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Chemical and Microbiological Analysis of Traditional Fermented Fish and Meat Products Collected from Battambang, Cambodia

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Abstract: *Cambodia is extremely reliant on biodiversity and aquatic ecosystems. Fish accounts for more than 80% of total animal protein intake, particularly in the rural community. Among fishery products in Cambodia, fermented fish called "Nem" is growing more popular and well-known to even outside of their original geographical regions. "Nem" is the kind of traditional lactic acid fermented freshwater fish of Cambodia that is consumed as raw. Three main types of ready-to-eat fermented fish and meat products named Nem Sach Chrouk, Nem Trey, and Sang Vak are formulated with ground pork skin and minced freshwater fish, respectively. Yet, reports of the chemical compositions, and microbiological aspect of these fermented products are limited. Therefore, the objectives of this study were to determine the physical characteristics, chemical compositions, and microbiological quality of 15 ready-to-eat fermented fish and meat samples collected from a well-known processing location, Prek Khporb village , Prek Khporb commune, Ek Phnom district, Battambang province, Cambodia. In this work, pH values were measured by using a pH meter and titratable acidity was done by titration method. Chemical compositions, including moisture, fat, ash, salt, and protein content, were examined by drying method, Soxhlet extraction, furnace, titration, and Kjeldahl method, respectively. As a result, the fermented fish and meat samples showed the pH and titratable acidity ranged from 4.52 to 5.12, and 1.31% to 2.47%. The chemical compositions such as moisture, fat, ash, salt, and protein contents of all samples were 58.65 to 68.07 g/ 100g, 0.40 to 5.45 g/ 100g, 3.01 to 4.40 g/ 100g, 1.40 to 2.31 g/ 100g, 11.97 g/100g to 13.82 g/100g, respectively. Bacterial viable count concentrations had a range between 5.85 to 7.98 log cfu/g. Among all samples, Sang Vak coded by BTB-SV1 provided the highest protein content with low salt concentration, high ash content, and acceptable microbial concentration detected. The results indicated that Cambodian fermented fish and meat products were produced with low salt content and contained high level of minerals as well as the good source of protein for human health.*

Keywords: Nem Trey; Nem Sach Chrouk; Sang Vak; Popular place of Nem processing

1. INTRODUCTION

Cambodia is extremely reliant on biodiversity and aquatic ecosystems. Evidently, fish, the abundant aquatic animal, has accounted for more than 80% of total protein intake in rural community [1]. As fish is a highly perishable resource, a great number of fishery products have been produced through many traditional preservation techniques. Those include salting, smoking, drying, and fermenting. Fermenting, in particular, has been identified as the process in which the raw materials are biochemically modified by a consortia of functional microorganisms to produce bioactive compounds that improve the organoleptic quality of the products preferably accepted by consumers for both cultural and social aspects [2]. During the process of fermentation, lactic acid bacteria could utilize carbohydrate substrates available in the fermenting matrix and produce organic acids, especially lactic acid, that not only contributes to the taste, aroma, and texture of the product but also

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lowers the product's pH. The decrease in pH is one of the critical factors in preserving and ensuring the quality and safety of food products. In East and Southeast Asian countries, fermented food products are gaining popularity due to their unique flavor, taste, and additional nutritional values [3].

In Cambodia, fish has also been preserved into various fermented fish products consumed daily as main dishes, side dishes, condiments, and seasonings. Most of them are cooked before consumption [4]. However, a ready-to-eat fermented fish product called "Nem" is routinely eaten after 2 or 3 days of fermenting. It should be noted that this type of fermented fish product, the "Nem", is also consumed in Vietnam and known as "Nem Chua", while in Thailand as "Nahm" [5]. Nem can be classified into many different types based on the raw materials used and the fermentation period [6,7] (Nguyen et al., 2010; Sangjindavong et al., 2005). In Cambodia, Nem is classified into three types: Nem Trey, Nem Sach Chrouk, and Sangvak. Nem Trey is processed mainly from minced fresh-water fish, fermented for 3 days; Nem Sach Chrouk is formulated with a combination between minced fresh-water fish and pork skin and fermented for 3 days; while, Sangvak is processed from minced fresh-water fish and formed into rectangular shapes by wrapping in banana leaves, then fermented for maximum only 2 days.

Nem has been traditionally produced in Cambodia and is one of the popular products for people of all age groups. As a traditional fermented food product, Nem is usually producing year-round by micro and small-scale processors in Battambang province, which is the most famous Nem production supplier to the whole country [8]. Thus the processing methods are usually unstandardized, and the quality might differ from one producer to another. Therefore, this study aims to conduct the physical characteristics, chemical compositions, and the concentration of bacterial variable count of the traditional fermented fish products in Cambodia with the purpose to assess and evaluate their current quality and to provide as database of the quality of fermented fish and meat products in Cambodia.

2. METHODOLOGY

2.1. Sample collection

A total of 15 samples (five for each fermented fish product, namely Nem Trey, Nem Sach Chrouk, and Sang Vak) were directly purchased from various producers in Prek Khporb village, Prek Khpob commune, Ek Phnom district, Battambang province as shown in Table 1.

The samples were freshly produced and packed with plastic or banana leaves. They were put in the zip-lock plastic bag, labeled with the sample's names, processing date and sampling location, then kept in curst ice and transported to the Institute of Technology of Cambodia laboratory for the analysis of physicochemical and microbiological properties.

Table 1 Characteristics of collected samples

Product types	Sample codes	Main ingredient	Fermentation period	Consumption habit
Nem Sach Chrouk	BTB-NC	Pork skin and minced freshwater fish	3 days	Ready to eat product
Nem Trey	BTB-NT	Minced freshwater fish	3 days	Ready to eat product
Sang Vak	BTB-SV	Mined freshwater Fish	2 days	Roast prior consumption

BTB-NC: Nem Sach Chrouk from Battambang province; BTB-NT: Nem Trey from Battambang province; BTB-SV: Sang Vak from Battambang province

2.2. Physicochemical analysis

All samples were analyzed for their physicochemical characteristics include pH, titrable acidity, fat, moisture, ash, and salinity. Methods for each parameters were described as follows.

2.2.1. pH and titratable acidity

Five grams of ground sample were diluted and homogenized by using an Ultra Turrax homogenizer. Then, it was measured by pH meter (LAQUA F-72-HORIBA, USA). Afterward, titratable acidity was determined by titration with 0.1 N of NaOH using phenolphthalein as the indicator (Sangjindavong et al., 2005). The percentage of lactic acid was calculated as given in Eq. 1.

$$\text{Acidity (\% as lactic acid)} = V \times N \times 9/m \qquad \textbf{(Eq. 1)}$$

Where V : volume of NaOH used (ml)
N : normality of NaOH (N)
m : mass of sample (g)

2.2.2. Fat content analysis

Five grams of each sample were ground and placed in an oven for 24 h at 105°C to reduce the moisture content. After that, the dried sample was packed with filter paper and placed directly inside the cellulose thimble. It was then inputted into the Soxhlet extraction unit (VELP SCIENTIFICA, Italy). Simultaneously, 50-70 mL of hexane was also added. Then, the extraction unit was closed, and started the cooling water flow and the heating. At the end of extraction, the extract collecting vessel was dried in an oven for 1h at 105 °C to completely evaporate the residue solvent. Last but not least, the desiccated glass was reweighted to obtain the extracted oil from the sample. The fat content was then calculated by Eq. 2.

% Fat = Extracted fat × 100/mass of sample **(Eq. 2)**

2.2.3. Moisture content analysis

Moisture content was analyzed by measuring the weight before and after drying in the oven. Five grams of each sample were grounded and weighted for its precise initial mass. Then, the sample was placed in the oven at 105 °C for 24 hours. After that, it was taken out, put in the dessicator for 15 mins, and reweighted [9]. The moisture content was calculated as the following formula (Eq. 3):

$$\% \text{ Moisture} = [(m_1+m_2)-m_3/m_1] \times 100 \quad \textbf{(Eq. 3)}$$

Where m_1 : mass of wet sample before drying (g)
m_2 : mass of empty plate (g)
m_3 : mass of plate and sample after drying (g)

2.2.4. Ash content analysis

Similarly, five grams of ground sample was placed in a crucible and introduced to the oven at 105 °C for 24 hours before transferring to the furnace at 550 °C for 3 hours until the gray content was obtained. It was then put in dessicator for 15 minutes and reweighted [9]. The ash content was calculated as the following formula (Eq. 4):

$$\% \text{ Ash} = (m_2-m_1) \times 100/m_3 \quad \textbf{(Eq. 4)}$$

Where m_1 : mass of empty crucible (g)
m_2 : total mass of crucible and ash (g)
m_3 : mass of sample (g)

2.2.5. Salinity

Five grams of sample (m_a) was weighed and filled with distilled water to 100 g (m_b). Then, 25 g (m_c) of the homogenized samples was transferred into a new glass beaker, and 25 mL of distilled water was added. Afterwards, 1 mL of K_2CrO_4 was added as indicator. The samples were then titrated with 0.1 M $AgNO_3$ until the reddish-brown precipitate was obtained [10]. The salt content was calculated as % as given in Eq. 5.

$$\% \text{ Salt} = V \times M \times 0.0584 \times 100/m \quad \textbf{(Eq. 5)}$$

Where:
V is the volume of the $AgNO_3$ (mL)
M is the molarity of the $AgNO_3$ (M)
the initial sample weight is $m= m_a \times m_c/m_b$

2.2.6. Protein analysis

The Kjeldahl method was used to estimate the nitrogen content. According to the AOAC 960.52, ISO 1871 manual was divided into three steps including digestion, distillation, and titration. Firstly, 100 mg of each grounded sample was placed into a Kjeldahl flask and mixed with 3.6 g of potassium sulfate, 0.2 g of copper sulfate, and 2.7 mL of sulfuric acid. The mixture was boiled for 2 h at 420 °C, then cooled at room temperature (solution A). Secondly, 40 mL of distilled water, 5 mL of 4% boric acid, and 3 drops of methyl blue were added into Erlenmeyer (Solution B). Then, the solution (A) and (B) were inputted into the distillation unit (Kjelflex, K-306). After that, 40ml of the distilled solution was collected and titrated with 0.02 M of HCl until its color changed to light pink. The protein content was then calculated by:

$$\% \text{ N} = (Vs-Vb)\times M\times F\times 14\times 100/m \quad \textbf{(E.q. 6)}$$

$$\% \text{ P} = \%\text{N} \times 6.25$$

Where Vs: volume of HCl (ml)
Vb: volume of HCl for for blank (ml)
M: molarity of HCl (mol/L)
F: Correction factor of HCl
m: mass of sample (mg)
14: mass molecule of nitrogen (g/mol)
6.25 was nitrogen factor conversion.

2.2.7. Microbiological analysis

From each fermented product, a 10 g sample was chopped, hand-homogenized for one minute and diluted with 90 mL of phosphate buffered saline (PBS) [11,12]. The enumeration of total plate count was done by using the spread plate technique. The volume of 0.1 ml of each diluted sample was spread onto the surface of Luria Bertani agar media until it completely absorbed. Plates were then placed in incubator at 37 °C for 24 h [13]. The colonies counting of total plate count were presented as logarithms colony forming units per gram (g) (log CFU/g).

$$N(CFU/g) = \sum C/Vd(n_1+0.1n_2) \quad \textbf{(Eq. 7)}$$

Where:

$\sum C$: sum of counting colonies on all retained plates
d : dilution factor
n_1 : number of plates retained for the first dilution
n_2 : number of plates retained for the second dilution
V : volume inoculated on each plate

2.2.8. Statistical analysis

The results were statistically analyzed by using the Statistical Package for the Social Sciences (SPSS, Version 25.0.0 for Windows, 2011; IBM Co., Somers, NY, USA). The Data were analyzed for the degree of variation by calculating the results' mean and standard deviations (SDs). The significance of differences was evaluated using analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant. All parameters were expressed as mean ± standard deviation and were analyzed using one-way ANOVA with 95% of confident level ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. pH and titratable acidity of fermented fish products

The fermentation process relies on pH, among other factors. The pH value measures the acidity and alkalinity of food products. As pH rises or falls, the environment for microorganisms in a food product changes [14]. During fermentation process, many lactic acid producing bacteria grow and produce organic acids, in particular, lactic acid, which forms somewhat favorable conditions for the meat protein degradation by lowering the pH value [15]. Fig. 1 represents the pH value and acidity in Cambodian fermented fish and meat products collected from Battambang province. Regarding the pH values, they ranged from 4.52 to 4.93, 4.54 to 5.05, 4.55 to 5.12 in Sang Vak, Nem Trey, and Nem Sach Chrouk, respectively, which were obtained after the short fermentation period. The pH and acidity value can give a knowledge on the acidity of product whether it is safe to consume as it is a natural preservation marker. Normally, the complete cured of fermented product should be less than 4.6 which is considered as safe for consumption recommended by Australian food safety authority.

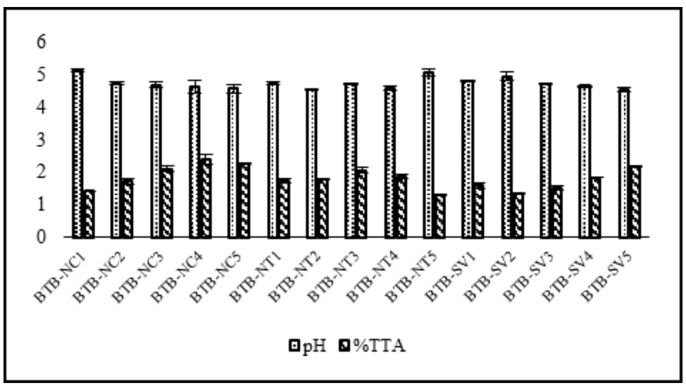

Fig. 1. pH and titratable acidity for each sample of fish products

Overall, the pH values of these products were higher than Nem Chua, the traditional fermented meat in Vietnam that was obtained on the third day of fermentation period. This might be due to the different raw material used and various environments applied fermentation took place [6].

During fermentation, many lactic acid-producing bacteria grow and produce organic acids, particularly lactic acid, which preserves the product by increasing the acidity value or lowering pH value [16]. This is responsible for the increased production of a variety of organic acids produced by natural starter cultures. TTA ranged from 1.33 to 2.15% in Sang Vak, 1.31 to 2.05% in Nem Trey, and 1.41 to 2.47% in Nem Sach Chrouk samples. TTA in fermented fish and meat products was expressed as total acid, which mainly focused on the lactic acid production of lactic acid bacteria present in the products. It has increased continuously which contradicts to pH throughout the fermentation process [17]. Thus, the drop of pH or increasing the amount of lactic acid in fermented fish products is relatable and beneficial for reducing or eliminating undesirable microorganisms for product preservation [18]. Furthermore, lactic acid is frequently reported as a critical contributor to the acid aroma and flavor of fermented meat products. It may play a significant part in creating its texture as a result of acid-induced alterations in fermented products [19].

3.2. Chemical compositions of fermented fish and meat products

Chemical compositions such as moisture, fat, salt, and ash content of 15 fermented fish and meat products were displayed in Table 2. The moisture content of all samples varied from approximately 58.65 to 68.07 g/ 100g. Moisture content refers to the amount of water in a food product and can be used to categorize product types based on its percentage. The moisture content in Nem production is quite essential, and it relies on the type of microbe and substrates involved in the fermentation process [20]. Theoretically, the higher moisture content level during the ripening stage of fermentation can be correlatable with water absorption by muscle tissue, for it swells when immersed in brine for an extended period [17]. In comparison, the moisture content of Cambodian fermented fish and meat products (Nem) was lower than Nham-Pla or sour fish cake from Thailand, which is one of the fermented fishery products processed from mostly fresh-water fish [21]. Based on the interview with 15 local producers at the sampling location, two types of fermented product (Nem Trey and Nem Sach Chrouk) were incubated for three days. In contrast, the fermentation period of Sang Vak took place only two days at room temperature, shorter than fermented fish in Thailand that were placed on trays and kept at ambient temperature (28 °C-30 °C) for 4 days [21].

Fat content varied from 0.40 to 5.45 g/100g of all samples. The lowest percentages were found in BTB-NT5 (0.40 ± 0.09) and BTB-NT4 (0.51 ± 0.09). Overall, the fat content in Nem Sach Chrouk was lower than other remaining fermented fish products (Nem Trey and Sang Vak). Based on a survey questionnaire with different local producers located in Battambang province (unpublished data), edible parts of fish have been rinsed with tap water to remove undesired parts especially fat. The rinsing time were varried from one producer to another producer. So, the amount of fat removed from fish was completely different. Besides that, there are many types of raw fishes used in their production that purchased from local producers and neighboring country, Thailand. The fat content of the fishes varied markedly among the fishes thereby resulting in different fat content within the final products [22]. Moreover, Kumar et al. [17] suggested that the decrease in fat content can be correlated with the increase in the peroxide value and free fatty acid value which suggests the degradation of lipid.

Additionally, the ash content referred to any inorganic materials found in food products, such as minerals. The finding showed the ash content found in three types of fish and meat fermented prodcuts ranged from 3.01% to 4.40%. The slight difference between the prodcuts could be attributed to the differences in the raw material types used, ingredients and processing techniques. According to the survey (unpublished data), producers at Ek Phnom distric have carried out their own recipes. Many types of fish are routinely used such as Trey Ros *(Chevron snakehead),* Trey Chhdor *(Giant snake head)*, and Trey Diep *(Channa micropeltes)* which are considered as the local fish while Trey Trasok *(Probarbus labeaminor)*, Trey Nuon Chan, Trey Proul *(Cirrhinus microlepis)*, Trey Kaek *(Labeo chrysophekadion)*, and Trey Slat *(Notopterus notopterus)* are imported from Thailand [8]. It can be concluded that the different raw materials used has a big influence on the ash content to the final products. The increase in the ash content of the edible parts of certain species of fish depending on the presence of total mineral varied on fish species [23].

Table 2 Chemical compositions of fermented fish products

Sample codes	Chemical compositions of fermented fish and meat products (Mean ± SD)			
	Moisture (%)	Fat (%)	Salt (%)	Ash (%)
BTB-NC1	58.65 ±0.52^{a}	1.29±0.13^{c}	2.34±0.01^{g}	4.40±0.06^{g}
BTB-NC2	66.70±0.20^{e}	1.71±0.04^{d}	1.91±0.01^{d}	3.24±0.16^{bcd}
BTB-NC3	66.87±0.44^{e}	2.65±0.21^{e}	2.12±0.01^{e}	2.70±0.29^{a}
BTB-NC4	68.07±0.67^{f}	1.47±0.17^{cd}	1.62±0.00^{bc}	3.13±0.01^{bc}
BTB-NC5	65.18±0.21^{c}	0.69±0.18^{b}	2.56±0.03^{h}	4.25±0.03^{g}
BTB-NT1	65.14±0.25^{c}	2.72±0.12^{e}	1.86± 0.03^{d}	3.15±0.05^{bc}
BTB-NT2	63.27±0.44^{b}	4.49±0.04^{h}	2.16±0.05^{e}	3.57±0.03 ^{def}
BTB-NT3	66.99±0.61^{e}	4.21±0.11^{gh}	1.89±0.01^{d}	3.54±0.03 ^{def}
BTB-NT4	64.60 ±0.22^{c}	0.51±0.09 ^{ab}	2.27±0.02^{f}	3.60±0.07^{ef}
BTB-NT5	65.46±0.35^{cd}	0.40 ± 0.09^{a}	2.16±0.06^{e}	3.01±0.33^{b}
BTB-SV1	66.27±0.23^{cd}	2.46±0.11^{e}	1.40±0.06^{a}	3.36±0.14^{b}
BTB-SV2	64.83±0.28^{c}	4.30±0.04^{gh}	1.60±0.01^{b}	3.79±0.06^{f}
BTB-SV3	62.70±0.17^{b}	5.45±014^{i}	1.60±0.02^{b}	3.43±0.23^{cde}
BTB-SV4	65.41±0.37^{cd}	3.60±0.21^{f}	2.10±0.02^{e}	3.59±0.02^{ef}
BTB-SV5	67.06±0.52^{e}	4.15±0.02 ^{g}	1.68±0.06^{c}	3.11±0.04^{bc}

* SD stands for Standard Deviation; Value with different superscript letter in the same column indicate significant differences (*$p<0.05$).*

Moreover, the higher ash content found in this current study might be due to the addition of ingredients such as galangal, red chili, garlic, salt, sugar, and monosodium glutamate (MSG). Among them, galangal is used in Nem processing as the flaovoring agent and to reduce undesirable order in meat products [24]. Previous work indicated that galangal rhizome are nutritionally valuable food spices which contained appreciable quantities of essential nutrients, especially minerals (5.38%) [25]. All Cambodian fermented fish and meat products contain high content of total minerals if compared to Nham Pla, Thai fermented fresh water fish product [21]. Moreover, the amount of ash content can be proportioned to the level of salt content due to the degradation of salt and protein by biochemical processes during fermentation [17]. The result of this current study illustrated that the salt content ranged from 1.40% to 2.31%. The different salt content in each product might be caused by the recipe of Nem processing from individual producer at Battambang province. This is not in agreement with a previous study, which reported that the salt content of Cambodian fermented fish products ranged from 6% to 34% [12].

3.3. Protein content

Fermentation of fish-based products produces preferable organoleptic properties, including desirable aroma, texture, and taste, but apart from these characteristics, fermented fish products have also been shown to have good nutritional value [26]. As illustrated in Fig. 2, the protein content varied widely among Nem products: 13.82 g/ 100g in BTB-SV, 11.97 g/100g in BTB-NT, and 12.33 g/100g in BTB-NC. In comparison, the protein content in all Cambodian fermented fish products above were lower than Nham-Pla (Thai fermented fish made from freshwater fish), ranging from 15.58 to 18.17 g/100g [21]. Although 15 local producers share common processes, the recipe from one producer to another producer of Nem processing can be varied as well as the variation in the initial protein in raw material, resulting in inconsistent protein content in the final products [15].

One review study provided an insightful review about protein that generated hydrophobic amino acids during fermentation, which plays a role as antioxidant activity. This means that fermented fish meat protein hydrolysates have hydrophobic amino acids that were produced during fermentation may have noticeable free radical-scavenging activity and can protect against oxidation by donating protons to the free radicals [27]. Additionally, the large number of small peptides released during fermentation also had hydroxyl and DPPH radical scavenging capacities. Therefore, this case study could provide us to conduct further studies after protein analysis (i.e. generation of hydrophobic amino acids during fermentation, amino acid profiles, and antioxidant activity generated from protein in fish during fermentation).

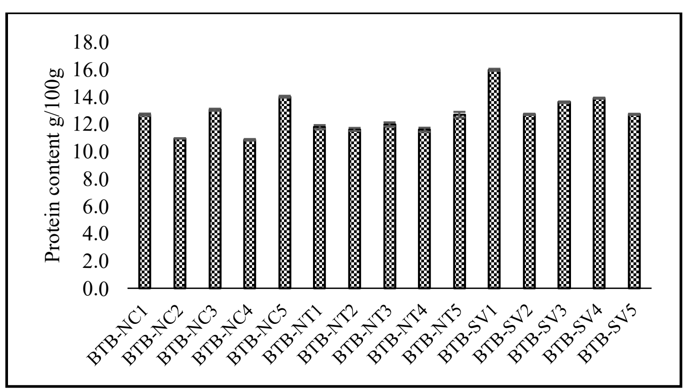

Fig. 2. Protein content in Sang Vak, Nem Trey, and Nem Sach Chrouk

3.4. Concentration of bacterial viable count

The results of microbial laods found in Nem Trey Nem Chrouk and Sang Vak are shown in Fig. 3. Overall, the concentration of bacterial viable count ranged from 5.85 log cfu/g to 7.98 log cfu/g. It points the level of all microbial population in Nem products. The highest TPC concentration was found in BTB-NT4. Based on survey and observation, BTB-NT4 producers were poor in sanitation and hygienic practices.

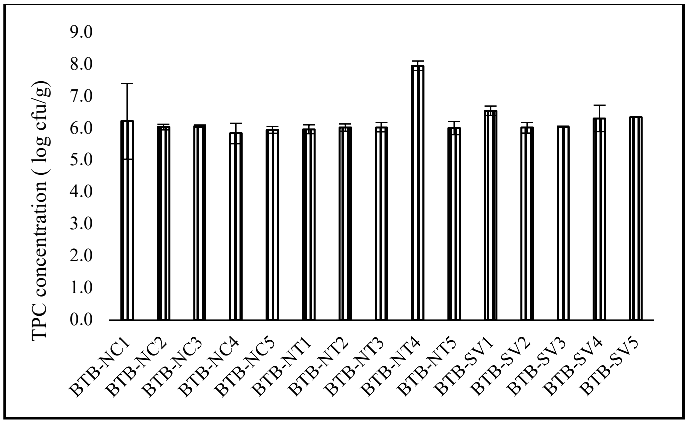

Fig. 3. Results of bacterial viable count for each sample

The presence of a high number of bacterial viable count indicates the poor quality of raw material, handling practice and storage condition. The results of TPC detected in ready-to-eat fermented fish and meat products in Battambang province were lower than other studies [7]. However, these products still have contaminated that could take place during the preparation process as well as the post-processing due to the inadequate hygience or packaging [28].

4. CONCLUSIONS

The chemical properties and nutritional values were assessed from 15 ready-to-eat fermented fish and meat products, Nem Trey, Nem Sach Chrouk, and Sang Vak from Battambang province. Among those samples, BTB-SV1 sample showed the high level of protein and ash content with low salinity among all samples. The current finding showed that the nutritional compositions provided a good source of protein as well as a high content of minerals which are beneficial to human health. The microbial concentration was also found with the lower level. This is the first report that provides basic information on various Cambodian fermented fish and meat products, and it will help to enhance the processing and manufacturing of these three types of products as well as develop a comprehensive scientific database to better understand fermented fish products. However, amino acid profiles and antioxidant activity generated from protein during fermentation would be investigated. Indentifciation of bacterial contamination would provide more information on safety profile of feremented and fish products. Furthermore, more research on shelf-life extension and improving the quality in term of packaging development will be needed to produce high-quality standardized fermented fish and meat products in Cambodia.

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REFERENCES

[1] Nam, S., & Bunthang, T. (2011). Fisheries Resources in Cambodia: Implications for Food Security, Human Nutrition and Conversation. Retrieved on 30 September 2022 from http://ifredi-cambodia.org/

[2] Gupta, S., Mohanty, U., & Majumdar, R. K. (2021). Isolation and characterization of lactic acid bacteria from traditional fermented fish product Shidal of India with reference to their probiotic potential. Lwt, 146, 111641.

[3] Narzary, Y., Das, S., Goyal, A. K., Lam, S. S., Sarma, H., & Sharma, D. (2021). Fermented fish products in South and Southeast Asian cuisine: indigenous technology processes, nutrient composition, and cultural significance. Journal of Ethnic Foods, 8(1), 1-19.

[4] Thompson, L., Vipham, J., Hok, L., & Ebner, P. (2021). Towards improving food safety in Cambodia: Current status and emerging opportunities. Global Food Security, 31, 100572.

[5] Anal, A. K., Perpetuini, G., Petchkongkaew, A., Tan, R., Avallone, S., Tofalo, R., Nguyen, H. Van, Chu-Ky, S., Ho, P. H., Phan, T. T., & Waché, Y. (2020). Food safety risks in traditional fermented food from South-East Asia. Food Control, 109, 1-40.

[6] Nguyen, H. T. H., Elegado, F. B., Librojo-Basilio, N. T., Mabesa, R. C., & Dizon, E. I. (2010). Isolation and characterisation of selected lactic acid bacteria for improved processing of Nem chua, a traditional fermented meat from Vietnam. Beneficial Microbes, 1(1), 67–74.

[7] Sangjindavong, M., Chuapoehuk, P., & Vareevanich, D. (2005). Studies on Nham-Pla's processing by using rock salt and solar salt. Kasetsart Journal, Natural Sciences, 39(2), 294–299.

[8] UNIDO. (2021). Profiling Of Post-harvest Fishery Value Chains And Market System Analysis. International Development Enterprise (iDE), Phnom Penh, Cambodia

[9] Sroy, S., Arnaud, E., Servent, A., In, S., & Avallone, S. (2021). Nutritional benefits and heavy metal contents of freshwater fish species from Tonle Sap Lake with SAIN and LIM nutritional score. Journal of Food Composition and Analysis, 96, 103731.

[10] Ly, D., Mayrhofer, S., Schmidt, J. M., Zitz, U., & Domig,

K. J. (2020a). Biogenic amine contents and microbial characteristics of Cambodian fermented foods. Foods, 9(2), 1–19.

[11] Desiree, K., Schwan, C. L., Ly, V., Hok, L., Bello, N. M., Nwadike, L., Phebus, R. K., & Vipham, J. L. (2021). Investigating Salmonella enterica, Escherichia coli, and coliforms on fresh vegetables sold in informal markets in Cambodia. Journal of Food Protection, 84(5), 843–849.

[12] Ly, D., Mayrhofer, S., Schmidt, J., Zitz, U., & Domig, K. J. (2020b). Characteristics of Cambodian Fermented Foods. Food, 9, 1–19.

[13] Macwilliams, M. P., & Liao, M. (2019). Luria Broth (LB) and Luria Agar (LA) Media and Their Uses Protocol Resource Type. Retrieved on 11 September 2022 from https://asm.org/

[14] Caert, I. (2006). pH and Fermentation. Retrieved on 13 October 2022 from http://agriculturewithmrsskien.weebly.com

[15] Stadnik, J., & Dolatowski, Z. J. (2014). Effect of inoculation with probiotics and ageing time on selected functional properties and oxidation of proteins in dry-cured pork loins. International Journal of Food Properties, 17(4), 866–876.

[16] Mahyudin, N. A., Ibadullah, W. Z. W., & Saadin, A. (2015). Effects of protein content in selected fish towards the production of lactic acid bacteria (Lactobacillus spp.) during the production of Pekasam. Current Research in Nutrition and Food Science, 3(3), 219–223.

[17] Kumar, G. P., Xavier, K. A. M., Nayak, B. B., Sanath Kumar, H., Gudipati, V., Senapathi, S. R., & Balange, A. K. (2021). Physicochemical and Microbiological Changes during Salt Fermentation of Pangasius Steaks, Journal of Culinary Science and Technology, 00(00), 1–17.

[18] Aro Aro, J. M., Nyam-Osor, P., Tsuji, K., Shimada, K. ichiro, Fukushima, M., & Sekikawa, M. (2010). The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. Food Chemistry, 119(1), 279–285.

[19] Aktaş, N., & Kaya, M. (2001). Influence of weak organic acids and salts on the denaturation characteristics of intramuscular connective tissue. A differential scanning calorimetry study. Meat Science, 58(4), 413–419.

[20] Sidi Ahmad, Z., & Abdul Munaim, M. S. (2017). Effect of Fermentation Time, Moisture Content, and Temperature on Sorbitol Production Via Solid State Fermentation Process. Journal of Chemical Engineering and Industrial Biotechnology, 1(1), 64–71.

[21] Sangjindavong, M., Chuapoehuk, P., & Raksakulthai, N. (2000). Quality characteristics of fermented sour fish cake (Nham-Pla). International Journal of Food Properties, 3(3), 399–406.

[22] Bimal Prasanna Mohanty, Satabdi Ganguly, Arabinda Mahanty, Tandrima Mitra, Sinjini Patra, D. Karunakaran, Suseela Mathew, Kajal Chakraborty, B.N. Paul, D. Sarma, Syama Dayal, Shivadhar Singh, & S. Ayyappan. (2019). Fish in human health and nutrition. Advances in Fish Research, 7, 189–218.

[23] Bogard, J. R., Thilsted, S. H., Marks, G. C., Wahab, M. A., Hossain, M. A. R., Jakobsen, J., & Stangoulis, J. (2015). Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. Journal of Food Composition and Analysis, 42, 120–133.

[24] Das, G., Patra, J. K., Gonçalves, S., Romano, A., Gutiérrez-Grijalva, E. P., Heredia, J. B., Talukdar, A. Das, Shome, S., & Shin, H. S. (2020). Galangal, the multipotent super spices: A comprehensive review. Trends in Food Science and Technology, 101, 50–62.

[25] Aljobair, M. O. (2022). Chemical composition, antimicrobial properties, and antioxidant activity of galangal rhizome. Food Science and Technology, 42, 1–8.

[26] Sezgin, A. (2019). Health bene fi ts of fermented foods. Critical Reviews in Food Science and Nutrition, 59(3), 1-22.

[27] Zang, J., Xu, Y., Xia, W., & Regenstein, J. M. (2020). Quality, functionality, and microbiology of fermented fish: a review. Critical Reviews in Food Science and Nutrition, 60(7), 1228–1242.

[28] Nout, M. J. R. (1994). Fermented foods and food safety. Food Research International, 27(3), 291–298.