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A Survey in Bacteriological Quality of Traditional Dried Seafood Products Distributed in Chon Buri, Thailand

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ABSTRACT

This study aimed to assess physicochemical and bacteriological qualities of dried seafood products distributed in Chon Buri province, Thailand. Forty-four dried seafood samples were collected from local markets and examined for pH, water activity (a_w), salt content, numbers of viable bacteria and halophilic and halotolerant bacteria, numbers of *Escherichia coli* and *Staphylococcus aureus* and the presence of *Salmonella*, *Vibrio parahaemolyticus* and *Vibrio cholerae* using a standard protocol. Values of pH, a_w and salt content in dried seafood products were 5.16 ± 0.04 – 8.63 ± 0.03, 0.221 ± 0.01 – 0.822 ± 0.02 and 0.28 ± 0.03 – 18.92 ± 0.10%, respectively. Numbers of viable bacteria, and halophilic and halotolerant bacteria were $10^2 – 10^9$ and $10^2 – 10^9$ CFU g^{-1}, respectively. Main compositions of total viable bacteria, and halophilic and halotolerant bacteria in dried seafood products were *Staphylococcus, Bacillus* and *Pantoae*. Based on regulatory standards for cooked ready-to-eat and prepared seafood products set by the Department of Fisheries, Thailand, nineteen (43.18%) samples contained viable bacteria numbers exceeding the allowable limit. Two dried shrimp products (4.55%) harbored *E. coli* over a regulatory acceptable value of 10 MPN g^{-1}. All dried seafood products had *S. aureus* numbers within allowable limit for good quality of

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processed seafood products. None of *Salmonella*, *V. parahaemolyticus* and *V. cholerae* was isolated from all dried seafood products. This study indicates that consumption of cooked ready-to-eat and prepared dried seafood products may constitute a potential hazard of foodborne infection.

Keywords: Dried seafood product; Viable bacteria, Halophilic and halotolerant bacteria; Water activity; Chon Buri

1. Introduction

Seafood products have been widely accepted as a good source of various essential nutrients such as proteins, vitamins, minerals, omega-3 fatty acid and taurine [1]. Dried processed seafood product, a popular food in Thai cuisine due to its accustomed taste and aroma, is one of the most important processed food products to the economy of Thailand with export revenue of $1,315 million in 2016 [2]. Major raw seafood materials, including shrimp, fish, crab, bivalve and cephalopod caught from farms and the Gulf of Thailand, have been supplying local facilities to produce a variety of dried seafood stuff. Traditional simple processes are applied to seafood, e.g. drying, salting, brining, marinating and fermenting, to retard spoilage and prolong the shelf-life [3]. Traditional dried seafood products are usually stable at high temperature, leading to storage and distribution at ambient temperature for 6 months or more [4]. However, they are also vulnerable to human intoxication and pathogen contamination. Despite high salt and low moisture contents limiting growth of most microorganisms, traditional dried seafood products have been also considered as a vehicle for a serious risk to human health in terms of contamination of indigenous and pathogenic bacteria derived from handling during production processes [1, 5-6]. Unfortunately, traditional dried seafood products in Thailand are produced under unhygienic processes and unawareness of proper sanitation during distribution leading to a relatively high contamination of microorganisms. They are typically preserved in plastic bags outside therefrigerators and distributed in open markets; consequently, they are easily exposed to bacterial contaminants that may contribute to morbidities and mortalities of consumers.

There have been a number of pathogenic bacteria found in dried seafood products such as *Clostridium botulinum*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Salmonella* Typhi and *Salmonella* Enteritidis [3-4, 7]. The pathogenic bacteria could threaten consumer health, in particular the food eaten without cooking before consumption. In addition, the high salt and low moisture content characteristics of the traditional dried seafood products have contributed to propagation of pathogenic halophilic and halotolerant bacteria [7, 8-9]. Diminished prevalence and growth prevention of pathogens is necessary for the food safety and quality. During recent years, dried seafood products have been confronted with stringent criteria enforced by regulatory authorities.

Chon Buri is a popular province in the eastern region of Thailand with 18.4 million of visitors in 2018. By 2017, 88 local factories associated with dried seafood production are found in Chon Buri province, which has a total processing capacity of ca. 4,841-t raw seafood materials to dried seafood products per year [10]. In the past decade, several reports stated that traditional dried seafood products distributed in Chon Buri province were not safe bacteriologically owing to contamination with high numbers of total viable count and

pathogenic bacteria e.g. *Salmonella*, *Staphylococcus aureus* and *Bacillus cereus* [11-12]. To date, there is little scientific information available
regarding bacteriological quality and safety of dried seafood products in Thailand. Therefore, the present study aimed to elucidate the physicochemical and bacteriological characteristics of traditional dried seafood products in Chon Buri province, Thailand.

2. Materials and Methods

2.1 Sample collection

Forty-four samples of a variety of dried seafood products were purchased monthly from five retail markets located in Chon Buri, Thailand between May 2011 and April 2012. Dried seafood samples were categorized into two groups: prepared products that need minimum heating before consumption (dried shrimp, dried seasoned whole fish, salted fish and dried mussels) and cooked ready-to-eat products (seasoned fish strips, crispy rolled seasoned squids, crushed squids in seasoned syrup and crispy dried seasoned crabs). All samples were wrapped in aseptic bags, placed in an icebox and immediately transported to the laboratory within 2 h.

2.2 Physicochemical characterization

The values of pH and water activity (a_w) of dried seafood samples were examined according to the AOAC [13] procedure. Dried seafood samples (10 g) were homogenized with distilled water (10 mL) in sterile sampling bags using a stomacher (AES Laboratories, Combourg, France). The homogenates were then measured directly using a regular calibrated pH meter (Sartorius, Professional Meter PP-50, Gottingen, Germany). Value of a_w was determined using a water activity meter (Novasina MS 1, Lachen, Switzerland) at 25 °C. Salt contents in dried seafood products were estimated using the Volhard method [14]. Each analysis was done in triplicate.

2.3 Bacteriological analysis

Numbers of total viable bacteria, and halophilic and halotolerant bacteria were enumerated using the spread plating method [15-16]. A portion of a sample (50 g) excised aseptically from each dried seafood product was homogenized vigorously with 450 mL Butterfield's Phosphate - Buffered Dilution Water (BPD) using a stomacher for 2 mins. Successive decimal dilutions were prepared by mixing 1 mL of the previous dilution with the same sterile diluents (10 mL). Then, an aliquot of each dilution (0.1 mL) was plated in triplicate onto Plate Count Agar (PCA; BD Biosciences, Sparks, Maryland, USA) and PCA containing 7.5% (w v^{-1}) NaCl for counting numbers of total viable bacteria, and halophilic and halotolerant bacteria, respectively. All petri dishes were incubated at 35 °C for 24 h. Petri dishes with 25-250 colonies were counted and calculated as CFU g^{-1}. Bacterial isolates were purified by re-streaking on Trypticase Soy Agar (TSA; BD Biosciences) or TSA added with 7.5% (w v^{-1}) NaCl. The purified isolates were characterized on the basis of morphological and biochemical characteristics [17-18].

Isolations of *V. parahaemolyticus*, *V. cholerae*, *Salmonella*, *S. aureus*, and total coliforms and *E. coli* were carried out according to the guidance of the US FDA [15].

Enumeration of *V. parahaemolyticus* was achieved by a three-tube MPN enrichment method. A portion (25 g) of samples was homogenized in 225-mL alkaline peptone water (APW), pH 8.5 ± 0.2 using a stomacher and serially diluted by 10-fold dilution technique. After incubation at 35 °C for 6-8 h, all turbid tubes were subcultured onto Thiosulfate Citrate Bile salt Sucrose (TCBS) agar (BD Biosciences) and incubated at 35 °C for 24 h. The green and/or blue-green colonies were picked from each sample and streaked on TSA containing 2% (w v^{-1}) NaCl. Confirmatory tests were done as follows: Gram's staining;

motility; presence of L-arginine dihydrolase, L-lysine decarboxylase and ornithine decarboxylase; salt tolerance; string test; oxidase reaction; cellobiose fermentation and growth at 43 °C [15].

To isolate *V. cholerae*, dried seafood samples (25 g) were homogenized in aseptic bags for 2 mins with 225-mL APW using a stomacher and then incubated at 35 °C for 6-8 h. A loopful of culture broth was streaked onto TCBS agar and incubated at 35 °C for 18-24 h. Yellow colonies with 1-3 mm diameter were subcultured on TSA containing 2% (w v^{-1}) NaCl for further confirmation as recommended by US FDA [15] including Gram's staining, motility, L-arginine dihydrolase production, salt tolerance, string test, oxidase reaction, serologic agglutination, and biotype classification.

A three-tube MPN protocol was used to enumerate *S. aureus* numbers in dried seafood products. Pre-enrichment of a 25-g sample in BPD (225 mL) was taken in aseptic bags and serially diluted with a 10-fold technique. An aliquot of each dilution (1 mL) was aspirated to Trypticase Soy Broth (BD Biosciences) plus 10% (w v^{-1}) NaCl and 1% (w v^{-1}) sodium pyruvate, and then incubated at 35 °C for 48 h. The culture broth was streaked onto Baird Parker agar (BD Biosciences) containing egg yolk and potassium tellurite. Suspected colonies (gray to black and halo surrounding colonies) were subsequently confirmed by coagulase test.

Presence of *Salmonella* in dried seafood products was determined using a three-tube MPN method. Briefly, a portion of each sample (25 g) was homogenized in aseptic bags with 225 mL lactose broth (BD Biosciences) for pre-enrichment. After incubation at 35 °C for 24 h, the culture broth was enriched with selective media as follows: Tetrathionate broth (BD Biosciences) at 35 °C for 24 h and Rappaport-Vassiliadis medium at 42 °C for 24 h. The turbid tubes were seeded onto Bismuth Sulfite agar (BD Biosciences), Xylose Lysine Desoxycholate agar (BD Biosciences), and Hektoen Enteric agar (BD Biosciences) and incubated at 35 °C for 24 h. Typical colonies of *Salmonella* grown on the media were characterized by selected biochemical tests (triple sugar iron, presence of urease, indole production and motility test). *Salmonella* isolate was serotyped according to the Kauffman-White scheme by seroagglutination using antisera [19].

Total coliform and *E. coli* numbers of dried seafood products were assessed using a three-tube MPN method. Briefly, a series of three fermentation tubes of Lauryl Tryptose Broth (BD Biosciences) and Brilliant Green Lactose Bile broth (BD Biosciences) were used for presumptive and confirmatory test of total coliform. In order to determine numbers of *E. coli*, EC broth was used and then the culture was confirmed by Eosin Methylene Blue agar (BD Biosciences) and IMViC reactions. The MPN tables were used to calculate the approximate numbers of bacteria per gram.

2.4 Statistical analysis

Each sample was done in triplicate. Data of physicochemical parameters and bacterial numbers were reported as mean ± standard deviation (SD).

3. Results and Discussion

Values of pH, a_w and salt content of 44 traditional dried seafood samples distributed in Chon Buri province, Thailand are illustrated in Table 1. The levels of pH, a_w and salt content were in the ranges of 5.16 ± 0.04 – 8.63 ± 0.03, 0.221 ± 0.01 – 0.822 ± 0.02 and 0.28 ± 0.03 – 18.92 ± 0.10%, respectively. The lowest a_w value was observed in crispy rolled seasoned squids (0.221 ± 0.01 – 0.313 ± 0.00) while dried shrimp exhibited the highest a_w value of 0.594 ± 0.00 – 0.822 ± 0.02. There were variations in salt content of each sample of crispy rolled seasoned squids (0.88 ± 0.08 –

2.15 ± 0.06%) and salted fish (11.33 ± 0.02 – 18.92 ± 0.10%). According to Rodrigues et al. [20] report, values of a_w and NaCl content of soaked cod produced from green salted and dried salted cod were 0.702–0.729 and 18.5–20%, respectively. Huang et al. [7] reported that values of pH, a_w and NaCl content in dried fish products ranged from 6.16–7.07, 0.68–0.87 and 4.07–16.80%, respectively. Kung et al. [21] also observed levels of pH, a_w and salt content in dried flying fish products ranging from 5.78–6.66, 0.61–0.84 and 1.38–7.60%, respectively. The different results may be due to processing techniques applied.

Table 1. Physicochemical characteristic of traditional dried seafood products distributed in Chon Buri, Thailand.

Dried seafood products	No. sample tested	pH	a_w	Salt content (%)
Prepared products [a]				
Dried shrimp	8	7.17±0.05-7.97±0.08	0.594±0.00-0.822±0.02	1.38±0.01-2.28±0.03
Dried seasoned whole fish	4	5.97±0.01-6.42±0.13	0.602±0.00-0.717±0.00	1.21±0.02-2.27±0.02
Salted fish	4	5.73±0.02-8.21±0.04	0.738±0.01-0.770±0.02	11.33±0.02-18.92±0.10
Dried mussels	8	5.35±0.03-6.42±0.18	0.473±0.01-0.744±0.00	1.40±0.07-2.27±0.01
Cooked ready-to-eat products [b]				
Seasoned fish strips	4	6.29±0.03-6.38±0.03	0.600±0.00-0.639±0.00	2.28±0.02-2.31±0.01
Crispy rolled seasoned squids	4	6.44±0.01-6.95±0.10	0.221±0.01-0.313±0.00	0.88±0.08-2.15±0.06
Crushed squids in seasoned syrup	4	5.16±0.04-6.56±0.02	0.695±0.01-0.748±0.01	0.63±0.03-1.36±0.04
Crispy dried seasoned crabs	8	7.05±0.07-8.63±0.03	0.470±0.01-0.642±0.00	0.28±0.03-0.38±0.02

[a] Prepared products that need minimum cooking before consumption, [b] Cooked ready-to-eat products (consumption without further cooking).

During the processing procedure, salt, condiments and other food preservatives are applied in order to season and prolong product shelf-life, thereby affecting variation in physical and chemical characteristics of dried seafood products.

All traditional dried seafood products contained numbers of viable bacteria in parallel with halophilic and halotolerant bacteria in the ranges of 10^2–10^9 CFU g^{-1} (Fig. 1(a) and 1(b)). Two dried shrimp samples represented 10^9 CFU g^{-1} of those bacteria numbers. Based on a bacteriological regulatory standard announced by Department of Fisheries, Thailand [22], the acceptable levels of viable bacteria in cooked ready-to-eat and prepared seafood products are 5 x 10^4 and 5 x 10^5 CFU g^{-1}, respectively. There were 19 (43.18%) samples containing viable bacteria numbers over the allowable limit of Thai regulatory standard (Fig. 1(a)): dried shrimp (6), dried seasoned whole fish (1), dried mussels (5) seasoned fish strips (1), crispy rolled seasoned squids (1), crushed squids in seasoned syrup (1), and crispy dried seasoned crabs (4). Kung et al. [21] observed viable bacterial numbers in dried flying fish products varying from 10^3–10^8 CFU g^{-1}. There were 10^4–10^8 CFU g^{-1} of viable bacterial numbers in dried fish products distributed in Penghu Island of

Taiwan [7]. Yam et al. [23] also revealed dried salted bacalao containing 10^3–10^4 CFU g^{-1} of viable bacteria and 10^2–10^3 CFU g^{-1} of halophilic bacteria numbers. These bacteria may cause damage to the products by changing texture, smell, taste, and appearance resulting in deteriorated organoleptic quality [24].

A total of 167 and 143 isolates of viable bacteria (Table 3) and halophilic and

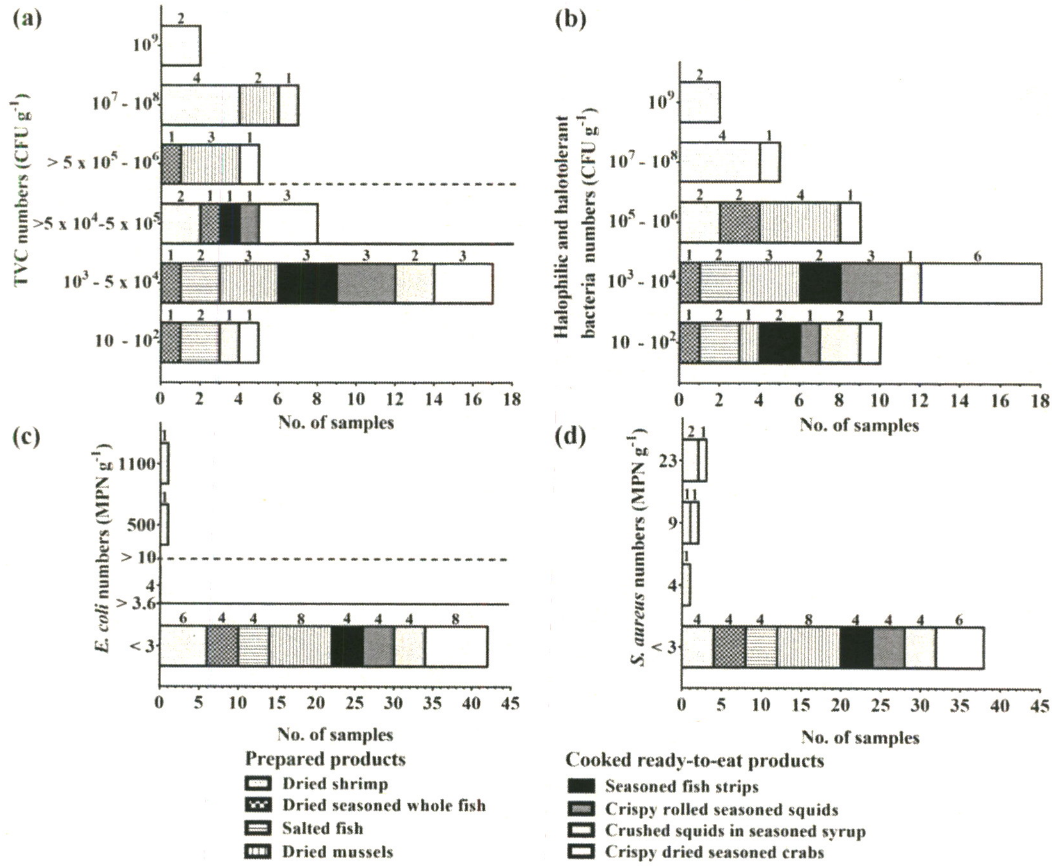

Fig. 1. Numbers of traditional dried seafood products distributed in Chon Buri, Thailand found to be contaminated with various types of bacteria. (a): total viable counts (TVC), (b): halophilic and halotolerant bacteria, (c): *E. coli* and (d): *S. aureus*. Dashed lines and solid lines indicate unacceptable standard values of prepared and cooked ready-to-eat products, respectively. Based on the Thai regulatory standard set by Department of Fisheries, Thailand, TVC number must be not exceeded 5 x 10^4 and 5 x 10^5 CFU g^{-1} in cooked ready-to-eat and prepared seafood products, respectively. *E. coli* number must be not exceeded 3.6 and 10 MPN g^{-1} in cooked ready-to-eat and prepared seafood products, respectively. *S. aureus* number must be not exceeded 100 and 1,000 MPN g^{-1} in cooked ready-to-eat and prepared seafood products, respectively.

halotolerant bacteria (Table 4) were isolated from traditional dried seafood products. Dominant viable bacteria found in this study were Gram-positive bacilli (77 isolates; 46.11%), followed by Gram-positive cocci (57 isolates; 34.13%) and Gram-negative bacilli (33 isolates; 19.76%), respectively. This can be probably explained as a better tolerance of Gram-positive bacteria under severe treatments like salting and drying [25]. In accordance with a previous report [26], almost all Gram-negative bacteria are generally inhibited at an a_w of less than 0.93 whereas Gram-positive bacteria can thrive down to 0.85. When considering an a_w value of traditional dried seafood products (Table 1), all products showed a_w values in the ranges of 0.221 ± 0.01 – 0.822 ± 0.02, which were lower than a minimum level (<0.85) for bacterial propagation. Therefore, the bacteria detected in these products perhaps do not grow actually, yet survive supported by an extremely low a_w value of our products and their growth on the culture media. In this study, Gram-positive bacteria were identified as *Bacillus cereus*, *B. coagulans*, *B. firmus*, *B. laterosporus*, *B. licheniformis*, *B. mycoides*, *Micrococcus agilis*, *Listeria ivanovii*, *Staphylococcus caprae*, *S. epidermidis* and *S. hominis,* while Gram-negative bacteria found in these products were designated into five species: *Bordetella holmesii*, *Burkholderia cepacia*, *Neisseria weaveri*, *Pantoea* sp. and *Serratia liquefaciens*. This indicated that most bacteria contaminated in traditional dried seafood products in this study were Gram-positive bacteria, especially genera of *Bacillus* and *Staphylococcus*. This result agreed with previous studies, which observed that *Bacillus* and *Staphylococcus* were major constitutes of processed seafood commodities sold in retail markets in Korea [27-28]. Six species of *Bacillus* (*B. cereus*, *B. coagulans*, *B. firmus*, *B. laterosporus*, *B. licheniformis* and *B. mycoides*) were most found in these products (65 isolates; 38.92%) (Table 3). Contamination of *Bacillus* may be associated with spices and condiments, i.e. pepper, chili and herbs used as food additives in the processed products. Fogele et al. [29] reported that the most predominant bacterium in spices and herbs was *Bacillus* sp., which could act as a vehicle of transmission of some pathogens and bacteria-derived toxins into foods. Fifty-five *Staphylococcus* isolates (32.93%) were detectable in all products in this study and recognized as *S. caprae*, *S. epidermidis* and *S. hominis* (Table 3). This result corroborated several reports carried out in soaked and salted cod [20] and Taiwanese dried seafood commodities [7]. *S. epidermidis* and *S. hominis* were found in dried salted cod [20] and salted Spanish anchovies [9]. *S. epidermidis*, an important opportunistic pathogen causing nosocomial infections, is usually found on human skin [30]. Typically, *Staphylococcus* has human and environmental origins; therefore, it possibly enters into these products during preparation through unhygienic handling [8]. In addition, *L. ivanovii* was also isolated in several dried seafood products, i.e. dried shrimp (1 isolate), crispy rolled seasoned squid (1 isolate), dried mussels (4 isolates), and crispy dried seasoned crabs (6 isolates). *L. ivanovii* is an enteric opportunistic human pathogen associated with gastroenteritis and bacteremia and has been occasionally isolated from processed food products [31]. The presence of *L. ivanovii* in these products may result from unhygienic contact by contamination and inadequate preservation of products leading to an increased risk of listeriosis.

In fact, five species of Gram-negative bacteria could be isolated from these products (Table 3). *Pantoae*, a member of Enterobacteriaceae, were the prevalent Gram-negative bacteria (21 isolates; 12.57%), which were recovered from nearly all products, except crispy dried seasoned crabs. *Pantoae* are naturally found in fresh fish [32], dried salted cod [20] and traditional dried fish [7]. *Bor. holmesii* were isolated from dried squids (1 isolate), crushed squids in seasoned syrup (1 isolate), dried mussels (1 isolate) and crispy dried seasoned crabs (6 isolates). *Bur. cepacia*, the opportunistic non-fermentative Gram-negative bacilli pathogen in human, was also recovered from dried shrimp and crushed squids in seasoned syrup (1 isolate each). An isolate of *N. weaveri* was found in a dried mussel sample. *N. weaveri* is regarded as normal oral flora in dogs and *Bor. holmesii* is likewise associated with dogs [18]. Meanwhile, *Bur. cepacia* can be isolated from numerous water and soil sources and is well-known as an important opportunistic pathogen associated with clinical infections, such as hemato-oncology infection in patients [33-34]. Their occurrences in some dried seafood products in the present study revealed unhygienic contact during processes, lack of hygienic and sanitary approaches, contamination by insects and animals and unsuitable preservation of products.

Table 3. Distribution of viable bacteria of traditional dried seafood products distributed in Chon Buri, Thailand[a]

Dried seafood products	No. sample tested	*Bacillus cereus*	*Bacillus coagulans*	*Bacillus firmus*	*Bacillus laterosporus*	*Bacillus licheniformis*	*Bacillus mycoides*	*Micrococcus agilis*	*Listeria ivanovii*	*Staphylococcus caprae*	*Staphylococcus epidermidis*	*Staphylococcus hominis*	*Bordetella holmesii*	*Burkholderia cepacia*	*Neisseria weaveri*	*Pantoea* sp.	*Serratia liquefaciens*	Numbers of total isolates
Dried shrimp [b]	8	-	-	-	3	1	2	1	1	6	5	3	-	1	4	-	-	27
Dried seasoned whole fish [b]	4	-	1	1	-	2	-	-	-	2	1	-	-	-	-	3	1	11
Salted fish [b]	4	2	-	1	1	-	-	-	-	4	-	3	-	-	-	2	-	13
Dried mussels [b]	8	3	3	4	1	4	-	-	4	6	-	3	1	-	1	7	-	37
Seasoned fish strips [c]	4	1	-	1	-	2	-	-	-	2	1	-	-	-	-	2	-	9
Crispy rolled seasoned squids [c]	4	-	1	1	1	3	1	-	1	1	1	1	-	-	-	1	-	12
Crushed squids in seasoned syrup [c]	4	-	-	-	1	-	-	-	-	3	-	1	1	1	-	2	-	9
Crispy dried seasoned crabs [c]	8	6	3	4	3	2	6	1	6	6	3	3	6	-	-	-	-	49
Numbers of total isolates	-	12	8	12	10	14	9	2	12	30	11	14	8	2	1	21	1	167

[a] Isolate number of each bacterial species recovered from traditional dried seafood products, [b] Prepared products that need minimum heating before consumption; [c] Cooked ready-to-eat products (consumption without further cooking)

High salt-containing foods can hamper growth of spoilage organisms; only halophilic and halotolerant microorganisms prosper under extreme condition [26]. The majority of bacteria isolated in this study were recognized as halotolerant bacteria due to growth under both saline and no saline conditions. All of *N. weaveri* and *Ser. liquefaciens* isolates were non-halophilic and halotolerant bacteria (Tables 3 and 4). Predominant halophilic and halotolerant bacteria in traditional dried seafood products were *Staphylococcus* (73 isolates; 51.05%), followed by *Bacillus* (34 isolates; 23.78%) and *Pantoea* spp. (23 isolates; 16.08%). A tolerant ability of Gram-positive bacteria, especially cocci, results in survival under extreme condition [26]. Incidence of halophilic and halotolerant bacteria can create health concerns and harm food safety. For example, there were several halotolerant bacterial species frequently being reported as active histamine-producing formers e.g. *S. epidermidis*, *S. xylosus*, *Pantoae agglomerans*, *Bur. cepacia* and *Bacillus*, isolated from various salted dried fish products [7, 9, 21]. In addition to histamine intoxication, some of the halophilic and halotolerant bacteria found in this study are recognized as foodborne pathogens, in particular, *B. cereus*, which is an infectious agent of diarrheal and vomiting illness [5].

Two (4.55%) samples of dried shrimp were found positive for *E. coli* in the

Table 4. Distribution of halophilic and halotolerant bacteria of traditional dried seafood products distributed in Chon Buri, Thailand[a]

Dried seafood products	No. sample tested	*Bacillus cereus*	*Bacillus coagulans*	*Bacillus firmus*	*Bacillus laterosporus*	*Bacillus licheniformis*	*Bacillus mycoides*	*Micrococcus agilis*	*Listeria ivanovii*	*Staphylococcus caprae*	*Staphylococcus epidermidis*	*Staphylococcus hominis*	*Bordetella holomesii*	*Burkholderia cepacia*	*Pantoea* sp.	Numbers of total isolates
Dried shrimp [b]	8	-	-	-	-	-	1	1	1	8	1	5	-	-	6	23
Dried seasoned whole fish [b]	4	-	-	-	1	-	-	-	-	4	1	-	-	-	1	7
Salted fish [b]	4	-	-	-	-	-	-	-	-	4	1	1	-	-	2	8
Dried mussels [b]	8	1	-	-	1	-	-	-	-	6	1	3	1	2	5	20
Seasoned fish strips [c]	4	-	-	1	-	2	1	1	-	3	-	2	-	-	3	13
Crispy rolled seasoned squids [c]	4	2	-	1	1	2	-	-	-	3	1	3	-	-	-	13
Crushed squids in seasoned syrup [c]	4	-	1	1	-	-	-	-	-	4	-	1	-	-	1	8
Crispy dried seasoned crabs [c]	8	-	2	3	4	-	3	-	1	5	6	6	1	1	3	35
Numbers of total isolates	-	4	4	8	8	5	5	3	3	40	12	21	3	4	23	143

[a] Isolate number of each bacterial species recovered from traditional dried seafood products, [b] Prepared products that need minimum heating before consumption; [c] Cooked ready-to-eat products (consumption without further cooking

ranges of 500 and 1,100 MPN g^{-1}, which were much higher than a bacteriological regulatory standard set by Department of Fisheries, Thailand [22] due to exceeding 10 MPN g^{-1}. The two dried shrimp samples having more than 0.75 of a_w contained ≥ 500 MPN g^{-1}, indicating that low a_w could inhibit the growth of *E. coli* (Fig. 1(c)). In accordance with a previous report [7], *E. coli* were often found in dried fish products with high moisture content in the ranges of < 3 – 210 MPN g^{-1}. *E. coli* is considered as an indicator of fecal contamination and can cause various diseases, including diarrhea, abdominal pain, fever, and sometimes vomiting [5]. Its presence in these products implied inadequate sanitary practice of producers and may cause illness in humans by consumption.

S. aureus was found in six (13.64%) samples: 4 dried shrimp samples containing over 3 MPN g^{-1} and 2 crispy dried seasoned crab samples containing 9 – 23 MPN g^{-1} (Fig. 1(d)). However, all products in this study contained *S. aureus* lower than the allowable limit of Thai regulatory criterion (100 MPN g^{-1} for cooked ready-to-eat seafood products and 1,000 MPN g^{-1} for prepared seafood products). *S. aureus* is not a natural bacterial flora of newly caught marine fish and cultivated fish [10]. However, *S. aureus* has been a major bacterial composition of human skin; therefore, it is reasonable to assume that its contamination in dried shrimp and crispy dried seasoned crabs in the present study would be possibly due to unhygienic direct contact during preparation and processing. Generally, *S. aureus* can proliferate well under high salt concentration up to 15% NaCl and low a_w content (0.65 – 0.85) in foods [35]. According to Yam et al. [23], *S. aureus* was isolated from dried salted Iranian fish products in the ranges of 10^1 – 10^2 CFU g^{-1}.

In the present study, none of *Salmonella, V. parahaemolyticus* and *V. cholerae* was isolated. *Salmonella* is among the most important causes of gastrointestinal disease and is a global public health concern [19] while *V. parahaemolyticus* and *V. cholerae* are usually present in marine and estuarine environments. However, no detection of these species may have been due to high levels of NaCl and limited amounts of a_w contained in foods [36].

In comparison with international standards of bacteriological regulation, nineteen (43.18%) or fourteen (31.82%) of traditional dried seafood products contained viable bacteria numbers over the allowable limits imposed by food safety authorities of either Australia and New Zealand (<10^5 CFU g^{-1}) or European Union (EU) (<5 x 10^5 CFU g^{-1}), respectively [37-38]. In addition, numbers of *E. coli* and *S. aureus* in some dried seafood samples were within the unacceptable levels announced by food administration agencies of the EU countries and China [38].

The presence of pathogenic and spoilage bacteria in traditional dried seafood products in our study indicates consequences of a combination of improper handling, unsuitable storage and unawareness of good hygiene and sanitation. Therefore, a decreased degree of contamination and control of bacterial growth are necessary for food safety. It is recommended to improve the preparation and processing procedures by adaptation of good manufacturing practice (GMP) and use of sanitary gloves for handling dried seafood products to diminish the contamination of harmful and spoiling organisms. Moreover, the active risk management in a farm-to-folk perspective is essential to further carry out in traditional dried seafood products for an effective surveillance of pathogen outbreak and a better health of the consumers.

4. Conclusion

This study concluded that levels of pH, a_w and salt content in traditional dried seafood products were 5.16 ± 0.04 – 8.63 ±

0.03, 0.221 ± 0.01 – 0.822 ± 0.02 and 0.28 ± 0.03 – 18.92 ± 0.10%, respectively. Of forty-four samples, there were 19 (43.18%) samples containing viable bacteria numbers over the allowable limit of Thai regulatory standard for cooked ready-to-eat and prepared seafood products. Most predominant viable bacteria, and halophilic and halotolerant bacteria isolated from traditional dried seafood products were *Bacillus*, *Staphylococcus* and *Pantoae*. Two dried shrimp samples (4.55%) contained *E. coli* that significantly exceeded the regulatory acceptable value of 10 MPN g^{-1}. All dried seafood products harbored *S. aureus* numbers within the allowable limit for good quality of processed seafood products. None of *Salmonella*, *V. parahaemolyticus* and *V. cholerae* was found in all products. This study reveals that traditional dried seafood products distributed in Chon Buri province pose a serious health concern and a public awareness issue for consumers. Therefore, in order to prevent the occurrence of foodborne illnesses, public education campaigns should be promoted to generate biosafety awareness and decrease improper behaviors during dried seafood preparation and handling that is common among the factory staffs, retailers and consumers.

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