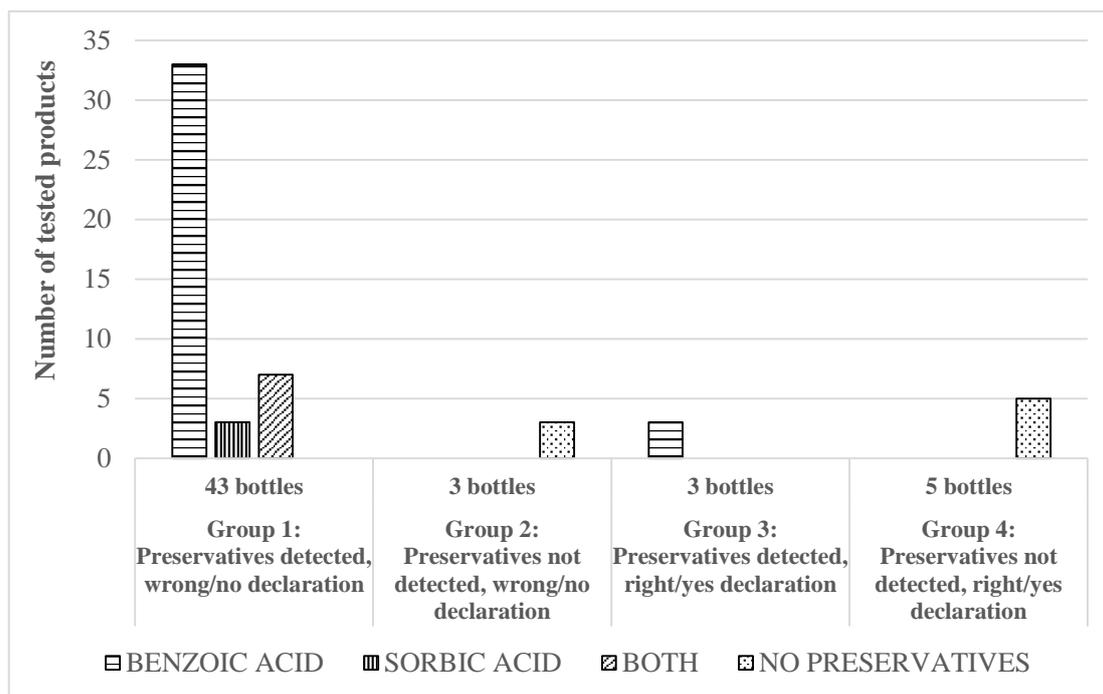


Figure 1 Preservatives detected in the 54 bottled *nam-pla-ra* products tested and the correspondence with their labels in 4 main groups.

It should be noted that the labeling of nutrition information occurred in only 4 products (7.4 %). This is because *nam-pla-ra* products are not in a food group which obliges the producers to declare nutritional content on the label, but it is optional. Recently, RDIs of sodium were reduced from 2,400 - 2,000 mg [26,33] due to their health effects on the Thai population. With today's greater health awareness, some food manufacturers, including *nam-pla-ra* industry have reduced the amounts of sodium, for example, and stated on their labels "reduced salt 10 - 20 %" to advise their customers.

Pathogenic prevalence

Table 4 shows the prevalence of foodborne pathogens, poor hygiene indicator microbes and total viable bacteria with their respective counts in bottled *nam-pla-ra* samples ($n = 54$) and *nam-pla-ra* obtained from *somtum* vendors ($n = 37$). The standards for fermented fish sauce products, according to the Thai Community Product Standard (NAM PLA RA) No. 1346/2557, specifies safety and quality-linked microbes including 4 main pathogens (i.e., *B. cereus*, *S. aureus*, *C. perfringens*, and *Salmonella* spp.), and poor hygienic indicator *E. coli* [2]. Almost 100 % of the *nam-pla-ra* samples complied with the regulations. Only one bottled *nam-pla-ra* sample (1.9 %) was found to contain *S. aureus* over the limit (3.2 log CFU/g) while 37 samples from the vendors contained *S. aureus* (5, 13.5 %), *E. coli* (1, 2.7 %), *C. perfringens* (1, 2.7 %), and *Salmonella* spp. (6, 16.2 %). Coliforms, which indicate poor hygiene, were not specified in the standards, but both types of samples contained 2.3 - 3.5 log CFU/g for bottles and 2.5 - 5.1 log CFU/g for *somtum* obtained from vendors.

Table 4 Prevalence of foodborne pathogens, poor hygiene indicators, total viable bacteria, and yeast/mold with their respective counts in 91 analyzed *nam-pla-ra* products sampling around Central Khon Kaen, Thailand, compared to Thai Community Product Standard No. 1346/2557 (NAM PLA RA).

Microorganisms	Thai community product standard No. 1346/2557 (NAM PLA RA)	Bottled <i>nam-pla-ra</i> sauce (54 samples)			<i>Nam-pla-ra</i> for vendor <i>somtum</i> ingredients (37 samples)		
		Positive samples (%)	Over standard	Range (log CFU/g)	Positive samples (%)	Over standard	Range (log CFU/g)
<i>B. cereus</i>	< 1,000 CFU/g sample	0/54 (0 %)	0/54	-	2/37 (5.4 %)	0/54	2.4
<i>S. aureus</i>	< 100 CFU/g sample	2/54 (3.7 %)	1/54	2.3 - 3.2	8/37 (21.6 %)	5/37	2.5 - 5.4
<i>E. coli</i>	< 3 MPN/g (equivalent to 3 CFU/g)	0/54 (0 %)	0/37	< 1	3/37 (8.1 %)	1/37	1.0 (equivalent to 10 MPN/g)
<i>Coliforms</i> *	Not specified	2/54 (3.7 %)	-	2.3 - 3.5	11/37 (29.7 %)	-	2.5 - 5.1
<i>C. perfringens</i>	< 1,000 CFU/g sample	1/54 (1.8 %)	0/54	2.1	3/37 (8.1 %)	1/37	2.1 - 3.2
<i>Salmonella</i> spp.	Not found in 25 g sample	0/54 (0 %)	0/54	ND (in 25 g sample)	6/37 (16.2 %)	6/37	Detected (in 25 g sample)
Total viable counts*	Not specified	5/54 (9.5 %)	-	2.3 - 4.3	18/37 (48.6 %)	-	2.7 - 5.3

*Not specified in Thai Community Product Standard No. 1346/2557 (NAM PLA RA).

There are few research studies on the prevalence of pathogenic bacteria in *pla-ra* products. Rattanasuk, Boonbao [34] stated that the most predominant foodborne pathogens isolated from *pla-ra* and Jaew bong comprise *S. aureus* at 100 % and *E. coli* as much as 72 % of the sample. Also, various institutions such as National Food Institute (NFI) have frequently reported that analyses of bottled *nam-pla-ra* products and *nam-pla-ra* in *somtum* have found *S. aureus*, *B. cereus* and *E. coli*. The present study also provides crucial information about the prevalence of key microbes which affect the safety and quality of *nam-pla-ra* products in samples obtained from 2 main consumer sources. It is important to draw attention to pathogenic bacteria in order to educate vendors in safe sanitary practices and warn consumers about the health risks of the consumption of *somtum* obtained from street and market sales. Moreover, this data could also help make small-scale manufacturers control the fermentation and pasteurization process of their products to ensure that unsanitary microbes are destroyed.

Conclusions

This study is the first evidence-based report to assess dietary exposure to sodium and preservatives and to evaluate the pathogenic prevalence of *nam-pla-ra* products in the rapidly developing city of Khon Kaen in Northeastern Thailand. The consumption of *nam-pla-ra* products, both pasteurized bottles and those obtained from *somtum* vendors is still safe for Thai consumers in terms of the health risks from preservatives and sodium exposure. However, it is important to pay more attention to those consumer groups of adults and elderly consumers who are at risk when exposed to 46.9 - 81.7 % sodium through the consumption of commercially pasteurized bottles of *nam-pla-ra* and *somtum* sauce. Moreover, the scientific data about preservatives and sodium obtained in this study should be used as a guide to safe consumption by means of better regulations and safety campaigns.

At present the labeling of *nam-pla-ra* products by manufacturers is inconsistent with inaccurate regulatory information (i.e., preservatives). This survey detected serious examples of the health risks of preservatives in samples, but it did not find any correct declarations in 26 samples (48.2 % of 54). Our findings may provide useful information for government data which should be used to inform the Thai manufacturers of these products. Interestingly, the survey also revealed that most pasteurized bottles of *nam-pla-ra* (92.6 %) did not have any declaration of contents as this is optional for this kind of product. It

was noted that, on the other hand, that higher quality brands showed the full nutritional details so that health-conscious consumers would be able to make informed decisions about their purchases.

Pathogenic and sanitary-indicator microbes found in the *nam-pla-ra* samples obtained from *somtum* vendors were in larger amounts than those found in the commercially produced bottles. This clearly indicates that good sanitation and practices of street vendors must be improved to reduce the risks of foodborne illnesses to the consumers of *somtum pla-ra*. The findings in this study should be integrated with the views of government, manufacturers, and consumers in all aspects in order to achieve the highest quality and safety of products for consumers.

Acknowledgements

This work was supported by Khon Kaen University, Thailand (Grant No. I62-00-24-07) and a University Scholarship for ASEAN and GMS Country Personnel 2018. We would like to thank University for supporting student's Master's study.

References

- [1] M Sangjindavong, P Chuapoehuk, J Runglerdkriangkrai, W Klaypradit and D Vareevanich. Fermented fish product (*Pla-ra*) from marine fish and preservation. *Kasetsart J. Nat. Sci.* 2008; **42**, 129-36.
- [2] Thai Industrial Standard Institute. *Thai community products standard*. Thai Industrial Standard Institute, Bangkok, Thailand, 2014.
- [3] Khon Kaen University, Available at: <https://plara.kku.ac.th/>, accessed July 2021.
- [4] National Bureau of Agricultural Commodity and Food Standards, Available at: http://www.thaincd.com/document/file/info/non-communicable-disease/Thai_Food_Consumption_Data_2016.pdf, accessed January 2019.
- [5] World Health Organization. *Guideline: Sodium intake for adults and children*. World Health Organization (WHO), Geneva, Switzerland, 2012.
- [6] GJ Jang, M Yoo and S Lee. Benzoic and propionic acids in fishery products on the Korean market. *Food Addit. Contam. B* 2020; **13**, 185-92.
- [7] DS Sharma. Food preservatives and their harmful effects. *Int. J. Sci. Res. Publ.* 2015; **5**, 1-2.
- [8] A Grillo, L Salvi, P Coruzzi, P Salvi and G Parati. Sodium intake and hypertension. *Nutrients* 2019; **11**, 1970.
- [9] JW Shin, JB Kim, HJ Cho and HJ Suh. Estimated daily intakes of benzoic acid and sorbic acid in South Korea. *J. Food Nutr. Res.* 2017; **56**, 244-54.
- [10] GBD 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2019; **393**, 1958-72.
- [11] Samui Times Editor, Available at: <https://www.samuitimes.com/low-salt-thailand-aims-to-cut-national-salt-intake-by-30/>, accessed March 2021.
- [12] L Kloss, JD Meyer, L Graeve and W Vetter. Sodium intake and its reduction by food reformulation in the European Union: A review. *NFS J.* 2015; **1**, 9-19.
- [13] Low Salt Thailand, Available at: <https://rb.gy/38h42m>, accessed March 2021.
- [14] ER Tavares, EA Esmerino, VD Santos, ACLD Silva and HMA Bolini. Dynamic aspects of salt reduction in tomato sauce by use of flavor enhancers and a bitter blocker. *Food Sci. Tech. Int.* 2020; **26**, 549-59.
- [15] Editorial department Chalard sue Magazine, Available at: <https://bit.ly/3wiWP9G>, accessed July 2021.
- [16] W Rimpeekool, SA Seubsman, C Banwell, M Kirk, V Yiengprugsawan and A Sleigh. Food and nutrition labelling in Thailand: A long march from subsistence producers to international traders. *Food Pol.* 2015; **56**, 59-66.
- [17] United States Department of Agriculture Food Safety and Inspection Service. *Determination of benzoic acid, sorbic acid, and methyl, ethyl, propyl, and butyl parabens by HPLC*. Office of Public Health Science, Connecticut, 2011, p. 1-15.
- [18] SS Nielsen. *Sodium and potassium determinations by atomic absorption spectroscopy and inductively coupled plasma-optical emission spectroscopy*. In: SS Nielsen (Ed.). *Food Analysis Laboratory Manual*. Springer International Publishing, New York, 2017, p. 171-77.
- [19] J Mbabazi. Principles and methods for the risk assessment of chemicals in food. *Int. J. Environ. Sci. Tech.* 2011; **68**, 251-2.

- [20] S Tallent, J Hait, RW Bennett and GA Lancette. *BAM Chapter 12: Staphylococcus aureus*. Bacteriological Analytical Manual (BAM) Main Page. Food and Drug Administration, Maryland, 2019.
- [21] International Organization for Standardization. *ISO 7932:2004-Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive bacillus cereus - Colony-count technique at 30 °C*. International Organization for Standardization, Geneva, Switzerland, 2004.
- [22] International Organization for Standardization. *ISO 7937:2004-Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Clostridium perfringens - Colony-count technique*. International Organization for Standardization, Geneva, Switzerland, 2004.
- [23] International Organization for Standardization. *ISO 9308-1:2014. Water quality - enumeration of escherichia coli and coliform bacteria - part 1: Membrane filtration method for waters with low bacterial background flora*. International Organization for Standardization. Geneva, Switzerland, 2014.
- [24] International Organization for Standardization. *ISO 6579:2017. Microbiology of the food chain - horizontal method for the detection, enumeration and serotyping of salmonella - part 1: Detection of Salmonella spp*. International Organization for Standardization. Geneva, Switzerland, 2017.
- [25] Food and Drug Administration Thailand. *Prescribing the principle, conditions, methods and proportion of food additives (No.2)*. Ministry of Public Health (No. 418) B.E. 2563, Bangkok, Thailand, 2020.
- [26] Food and Drug Administration Thailand. *Nutrition labelling (No.3)*. Ministry of Public Health (No. 392) B.E.2561, Bangkok, Thailand, 2018.
- [27] Codex Alimentarius, Available at: <http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/gsfa/en/>, accessed August 2021.
- [28] A Ardiansyah, S Puat, N Huda, W Wan Nadiyah, A Alkarkhi and A AlKharki. Chemical composition and protein quality of fish sauces (Kecap Ikan and Nampla). *Asia Pac. J. Sustain. Agr. Food Energ.* 2015; **3**, 2-9.
- [29] Wolfson Institute of Preventive Medicine, Available at: <http://www.worldactiononsalt.com/worldaction/asia/thailand/>, accessed February 2020.
- [30] Food and Drug Administration Thailand. *Labeling of Prepackaged foods*. Ministry of Public Health (No. 367) B.E. 2557, Bangkok, Thailand, 2014.
- [31] PF Ababio, DD Adi and M Amoah. Evaluating the awareness and importance of food labelling information among consumers in the Kumasi metropolis of Ghana. *Food Contr.* 2012; **26**, 571-4.
- [32] H Mai, S Shakur and KHP Do. Consumer concern about food safety in Hanoi, Vietnam. *Food Contr.* 2018; **98**, 238-44.
- [33] Food and Drug Administration Thailand. *Nutrition labelling*. Ministry of Public Health (No. 182) B.E.1998, Bangkok, Thailand, 1998.
- [34] S Rattanasuk, J Boonbao, N Sankumpa and T Surasilp. Foodborne pathogens in fermented fish purchased in Selaphu, Roi Et. *In: Proceedings of the 2015 International Conference on Science and Technology, Pathum Thani, Thailand.* 2015, p. 178-81.