Vietnam Journal of Food Control
A Scientific Journal of National Institute for Food Control
Journal homepage: https://vjfc.nifc.gov.vn

Research Article

Quality evaluation of Vietnam traditional soy sauce concerning nutritional composition and aflatoxin criteria

Le Thi Hoang Yen[1*], Nguyen Thi Minh Huyen[2], Nguyen Thi Thanh Mai[1], Phan Diep Tu[1], Pham Thi Bich Dao[3], Nguyen Ngoc Linh[3]
[1]*Center for Experimental Biology, Institute of Technology Application, Thanh Xuan, Hanoi, Vietnam*
[2]*University of Science and Technology of Hanoi (USTH), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[3]*Institute of Medicine and Pharmacy, Thanh Do University, Kim Chung, Hanoi, Vietnam*

(Received: 23 Sep 2025; Revised: 10 Nov 2025; Accepted:11 Nov 2025)

Abstract

Soy sauce (Tương) is a traditional Vietnamese fermented dipping sauce, known for its high nutritional value and cultural significance. However, the production process poses potential risks of contamination by pathogenic microorganisms and mycotoxins (aflatoxins). This study aimed to evaluate the quality and safety of *tương* through three main indicators: reducing sugar content, soluble protein content, and total aflatoxin levels in 38 samples collected from Hung Yen, Hanoi, Ha Nam, and Nghe An. The results showed that 35 out of 38 samples had aflatoxin levels below the detection limit (LOD = 0.74 µg/kg), while 3 samples from small-scale producers had levels above the LOD but still within the permissible limits set by QCVN 8-1:2011/BYT. Soluble protein content in the completed products ranged from 5.05% to 6.61%, and reducing sugar varied widely between 2.26% and 10.04%. These findings provide a scientific evidence for assessing the current quality status of traditional *tương* products and suggest directions for improving the production process to enhance and stabilize nutritional value and food safety of the product.

Keywords: *Aflatoxin, ELISA with the RIDASCREEN® Aflatoxin Total kit, total sugar, protein, Tuong Ban.*

1. INTRODUCTION

"Tương" (Vietnamese fermented soybean paste) is a traditional fermented product of Vietnam, produced from soybeans and glutinous rice, and it plays an important role in both cuisine and folk culture. In addition to its basic nutritional value, tương and fermented soybean products in general have been proven to provide multiple health benefits, including anti-inflammatory, antioxidant effects, support in reducing insulin resistance, regulation of gut microbiota, and potential for preventing of chronic diseases such as diabetes and cancer [1, 2]. These benefits largely arise from the fermentation process with the presence of beneficial microorganisms which can generate bioactive compounds, such as metabolized isoflavones, bioactive peptides, and so on [3].

In Vietnam, "tương" is widely produced with an estimated total output of roughly 100 million liters per year. "Tương" is traditionally producted on a small household scale, following a self-production and self-consumption model and only once a year during the summer, makes the fermentation process difficult to stabilize. Under uncontrolled conditions that depend heavily on the natural environment, the chance of retaining the beneficial mold strain *Aspergillus oryzae* is very low. In contrast, the product is highly susceptible to contamination by undesirable airborne fungal species such as *Aspergillus flavus* (toxic green mold),

*Corresponding author: Le Thi Hoang Yen (E-mail: yenlthvtcc@gmail.com)
https://doi.org/10.47866/2615-9252/vjfc.4588

Cladosporium spp, *A. niger* (black mold), and red molds. These fungi can spoil the product, reduce its quality, and pose risks of food poisoning due to mycotoxin production [4].

Among traditional tương production villages, "Tương Bần" Hung Yen (Ban soy paste) is manufactured on a larger scale, with many facilities having separated production zones and operating year-round. The *A. oryzae* mold strain is maintained and reused stably over many years, helping to preserve the beneficial microbial community, limit contamination from the environment, and contribute to improved food safety compared with smaller household-level facilities. However, even within craft villages, it remains unclear whether the quality of tương products is truly consistent in terms of nutritional composition and safety indicators such as aflatoxin content, as these factors have not yet been systematically monitored and reported.

Based on these concerns, we carried out a study to evaluate the current state of quality and safety of traditional "Tương" in Vietnam, through indicators such as protein content, reducing sugar level, and total aflatoxin. The findings will clarify the advantages of the semi-industrial production model in craft villages and provide scientific evidence for establishing standards and improving the production process of traditional tương toward enhanced safety, stability, and quality.

2. MATERIALS AND METHODS

2.1. Research materials

- Three raw material samples used for tương production were collected from the Thanh Dat traditional tương production facility (Ban Yen Nhan – Hung Yen), including soybeans and glutinous rice; and one corn sample purchased from Thanh Xuan Bac Market (Hanoi).

- Traditional tương samples: A total of 38 samples were collected: 29 samples were obtained from 10 production facilities in Hung Yen (coded S1–S29), comprising 22 finished products (S1–S21 and S23) and 7 semi-finished samples including rice fermented with *A. oryzae* and soybeans at the 5-day acid-fermentation stage (S22, S24–S29).

- Additionally, 5 finished tương products manufactured in Hanoi (HNS1, HNS2, HNS4, HNS5, HNS6), 1 semi-finished sample from Hanoi (HNS3), 1 sample from Nghe An (NgA), 1 sample from Ha Nam (Hna), and 1 sample from Trung Thanh Company (TT) were collected.

All samples were stored in PPE plastic bottles, transported to the laboratory, subsampled into 15-mL Falcon tubes, and preserved at 4°C until analysis.

2.2. Chemicals

RIDASCREEN® Aflatoxin Total kit (R-Biopharm, Germany); aflatoxin standards (R-Biopharm, Germany); Aflatoxin B1 and B2; Folin reagent; 0.2-µm cellulose membrane filter (Merck, Germany); Na_2CO_3 (Himedia, India); $NaHCO_3$; NaOH; phenol; tyrosine (Merck, Germany); 3,5-dinitrosalicylic acid (DNS) (Merck, Germany); $NaC_4H_4O_6 \cdot 4H_2O$ (Rochelle salt) (Merck, Germany); Na_2SO_3 (sodium sulfite) (Merck, Germany).

2.3. Equipment

UV–Vis spectrophotometer (UV-1601; Shimadzu, Tokyo, Japan); LC-MS/MS 6460 (Agilent Technologies, Germany); Spectra MaxID5 ELISA microplate reader; centrifuge (Germany); biological safety cabinet Labtech LCB-1121 VE (South Korea); etc.

2.4. Methods

2.4.1. Determination of pH

The pH was measured using a pH meter (Hanna Instruments HI2030-01, USA) following the standard protocol calibrated with pH buffer solutions 4.0 and 7.0. Samples were centrifuged at 10,000 rpm for 3 min and the supernatant was collected for measurement. Results were compared to the titration-based reference method according to TCVN 5516:2010 [5].

2.4.2. Determination of salt content

Salt concentration was determined using a handheld electronic salinity meter (Eutech ECSALT603PLUSK, Singapore), calibrated with standard NaCl solution. Results were compared to the reference titration method according to TCVN 3701:2009 [6].

2.4.3. Determination of total sugar content

A 1.0-mL reaction mixture containing 0.2 mL filtered and centrifuged tương sample and 0.6 mL DNS reagent was boiled at 100°C for 10 min. The mixture was rapidly cooled on ice and diluted with 4.2 mL of water. The reducing sugar content was measured using a UV–Vis spectrophotometer (UV-1601; Shimadzu, Tokyo, Japan) at 540 nm [7].

2.4.4. Determination of soluble protein content

Soluble protein was determined using the Folin reagent. Briefly, 1 mL of clarified sample was transferred into a test tube and mixed with 4 mL of 6% Na_2CO_3. Then, 1 mL of 0.2 N Folin reagent was added, followed by gentle mixing, and the mixture was incubated for 30 min at room temperature. The optical density (OD) of the reaction mixture was measured at 750 nm. The soluble protein content (expressed as µg tyrosine/mL) was calculated based on the tyrosine/albumin standard calibration curve [8].

2.4.5. Determination of aflatoxin

Aflatoxin content was measured using the ELISA method with the RIDASCREEN® Aflatoxin Total kit (R-Biopharm, Germany) [9]. The limit of detection (LOD) was 0.74 µg/kg and the limit of quantification (LOQ) was 3.43 µg/kg. All 38 tương samples were analyzed for aflatoxin content using the RIDASCREEN® Aflatoxin Total ELISA kit (R-Biopharm, Germany). Three aflatoxin concentrations (0; 0.15; 0.45 µg/L) were used to construct the calibration curve. The linear relationship between aflatoxin concentration (x) and optical density (OD, y) was described by the regression equation: $y = -1.3481x + 1.7223$ (**Figure 1**). The limit of detection (LOD) and limit of quantification (LOQ) were 0.74 µg/kg and 3.43 µg/kg, respectively.

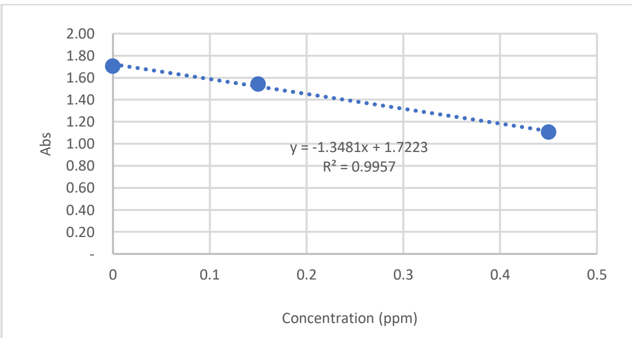

***Figure 1**. Calibration curve of aflatoxin concentration*

-The Hung Yen soy sauce sample which showed no detectable aflatoxin by the ELISA method - was re-examined using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS) for comparison, with a limit of detection (LOD) of 0.3 µg/g.

- The analysis was carried out using an LC-MS/MS 6460 system (Agilent Technologies, Germany). Chromatographic separation of aflatoxins was performed on an XDB-C18 column with an internal diameter of 2.1 mm, length of 100 mm, and particle size of 1.8 µm (Agilent Technologies, USA). The gradient mobile phases consisted of methanol (A) and 10 mM ammonium acetate (B). The flow rate of the mobile phase was maintained at 0.6 mL/min, and the injection volume was 5.0 µL. The column oven temperature was kept constant at 40°C [10].

3. RESULTS AND DISCUSSION

3.1. pH value and salinity in soy sauce samples

pH is one of the important characteristics to ensure the quality of soy sauce. If the pH value is too low, the growth of harmful microorganisms can be minimized; however, it has the drawback of creating an excessively sour taste, reducing the sweet and savory flavor of the sauce. In contrast, if the pH is too high, it diminishes the natural mild acidity, resulting in a bland and unbalanced taste, and may also cause changes in color (loss of the characteristic brown color) and the appearance of unusual or unpleasant odors. Additionally, a high pH (> 4.5) creates favorable conditions for the growth of harmful microorganisms (such as *Salmonella, E. coli*, *Clostridium botulinum*). Therefore, some countries have established standards requiring soy sauce to have an appropriate pH range. For example, the Cambodian standard CS 066:2011 for soy sauce specifies that the acceptable pH range is 4.2–4.8 [11].

In our study, the results showed that the pH values between finished soy sauce samples and soybeans at the acid-fermentation stage differed at a statistically significant level $\alpha = 0.05$ ($p < 0.05$), ranging from 3.7 to 4.8 (with an average value of 4.18) (**Figure 2**). These results are consistent with those reported by Ly *et al.* (2020) when studying the salinity of soy sauce from Cambodia, Vietnam, Thailand... noting that Vietnamese soy sauce samples have a pH range of 3.79–4.5, which is lower compared to countries such as Thailand, China, Singapore, Japan, Malaysia, Hong Kong, Taiwan, and France (pH 4.2–4.8) [11].

Among the 38 samples analyzed, 16 samples had pH < 4.2 - not meeting the pH standard for soy sauce set by Cambodia. This finding aligns with the study of Ly *et al.* (2020), which reported that 2/3 of Vietnamese soy sauce samples had pH < 4.2 - not compliant with Cambodia's pH standard [11]. Excessively low pH may result from prolonged fermentation or the adjustment of acidity to artificially increase sourness, which can affect flavor, microbiological quality, and may be related to the uncontrolled use of additives.

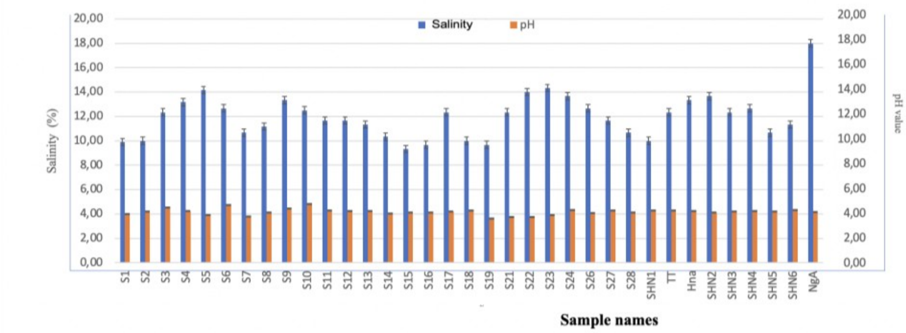

***Figure 2.** pH values and salinity concentration of soy sauce samples*

Salt, added from the beginning of the fermentation process at a proportion of approximately 12–18% of the final product weight. Salt is not added solely to add flavor or impart a salty taste; it also plays a crucial role in the natural preservation of soy sauce. By establishing a suitable chemical environment, it enables lactic acid bacteria and fermentation fungi to function properly, thereby protecting the product from contamination [2].

The results of salinity measurements obtained using two methods (electronic salinity meter and titration based on TCVN 3701:2009) showed no significant difference; therefore, the salinity values in this report were derived from the average of the two measurements. This ensured the accuracy and reliability of the results. Two samples, S25 and S29, at the koji mold fermentation stage (prior to acid fermentation and without salt supplementation), had salinity = 0 (not shown in **Figure 2**). The remaining samples, both finished and semi-finished, ranged from 10–15% (**Figure 2**). Only soy sauce from Nghe An showed a high salinity level (18.0%). This salinity level is still lower than soy sauce produced in China and Cambodia, where salt concentrations can reach up to 23.4% [11]. These results indicate that the salinity of Vietnamese soy sauce lies within a moderate range - suitable for the taste preferences of both domestic and international consumers - while still ensuring a certain level of preservation without being excessively salty or posing health risks.

3.2. Sugar and protein content in soy sauce samples

Soy sauce is a traditional fermented product prepared from soybeans and glutinous rice - both of which are nutritionally rich raw materials. Soybeans are an abundant source of plant protein, accounting for approximately 40.3%, along with fat (21%) and carbohydrates (33.9%) [12]. During fermentation, under the protease activity of microbial systems (*Aspergillus oryzae, Bacillus* spp., *Lactobacillus* spp., etc.), proteins in soybeans are hydrolyzed into short peptides and amino acids, increasing the soluble nitrogen (amino nitrogen) content - an important indicator reflecting nutritional value and fermentation degree [3, 12].

Among the 38 analyzed samples, soluble protein content in semi-finished products ranged from 3.15–4.28%. In contrast, this value in finished soy sauce samples was 5.05–6.61% (**Figure 3**). The average protein content of the 29 samples of Ban soy sauce (Hung Yen) was higher (5.43%) compared to the remaining samples (5.17%). This value is higher than the protein content reported for soy sauce from Myanmar (2.05–3.88%)

[13], but lower than that of Thai soy sauce (7.8%). The soluble protein content in the Ban Yen Nhan region (Hung Yen) was also relatively high (5.43%), with a standard deviation of 0.84% among samples; meanwhile, the deviation in other regions reached 1.28%. Therefore, Ban soy sauce products not only contain higher soluble protein contents but also demonstrate more stable quality compared to soy sauce produced in Hanoi, Nghe An, and Ha Nam.

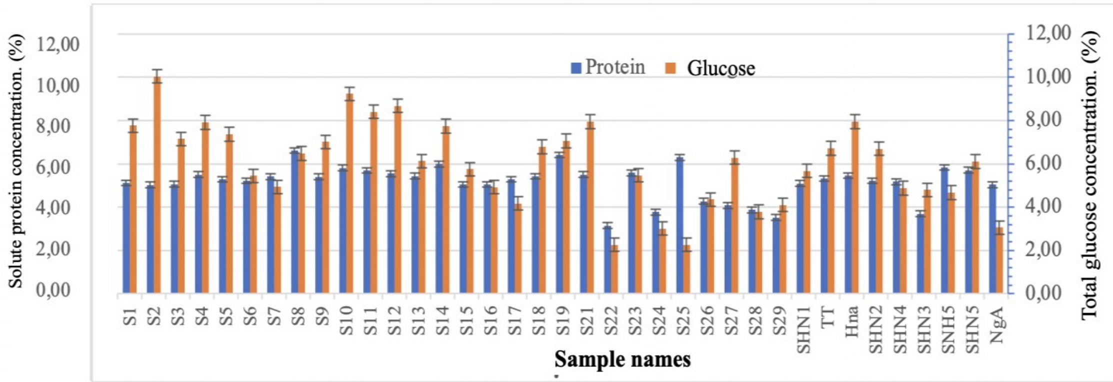

Figure 3. *Total sugar and soluble protein concentration in "tương"samples*

If soybean is the key protein component then glutinous rice is the key - starch content (mainly amylopectin) for producing "Tương". During the fermentation process, under the activity of microbial systems (such as molds, lactic acid bacteria, and yeasts), the starch from glutinous rice is hydrolyzed by amylase enzymes into simple sugars like glucose and maltose. This increases the natural reducing sugar content in tương, creating the characteristic natural sweetness and aroma as well as enhancing the nutritional value of the product.

In our study, the reducing sugar content among the 38 tương samples ranged from 2.26% to 10.04%, with an average value of 5.97%. Notably, the traditional Hung Yen tương samples (29 samples) exhibited a slightly higher average reducing sugar level (6.08%) compared to the overall mean of samples from other regions (5.97%) (**Figure 3**). Therefore, the products of traditional Hung Yen tương not only contain soluble protein but also possess higher total sugar content compared with "tuong" samples collected in other areas (Hanoi, Ha Nam and Nghe An).

3.3. Aflatoxin content in "tuong"

Among the 38 traditional tương samples analyzed (29 samples collected in Hung Yen, 6 samples of commercially produced products from Hanoi, 1 sample from Nghe An, 1 sample from Ha Nam, and 1 sample from Trung Thanh), 35 out of 38 samples showed no detectable aflatoxin contamination. Three samples were found to contain aflatoxins (one sample collected in Ha Nam and two samples collected in Hanoi), with concentrations of 0.82–0.85 µg/L (the contaminated samples originated from households using their own mold starter cultures that had not been standardized). None of the samples collected from Hung Yen contained aflatoxins when tested by the ELISA method.

These results were consistent with the subsequent HPLC–MS/MS aflatoxin analysis, in which no aflatoxin B1 was detected in the Hung Yen tương samples. The LC–MS/MS chromatographic transitions monitored included 315 → 287 m/z (aflatoxin B2), 329 → 243 m/z (aflatoxin G1) and 331 → 313 m/z (aflatoxin G2). Although a number of peaks were observed near the detection limit (0.3 µg/g), none of these peaks met the identification criteria (no characteristic peak pattern, retention time mismatch relative to the reference standard, and signal-to-noise ratio below the acceptable threshold). Therefore, the samples were classified as ND – Not Detected for aflatoxins upon LC–MS/MS confirmation. These findings are in agreement with the study conducted by Doan Van Thuoc *et al.* (2018), which reported that although 1/5 fungal strains isolated from tương Ban Hung Yen could produce aflatoxins at levels >5 ppm, the final corresponding tương products were aflatoxin-free [14].

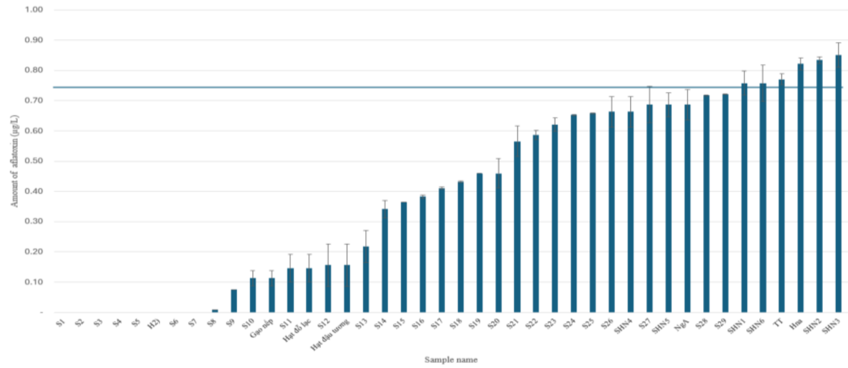

***Figure 4**. Aflatoxin content in traditional tương samples (the horizontal line indicates the detection threshold at 0.74 µg/L)*

Vietnam currently does not specify maximum permissible aflatoxin levels for tương products. However, QCVN 8-1:2011/BYT [15] regulates the allowable aflatoxin B1 limit for peanuts and other oilseed-based products intended for food processing at 8 µg/kg, and for peanuts and oilseeds intended for direct consumption without processing at 2 µg/kg. For cereal and cereal-based products (processed or unprocessed), the regulatory limits are 2 µg/kg for aflatoxin B1 and 4 µg/kg for total aflatoxins.

As shown, all 38 tương samples and 3 raw ingredient samples complied with QCVN 8-1:2011/BYT [15]. Notably, the samples that tested positive for aflatoxins at concentrations near the ELISA detection threshold (0.82–0.85 µg/L) were exclusively collected from small-scale household producers, highlighting potential food-safety risks associated with uncontrolled microbial inocula and inadequate fermentation and storage management. Therefore, there is a need to strengthen quality monitoring and provide technical guidelines to ensure product quality and safety for traditional tương production.

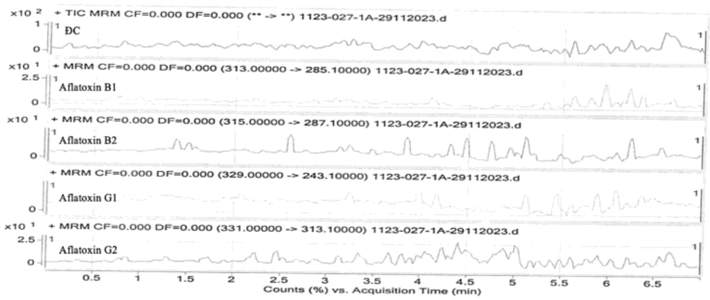

***Figure 5.** Aflatoxin analysis using LC–MS/MS*

4. CONCLUSION

This study surveyed and evaluated the quality of 38 traditional tương samples, focusing on sensory properties, pH, salinity, reducing sugar content, soluble protein content, and aflatoxin levels. The results show that tương Ban possesses distinctive quality characteristics, with higher protein and reducing sugar contents than other tương varieties, demonstrating its superior nutritional quality and product identity. pH and salinity values fell within appropriate ranges, contributing to microbial stability and product safety. Importantly, aflatoxins were absent or detected only at very low levels, meeting the requirements of QCVN 8-1:2011/BYT. However, the detection of aflatoxins in a small number of commercially marketed household samples suggests the necessity to tighten control of raw material quality, fermentation conditions, and storage practices. The findings of this study provide a scientific basis for developing technical standards for traditional Vietnamese tương products, contributing to quality assurance and the sustainable development of the industry.

ACKNOWLEDGEMENT

This study was funded by the project “Isolation, selection of beneficial microbial strains for safe production of traditional tương Ban and enhancement of product value”, supported by the Department of Science and Technology of Hung Yen Province.

The authors thank industrial producers in Trieu Son and traditional tương craft villages (Ban Yen Nhan, Hung Yen) and producers in other provinces (Duong Lam – Hanoi, Nam Dan Nghe An, Ha Nam) for providing representative samples and information for the study.

We also thank the Experimental Biology Center, Institute of Applied Technology; Hanoi University of Science and Technology; and the Institute of Biotechnology – Vietnam National University for supporting equipment and experimental facilities throughout the research process.

REFERENCES

[1]. Z. H. Cao, J. M. Green-Johnson, N. D. Buckley, Q. H. Lin, "Bioactivity of soy-based fermented foods: A review," *Biotechnology Advances*, vol. 37, no. 1, pp. 223–238, 2019.

[2]. M. Jayachandran and B. Xu, "An insight into the health benefits of fermented soy products," *Food Chemistry*, vol. 271, pp. 362–371, 2019.

[3]. N. T. V. Anh, "Research on technology and equipment for microbial preparations applied in industrial-scale traditional fermented food production," *Research Project KC07.12.06-10, Food Industry Research Institute*, Hanoi, Vietnam, 2010.

[4]. N. L. Dung, "The risk of liver cancer from traditional soybean paste," 2016. [Online] https://vietnamnet.vn/gs-nguyen-lan-dung-nguy-co-ung-thu-gan-tu-mon-tuong-truyen-thong-303638.html

[5]. Directorate for Standards, Metrology and Quality, National Standard TCVN 5516:2010 - Food Additive – Citric Acid. Hanoi: Science and Technics Publishing House, 2010. [Online]. Available: https://caselaw.vn/van-ban-phap-luat/252550-tieu-chuan-quoc-gia-tcvn-5516-2010-ve-phu-gia-thuc-pham-axit-citric-nam-2010

[6]. Directorate for Standards, Metrology and Quality, National Standard TCVN 3701:2009 - Fishery and Fishery Products - Determination of Sodium Chloride Content. Hanoi: Science and Technics Publishing House, 2009. [Online]. Available: https://thuvienphapluat.vn/TCVN/Cong-nghe-Thuc-pham/TCVN-3701-1990-thuy-san-phuong-phap-xac-dinh-ham-luong-natri-clorua-902529.aspx

[7]. W. Marsden, P. Gray, G. Nippard, and M. H. Quan, "Evaluation of the DNS method for analysing lignocellulosic hydrolysates," *Journal of Chemical Technology & Biotechnology*, vol. 32, no. 7, pp. 1016–1022, 2007.

[8]. L. T. Mai, N. T. Hien, P. T. Thuy, N. T. Hang, L. T. L. Chi, *Analytical Methods in Fermentation Technology*. Hanoi: Science and Technics Publishing House, 2009.

[9]. A. Ouakhssase, A. Chahid, H. Choubbane, A. Aitmazirt, and E. Ait Addi, "Optimization and validation of a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of aflatoxins in maize," *Heliyon*, vol. 5, no. 5, 2019.

[10]. H. Tanveer, H. Glesener, B. Su, N. Johnson, and D. L. M. N. H. Smith, "Evaluating methods for aflatoxin B1 monitoring in selected food crops within decentralized agricultural systems," *Toxins*, vol. 17, no. 1, 2025.

[11]. L. Ly, C. Peng, and R. Tan, "Price evaluation and quality control of different soy sauces sold in the markets," in *Proc. 13th AUN/SEED-Net Regional Conference on Chemical Engineering (RCChE-2020) jointly held with the 5th International Symposium on Conservation and Management of Tropical Lakes: Insights and Challenges toward Achieving SDGs*, Phnom Penh, Cambodia, 2020.

[12]. Bui The Truong, "A study of Vietnamese soy sauce fermentation," M.S. thesis, School of Environment and Agriculture, Univ. of Western Sydney, Penrith, Australia, 2003.

[13]. T. M. Lynn, K. Nyein Aye, and K. Khaing, "Study on the production of fermented soybean sauce by using Aspergillus oryzae and Aspergillus flavus," *Journal of Scientific & Innovative Research*, vol. 2, no. 1, pp. 1–10, 2013.

[14]. V. T. Doan, T. X. Dang, T. V. H. Le, and D. T. Nguyen, "Fungal and aflatoxin contamination in soybean paste collected from Ban village, Hung Yen province, Vietnam," *American Journal of Microbiology and Biotechnology*, vol. 5, no. 2, pp. 25–28, 2018.

[15]. Ministry of Health, National Technical Regulation QCVN 8-1:2011/BYT – Limits of Mycotoxins (Aflatoxin) in Food. Hanoi, Vietnam, 2011.