Research Note

Determination of biogenic amines and total aflatoxins: Quality index of starter culture soy sauce samples

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Abstract This study deals with understanding the effects of starter cultures on the safety aspects of soy sauce fermentation and their application to compensate undesirable changes occurring during fermentation processes. Various starter cultures of bacterial and fungal combinations with different microbial strains were used for the production of soy sauce samples under standardized fermentation/manufacturing conditions, and contents of biogenic amines (BAs) and aflatoxins were determined. Quantitative analysis of BAs and aflatoxins was performed using HPLC and immunoassay-based total aflatoxin detection kit, respectively. The amount of total BAs in all laboratory-made starter culture soy sauce samples was quantified in the range of 2.80-20.42 mg/L, which was within the limit of human consumption safety level. In addition, the level of total aflatoxin was under the limit (0.00 to 4.80 µg/kg), according to the standard parameters of food and agricultural organization (FAO) and world health organization (WHO).

Keywords: soy sauce, starter culture, aflatoxin, biogenic amines, food safety

Introduction

Soy sauce is a commonly known condiment that has been utilized for marinade and seasoning purposes in a variety of meat-based cuisines in China, Japan, Korea, and Thailand (1). Soy sauce, mainly used as a flavor and taste enhance ingredient, is a fermented food derived from the fermentation of soybean or wheat and has become increasingly popular because of its unique salty taste and sharp flavor as well as antioxidant properties and health benefits (2). The quality of soy sauce is mainly determined based on its sensory attributes and biochemical compositions (3).

Traditionally, Korean soy sauce is produced during post-fermentation of *Meju* (as a starter culture) in a salty environment (15-20%) by a combined action of different microbial strains, including bacteria and fungi such as *Bacillus*, *Mucor*, *Rhizopus*, *Penicillium*, *Syncephalastrum*, *Eurotium*, and *Aspergillus* species, which significantly contributes to its distinctive sensory characteristics (4). Production of hazardous substances, including mycotoxins and biogenic amines (BAs) produced by microorganisms during fermentation, has been a serious concern for human health. Production of aflatoxins is mainly attributed to *Aspergillus flavus* and *Aspergillus parasiticus*, which cause contamination of food and feed including corn, rice, wheat, spices, soybean, and nuts (5). As previously reported, the consumption of a number of fermented soy products contaminated with aflatoxin producing fungi and/or aflatoxins has

been found to have caused several cases of stomach cancer in the Korean population (6).

In addition, a level of BAs above the recommended limit can cause a significant threat to human and animal health because of the potent carcinogenic, teratogenic, and mutagenic nature of BAs, which may also cause severe complications such as neurotransmission diseases (7). Formation of BAs and aflatoxins in soy sauce depends on various factors, including the ratio of soybean in the raw material, microbial flora, fermentation conditions, and salt content (8,9).

Previously, we reported reduction of aflatoxins and BAs in fermented soybean paste samples (*Doenjang*) using various starter cultures and observed that *Doenjang* samples produced using a few combinations of starter cultures accomplished a threshold limit of human consumption based on their quality and safety assessments (10). Therefore, in the present study, we aimed to produce soy sauce using various similar combinations of starter culture strains (bacterial and fungal strains) and to determine the content of BAs and aflatoxins in soy sauce samples in order to confirm their food safety from a hygiene point of view.

Materials and Methods

Production of starter culture-based soy sauce We used our previously reported method for making starter culture-based soy

sauce samples (9). Microbial strains were selected based on their content in traditional *Meju* samples and were identified previously by our research group. Briefly, starter culture-based soy sauce was made by fermenting the *Meju* (different microbial flora in various ratios such as 1:1 and 1:1:1) with 15% (w/v) sodium chloride solution for 1 month at 30°C. After 1 month of fermentation, fermented samples were separated in liquid and solid phases. The separate liquid phase is referred to as soy sauce. A list of all starter culture soy sauce samples analyzed in this study is presented in Table 1. All fermented soy sauce samples were stored at 4°C until analyzed.

Measurement of pH pH measurement of the starter culture-based soy sauce samples was performed according to the procedure described previously (10) using a pH meter (Orion 35 Star pH Benchtop; Thermo Electron Corporation, Beverly, MA, USA).

Determination of total aflatoxin Extraction of aflatoxin from starter culture soy sauce samples was performed as per the producer's (Neogen Corp., Lansing, MI, USA) instructions supplied with the quantitative aflatoxin test kits (Veratox for total aflatoxin). Briefly, 10 mL of each soy sauce sample was mixed and vortexed with 50 mL of 70% (v/v) methanol for 2–3 min at room temperature and filtrated with Whatman filter paper No. 2 (Advantec, Tokyo, Japan). Each filtrate was subjected to an enzyme-linked immunosorbent assay (ELISA) analysis using a Neogen Veratox kit without further purification. Optical densities of the developed color were measured in comparison to known concentrations of four reference standards (0, 5, 15, and 50 ppb) using an ELISA reader (Tecan, Mannedorf, Switzerland) at 650 nm. The results were calculated using Neogen's Veratox data reduction software to convert the absorbance values into concentration values.

Determination of BAs Solutions of all nine standard biogenic

amines, agmatine sulfate, tryptamine hydrochloride, 2-phenylethylamine, putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride, spermidine trihydrochloride, and spermine tetrahydrochloride, were separately prepared at 50 mg/50 mL concentration in distilled water. A working solution was prepared by diluting 1,000 µL of each stock solution in distilled water to obtain a final volume of 10 mL. These solutions were stored at 4°C until used. Extraction of fermented soy sauce samples and HPLC determination of BAs in soy sauce samples were performed according to the modified procedure developed by Shukla *et al.* (8).

Statistical analysis All data were analyzed and presented as mean ± standard deviation of two independent experiments.

Results and Discussion

pH measurement The pH values of all tested starter culture soy sauce samples are shown in Table 1. The pH is a limiting factor that significantly influences several reactions, the presence of microflora, and the decarboxylase activity of amino acids of soy sauce products. In the present study, all tested starter culture soy sauce samples showed pH in the range of 4.9–6.4. Yongmei *et al.* (11) also reported similar pH range in Chinese soy sauce samples. Xu *et al.* (12) also studied the improved fermentation of soy sauce by *Aspergillus oryzae* based-starters and reported that at the initial stage of soy sauce fermentation, the value of pH gradually decreased to 4.5–5.0, which might be due to the degradation of proteins and accumulation of organic acids.

Total aflatoxin The results of the quantitative analysis of total aflatoxin levels, determined in soy sauce starter culture samples, are

Table 1. List of Korean soy sauce samples fermented with starter culture made of various microbial strains

Soy sauce samples	Microbial strains used	pH
1	<i>Bacillus subtilis</i>	5.0
2	<i>Aspergillus oryzae</i>	5.3
3	<i>Penicillium chrysogenum</i>	6.4
4	<i>Mucor racemosus</i> -1	5.6
5	<i>Cladosporium uredinicola</i>	5.8
6	<i>Penicillium fellutanum</i>	5.3
7	<i>Mucor racemosus</i> -2	5.6
8	<i>Bacillus subtilis</i> + <i>Aspergillus oryzae</i>	5.0
9	<i>Bacillus subtilis</i> + <i>Penicillium chrysogenum</i>	5.7
10	<i>Bacillus subtilis</i> + <i>Mucor racemosus</i> -1	5.5
11	<i>Bacillus subtilis</i> + <i>Cladosporium uredinicola</i>	5.0
12	<i>Bacillus subtilis</i> + <i>Penicillium fellutanum</i>	5.0
13	<i>Bacillus subtilis</i> + <i>Aspergillus oryzae</i> + <i>Penicillium chrysogenum</i>	5.2
14	<i>Bacillus subtilis</i> + <i>Aspergillus oryzae</i> + <i>Mucor racemosus</i> -1	5.2
15	<i>Bacillus subtilis</i> + <i>Aspergillus oryzae</i> + <i>Cladosporium uredinicola</i>	4.9
16	<i>Bacillus subtilis</i> + <i>Aspergillus oryzae</i> + <i>Mucor racemosus</i> -2	4.9
17	<i>Bacillus subtilis</i> + <i>Aspergillus oryzae</i> + <i>Penicillium fellutanum</i>	5.0

Table 2. Analysis of biogenic amine content (mg/L) and aflatoxin ($\mu\text{g/kg}$) in soy sauce samples fermented with starter culture *Meju*

Soy sauce samples	Agmatine	Tryptamine	Phenyl-ethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	Total	Aflatoxin
1	1.93 $\pm$ 0.01 ¹⁾	0.48 $\pm$ 0.00	0.35 $\pm$ 0.01	3.22 $\pm$ 0.00	0.11 $\pm$ 0.00	0.59 $\pm$ 0.00	2.14 $\pm$ 0.02	0.23 $\pm$ 0.00	0.05 $\pm$ 0.02	9.09	3.80
2	1.42 $\pm$ 0.00	0.06 $\pm$ 0.00	ND ²⁾	6.33 $\pm$ 0.03	0.07 $\pm$ 0.00	7.78 $\pm$ 0.02	0.05 $\pm$ 0.00	0.15 $\pm$ 0.00	ND	15.87	3.80
3	0.54 $\pm$ 0.02	1.00 $\pm$ 0.01	0.95 $\pm$ 0.01	6.86 $\pm$ 0.06	0.09 $\pm$ 0.00	8.19 $\pm$ 0.15	2.44 $\pm$ 0.04	0.12 $\pm$ 0.00	0.10 $\pm$ 0.00	20.28	0.20
4	0.63 $\pm$ 0.02	0.71 $\pm$ 0.01	1.29 $\pm$ 0.01	5.29 $\pm$ 0.06	Trace ³⁾	4.75 $\pm$ 0.03	2.00 $\pm$ 0.04	0.08 $\pm$ 0.01	0.26 $\pm$ 0.01	15.06	2.60
5	0.74 $\pm$ 0.04	0.77 $\pm$ 0.01	1.67 $\pm$ 0.01	5.14 $\pm$ 0.01	0.12 $\pm$ 0.00	4.76 $\pm$ 0.02	2.24 $\pm$ 0.01	0.27 $\pm$ 0.01	ND	15.71	1.80
6	0.90 $\pm$ 0.01	0.55 $\pm$ 0.00	0.70 $\pm$ 0.01	7.34 $\pm$ 0.05	0.21 $\pm$ 0.00	0.56 $\pm$ 0.00	1.5 $\pm$ 0.00	0.09 $\pm$ 0.03	ND	11.94	3.00
7	0.65 $\pm$ 0.01	0.74 $\pm$ 0.01	2.46 $\pm$ 0.01	0.11 $\pm$ 0.00	Trace	4.39 $\pm$ 0.04	1.25 $\pm$ 0.02	0.13 $\pm$ 0.01	ND	9.76	2.60
8	1.51 $\pm$ 0.03	0.44 $\pm$ 0.00	0.56 $\pm$ 0.02	4.65 $\pm$ 0.02	0.07 $\pm$ 0.00	2.66 $\pm$ 0.00	1.61 $\pm$ 0.01	0.14 $\pm$ 0.00	ND	11.64	0.00
9	0.99 $\pm$ 0.00	0.78 $\pm$ 0.00	1.13 $\pm$ 0.00	5.19 $\pm$ 0.03	0.07 $\pm$ 0.02	5.47 $\pm$ 0.01	2.19 $\pm$ 0.04	0.19 $\pm$ 0.01	ND	16.01	0.00
10	0.74 $\pm$ 0.05	0.55 $\pm$ 0.00	0.72 $\pm$ 0.00	4.63 $\pm$ 0.01	0.09 $\pm$ 0.01	3.74 $\pm$ 0.01	2.01 $\pm$ 0.01	0.14 $\pm$ 0.00	ND	12.62	0.00
11	1.79 $\pm$ 0.01	0.48 $\pm$ 0.00	0.42 $\pm$ 0.00	3.41 $\pm$ 0.04	0.09 $\pm$ 0.01	2.73 $\pm$ 0.03	2.25 $\pm$ 0.01	0.25 $\pm$ 0.00	ND	11.41	0.80
12	1.27 $\pm$ 0.00	0.46 $\pm$ 0.00	Trace	4.64 $\pm$ 0.02	0.07 $\pm$ 0.00	6.69 $\pm$ 0.03	0.35 $\pm$ 0.01	0.09 $\pm$ 0.00	ND	13.62	2.60
13	1.72 $\pm$ 0.02	0.59 $\pm$ 0.00	0.42 $\pm$ 0.02	0.34 $\pm$ 0.00	0.06 $\pm$ 0.02	0.36 $\pm$ 0.00	1.87 $\pm$ 0.00	0.15 $\pm$ 0.02	ND	5.51	3.00
14	1.10 $\pm$ 0.06	0.50 $\pm$ 0.00	1.54 $\pm$ 0.01	7.57 $\pm$ 0.05	0.15 $\pm$ 0.03	7.60 $\pm$ 0.03	1.82 $\pm$ 0.00	0.13 $\pm$ 0.03	ND	20.42	2.60
15	1.36 $\pm$ 0.06	0.52 $\pm$ 0.01	0.13 $\pm$ 0.02	0.31 $\pm$ 0.00	0.05 $\pm$ 0.01	0.22 $\pm$ 0.00	Trace	0.18 $\pm$ 0.01	ND	2.80	3.40
16	1.17 $\pm$ 0.00	0.48 $\pm$ 0.02	0.53 $\pm$ 0.00	0.15 $\pm$ 0.00	Trace	0.40 $\pm$ 0.01	0.19 $\pm$ 0.00	0.09 $\pm$ 0.02	ND	3.04	4.80
17	1.51 $\pm$ 0.01	0.04 $\pm$ 0.00	Trace	7.22 $\pm$ 0.07	0.19 $\pm$ 0.00	Trace	ND	0.14 $\pm$ 0.00	ND	9.14	1.80

¹⁾Results are given in mean values of two determinations.²⁾ND, not detected.³⁾Trace indicates less than 0.01 mg/mL BA content.

presented in Table 2. In this study, the majority of samples contained total aflatoxin levels in the range below the quantitation limit (<5 ppb), as described in the Veratox total aflatoxin test kit used for aflatoxin detection. The amount of total aflatoxin levels in the starter culture of soy sauce samples was found to be in the range from 0.00 to 4.80 $\mu\text{g/kg}$. According to Korean Food and Drug Administration, the sample having 10 ppb (10 $\mu\text{g/kg}$) aflatoxin is assumed to be hazardous (13). However, the Codex Alimentarius Commission, the joint food and agricultural organization (FAO) and world health organization (WHO) food standards program, adopted a consumption limit of 15 ppb (15 $\mu\text{g/kg}$) for total aflatoxins (14).

Kim (15) studied the combined effect of starter cultures on growth reduction and aflatoxin inhibition in Korean fermented foods by co-inoculating *Aspergillus* species along with several bacterial strains including *Bacillus subtilis* (*B. subtilis*). Aflatoxigenic fungi when combined with *B. subtilis* as starter culture exhibited maximum inhibition of aflatoxin production because of the versatile antifungal spectrum of *Bacillus* species against aflatoxin producing fungi (16). Several other factors, including production of ammonia during fermentation, light, microbial competitions with *Bacillus* species, and the addition of charcoal or vitamin C may also degrade aflatoxins in soybean products during fermentation (17).

BA content The BA contents determined quantitatively in tested starter culture soy sauce samples are listed in Table 2. All the tested sauce samples made with starter culture showed minimum total BA formation, ranging from 2.80–20.42 mg/L. The chromatograms demonstrate comparative values of the nine BAs determined in test soy sauce samples (Fig. 1). As shown in Table 2, among the tested soy

sauce samples, putrescine and histamine (majorly important classes of BAs) represented the highest value, ranging from 0.11 $\pm$ 0.00 to 7.57 $\pm$ 0.05 mg/L and from 0.22 $\pm$ 0.00 to 8.19 $\pm$ 0.15 mg/L, respectively; spermidine was absent in almost all the tested samples (Fig. 1 and Table 2).

The variability of the levels of BAs in the fermented soy sauce samples might be attributed to the variations in the manufacturing processes (18). According to the results of the present study, none of the BAs was found to be above the toxicity limit in the starter culture soy sauce samples. This may be because of the use of specific starter cultures that could stop or inhibit the formation of BAs (19), suggesting that different active starter cultures and/or processing regimes could control the formation of these BAs. As reported, 40 mg BAs intake is considered highly toxic (18). In contrast, Brink *et al.* (18) reported that 100–800 mg/kg of tyramine in foods may be toxic, while Shalaby (20) suggested that a content of >1000 mg/kg of total BAs in food might be threatening to human health. Yongmei *et al.* (11) reported a higher content of BAs in Chinese soy sauce samples, ranging from 41.7 to 1,357 mg/L, which was above the limit set by regulatory authorities. In addition, Guidi and Gloria (21) observed the presence of certain amines in Chinese soy sauce, which indicated poor sanitary conditions during processing or the use of low-quality ingredients. The results of the present study confirmed that the total content of BAs in our tested starter culture soy sauce samples was under the safety limit stipulated by safety regulations.

This study determined the overall content of BAs and total aflatoxins in starter culture soy sauce samples made using various combinations and ratios of bacterial and fungal strains under laboratory sanitized conditions. The results of this study confirmed

Fig. 1. Chromatograms of biogenic amines. (A) standard biogenic amines solution. (B) starter culture soy sauce sample extract solution, containing agmatine (1), tryptamine (2), 2-phenylethylamine (3), putrescine (4), cadaverine (5), histamine (6), tyramine (7), spermidine (8), and spermine (9).

that none of the tested starter culture soy sauce samples contained any of the described BAs and aflatoxins above the threshold limit of human consumption as defined by FAO/WHO. Based on the findings of this study, it can be concluded that an approach of using microbial starter culture for the production of good quality soy sauce could be recommended as a safe alternative for its commercial use.

Disclosure The authors declare no conflict of interest.

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