

Article

Proanthocyanidins from Chinese Bayberry (*Myrica rubra* Sieb. et Zucc.) Leaves Effectively Inhibit the Formation of Biogenic Amines in the Brewing Soy Sauce

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Abstract: Biogenic amines are a group of metabolites generated in the process of soy sauce brewing, which can result in severe negative impacts on human health at high concentrations. In this study, we innovatively proposed natural extracts (0.1 wt%), of proanthocyanidins, from Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) leaves to alternate commercial additives (0.1 wt%), i.e., sodium benzoate and potassium sorbate, for lowering the harmful biogenic amine formation during the fermentation of soy sauce. HPLC results showed that natural extracts from Chinese bayberry leaves could effectively decrease the content of cadaverine, putrescine, histamine, tyramine, phenylethylamine, and agmatine ($p < 0.05$). In contrast to the inhibitory influence exhibited by commercial sodium benzoate and potassium sorbate, extracts from bayberry leaves can maintain spermidine, spermine, and tryptamine at certain concentrations. Furthermore, both sodium benzoate and potassium sorbate significantly reduced the production of ammonium salt and characteristic components ($p < 0.05$), like soluble saltless solids, total nitrogen, and amino acid nitrogen, during the 40-day fermentation of soy sauce, whereas proanthocyanidins extracted from Chinese bayberry leaves slightly inhibited the content of ammonium salt. Thus, we can conclude that, while inhibiting the biogenic amine and ammonium salt production, extracts from Chinese bayberry leaves facilitate or maintain the production of characteristic indicators compared to commercial sodium benzoate and potassium sorbate. Taken together, natural extracts from Chinese bayberry leaves can be considered a natural additive to significantly improve the quality of traditional brewing soy sauce.

Keywords: soy sauce; biogenic amines; proanthocyanidins; food quality



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1. Introduction

Soy sauce is a liquid seasoning extensively consumed in most Asian countries [1]. It is made from fermented soybeans and is enriched in organic compounds, minerals, and nutrients [2]. Additionally, it imparts a unique color, taste, and flavor to food during cooking because of its special fermentation process and chemical composition [3,4]. However, the microbes in the process of soy sauce brewing decarboxylate amino acids or transaminate aldehydes, leading to the production of potentially harmful substances like biogenic amines (BAs) [5].

BAs are basic nitrogenous organic substances that can be aliphatic-aromatic and heterocyclic in structure and respond to putrescine (Put) and histamine (His) [6]. In addition, according to the number of amines, BAs are classified into three groups, among which spermine (Spm) and spermidine (Spd) are recognized as polyamines (PAs), phenylethylamine (Phe) and tyramine (Tyr) as mono-amines, and cadaverine (Cad) and putrescine

(Put) as di-amines [7]. Generally, low concentrations of BAs do not lead to serious risks to human health because special mono- and diamine oxidases can detoxify the BAs in the body [8]. Even low levels can enhance the physiological functions in humans and animals, such as arterial pressure regulation, relief of pain and anxiety, gastric acid secretion, muscle development, and thermoregulation [9]. In contrast, high levels of BAs would bring about “BA poisoning,” further generating toxicological influences harmful to human health [10]. The symptoms of “His poisoning” generally manifest as low blood pressure, skin irritation, headache, edemas, and rashes typical of allergic reactions [11].

In contrast, “Try poisoning” can cause hypertension, migraine, neurological disorders, nausea, and vomiting [12]. Furthermore, some BAs, like Cad and Put, cannot directly lead to the above symptoms but can markedly strengthen His toxicity and decrease His catabolism by interacting with amine oxidases, thereby facilitating intestinal absorption and impeding His detoxification [13]. Therefore, it is necessary to maintain the BA content within safe limits.

To the best of our knowledge, there are a few factors affecting the BA concentration in food, such as product size, packing materials, processing methods, storage environment, and food additives [14]. Grazyna (2020) found that storage of *Agaricus bisporus* fruiting bodies in perforated packaging resulted in a reduction in the total content of biogenic amines [15]. In addition, low temperatures (0 and 4 °C) have been demonstrated to inhibit the BAs formation in the *Gonatopsis borealis* muscle during storage [16]. Apart from that, the application of food additives, including commercial and natural substances, is one of the most common approaches because of their money-saving and energy-saving properties. Mah and Hwang (2009) found that glycine could significantly reduce the BA production by over 60% compared with the control Myeolchi-jeot [17]. Similarly, it was found that three sodium salt additives were found to exert significant inhibitory effects on BA formation in animal products [18]. Furthermore, the mixtures of catechins and grapefruit seed extracts could also be utilized to decrease the contents of Put and Cad during the soybean paste formation [19]. However, few studies have focused on the inhibition of BA biosynthesis during soy sauce brewing by using natural extracts.

The Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) has been cultivated in the south of China for over 2 centuries, with an annual production of approximately 300,000 tons [20]. Chinese bayberry trees are lush and evergreen; however, the low utilization of bayberry leaves results in significant ecological waste. Some research found that the extracts from Chinese bayberry leaves contain high levels of proanthocyanidins and possess excellent antioxidant, antimicrobial, and antiviral properties [21]. However, studies have failed to apply natural proanthocyanidins extracted from Chinese bayberry leaves (BPLs) to the food industry.

In this study, BPLs as alternative food additives were innovatively introduced to reduce the formation of BAs during soy sauce brewing. Specifically, two common additives in soy sauce fermentation, sodium benzoate (SB) and potassium sorbate (PS), and a nature-derived additive, BPLs, were added to the finished koji during the soy sauce brewing. After the complete fermentation for 40 days, different BAs were determined using the HPLC method. Furthermore, soluble saltless solids (SSS), total nitrogen (TN), amino acid nitrogen (AAN), and ammonium salt (AS) were established to verify the potential of BPLs for application in soy sauce fermentation to improve product quality.

2. Materials and Methods

2.1. Materials

Putrescine (Put), cadaverine (Cad), spermidine (Spd), spermine (Spm), tyramine (Tyr), phenylethylamine (Phe), tryptamine (Try), histamine (His), serotonin (Ser), agmatine (Agm), and benzoyl chloride (Dns-Cl) were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). The two common additives used in soy sauce fermentation, sodium benzoate (SB) and potassium sorbate (PS), were of food-grade quality and acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. The Preparation of the BPLs

The BPLs were prepared according to a previous report [22]. In brief, the dried bayberry leaves were ground into powder and extracted twice with 70% aqueous acetone (1 L) containing 0.1% (*w/v*) ascorbic acid at 25 °C for 1 day. The extracts were mixed and then evaporated to remove the acetone. The aqueous phase was recovered and washed with hexane to remove nonpolar substances, and the organic solvent components were evaporated under a vacuum. Afterward, they were transferred to freeze-drying equipment and lyophilized to obtain the BPLs.

2.3. Soy Sauce Brewing

The soy sauce process shown in Figure 1 was performed in a fermentation factory in Hangzhou, China. This process was performed according to previously reported studies [14,23]. In brief, the steamed soybean and wheat flour were mixed at a ratio of 7:3, and 0.3–0.5% of seed koji (*Aspergillus oryzae* AS3.951) was added at 30 ± 2 °C. Then, the mixture was transferred to a sealed koji machine and paved evenly at a height of 25 cm. When the color of the koji became yellow-green, it meant that the process of koji making was complete, which normally took 2 days. Afterwards, the as-prepared koji was blended with NaCl solution (19%, *w/w*) containing different additives (1‰ SB, 1‰ PS, and 1‰ BPLs) at a ratio of 1:2 and finally moved to a brewing tank at 38 °C for 40 days.

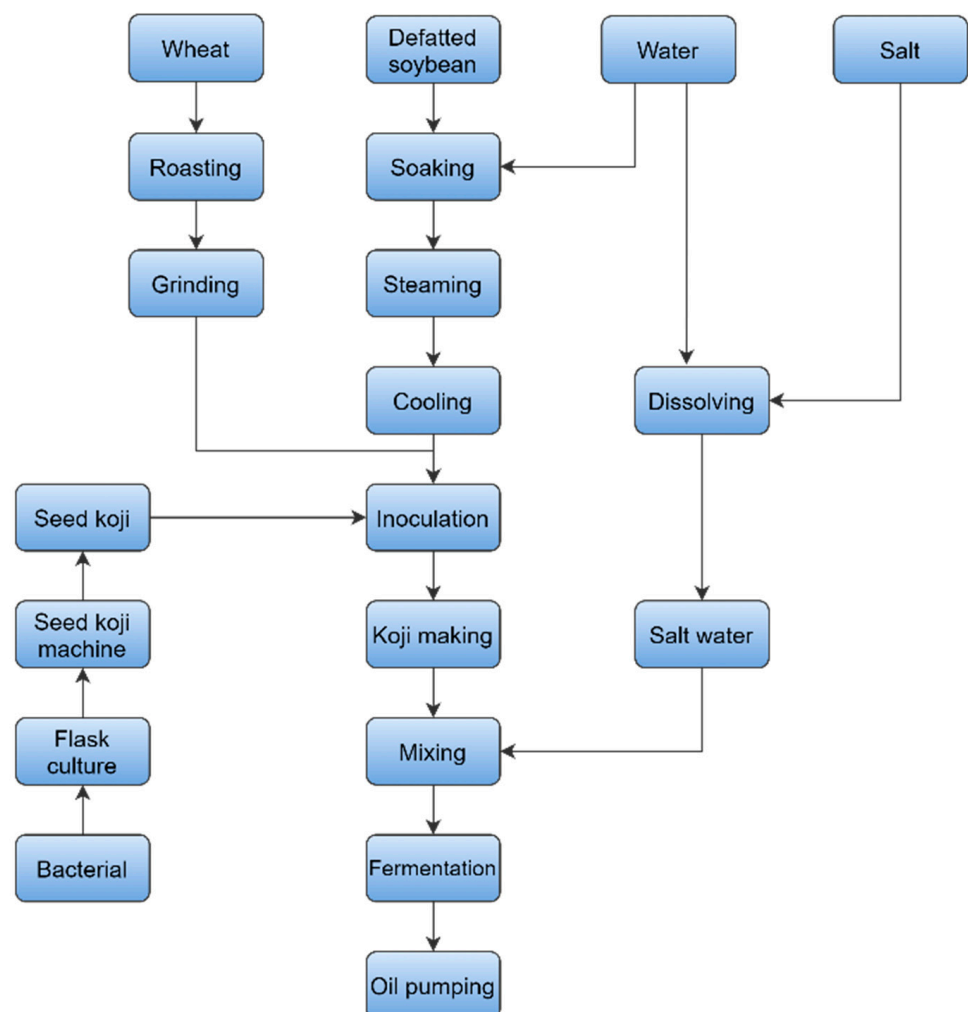


Figure 1. The traditional brewing process of soy sauce.

2.4. Sampling and Pretreatment

The sampling times were on days 0, 2, 5, 15, 18, 26, 33, and 40 of brewing. The sampled soy sauce mash was filtered using a 100-mesh gauze to remove impurities for further analysis.

2.5. Determination of Biogenic Amines (BAs)

The determination method was based on our previous studies with slight modifications [14,24].

2.5.1. Standard Curve Drawing

A standard curve was drawn with slight modifications to calculate the BA content [25]. The BA standards were added to an HCl solution (0.1 mol/L) to formulate a stock solution of 1 mg/mL. By matching the same concentration of 10 BAs, a solution containing 100 µg/mL of mixed standards was acquired. A heptamine (Hep) solution (100 µg/mL) was used as the internal working solution. The standard solution was diluted to different concentrations (0.1–80 µg/mL), after which the Hep solution (100 µL), NaOH solution (1 mL), and Dns-Cl solution (10 µL) were blended well with different concentrations of standard solutions and kept standing at 30 °C for 50 min. NaCl solution (26%, *w/w*) was added to halt the reaction, followed by the addition of an anhydrous solution and centrifugation at 1200 rpm for 5 min at 5 °C for extraction. The ether phase was collected and dried using a nitrogen blower. The dried samples were dissolved in 1 mL of acetonitrile and filtered with 0.45 µm PVDF for further analysis.

2.5.2. Sample Pre-Treatment

The perchloric acid solution (20 mL), heptylamine solution (100 µL), and soy sauce samples (2 mL) were mixed thoroughly. After oscillating and resting for 5 min, the Dns-Cl solution (10 µL) and NaOH solution (10 µL) were dropped into the mixture (1 mL) at 30 °C for 50 min. Then, saturated sodium chloride was added to terminate the reaction. Afterward, anhydrous ether was mixed with the above solution and centrifuged at a speed of 1200 rpm for 5 min at 5 °C, which was carried out 3 times. The ester layers were collected via nitrogen blow-drying. Acetonitrile (1 mL) was used to dissolve the dried samples, followed by filtering with 0.45 µm PVDF for further determination.

2.5.3. HPLC Detection

A Waters 1525 HPLC system with an ODS-3 column (250 × 4.6 × 5 mm) was used to analyze the BA content according to our previously developed method. A continuous gradient elution with 0.005 mol/L ammonium acetate solution (eluent A) and HPLC-grade acetonitrile (eluent B) was used for the chromatographic separation. The flow rate of elution was 1.0 mL/min, and the injection volume was 20 µL. The derivatives of the mixed standards (0.1 µg/mL) were appropriately diluted with acetonitrile. The criterion for the detection limits of the signal-to-noise ratio (S/N) was set at a minimum of 3, while the quantitation limit criterion was established at a minimum of 10.

2.6. Determination of Total Nitrogen (TN) and Amino Acid Nitrogen (AAN)

The content of TN and AAN was measured using the Kjeldahl method. Their detailed operation steps are referred to as GB 18186-2000 and GB/T 5009.39-2003.

2.7. Determination of Soluble Saltless Solids (SSS)

The SSS was measured based on GB 18186-2000 for soybean sauce (China National Standards). Six milliliters of samples were evaporated in a glass dish and dried for 2 days at 80 °C, followed by a cooling in a dry environment for 6 h. The total salt content was the remaining weight divided by the original weight, whereas the SSS concentration was the total salt content minus the NaCl content, which was determined using the silver nitrate (AgNO₃) titration method.

2.8. Determination of Ammonium Salt (AS)

The AS determination was based on the GB/T5009.39–2003 method for the analysis of the hygienic standard of soybean sauce (China National Standards). Liquid samples (2 mL) were transferred into a distilling flask with the addition of water (150 mL), MgSO_4 (1 g), and a few drops of ethanol solution (2 g/L) containing methyl red-bromocresol green (1/5) and then heated to boiling for half an hour. The mixture was blended with a 20 g/L boric acid solution (10 mL) in a flask and titrated with HCl (0.1 mol/L). The same amounts of distilled water, MgSO_4 , and H_3BO_3 solutions were used as controls.

2.9. Statistical Analysis

The above experiments were performed in triplicate, and all data are presented as mean $\pm$ standard deviation (SD). GraphPad Prism 8 was used to analyze the acquired data, and two-way ANOVA was used to calculate the significance.

3. Result and Discussion

3.1. Effect of Different Additives on Biogenic Amines

As shown in Figure 2a, SB, PS, and BPLs had a significant inhibitory effect on the Put generation during soy sauce brewing. The content of Put in the control group first decreases to 11.82 ± 1.73 mg/L and then increases to 19.01 ± 0.17 mg/L at the end of the 40-day fermentation, while the levels of Put in the soy sauce with SB, PS, and BPLs continuously decrease to 3.02 ± 0.64 mg/L, 2.40 ± 0.52 mg/L and 0.61 ± 0.23 mg/L, respectively. This may be explained by the fact that SB, PS, and BPLs can inhibit the activity of microorganisms to some extent, thereby inhibiting the production of Put. However, there were some variations in the inhibitory effects between them, consistent with a previous report [18]. In addition, Aneta et al. found that different additives, such as sorbic acid, lactic acid, and disodium dihydrogen pyrophosphate, exhibited different inhibitory capacities against the Put formation [26].

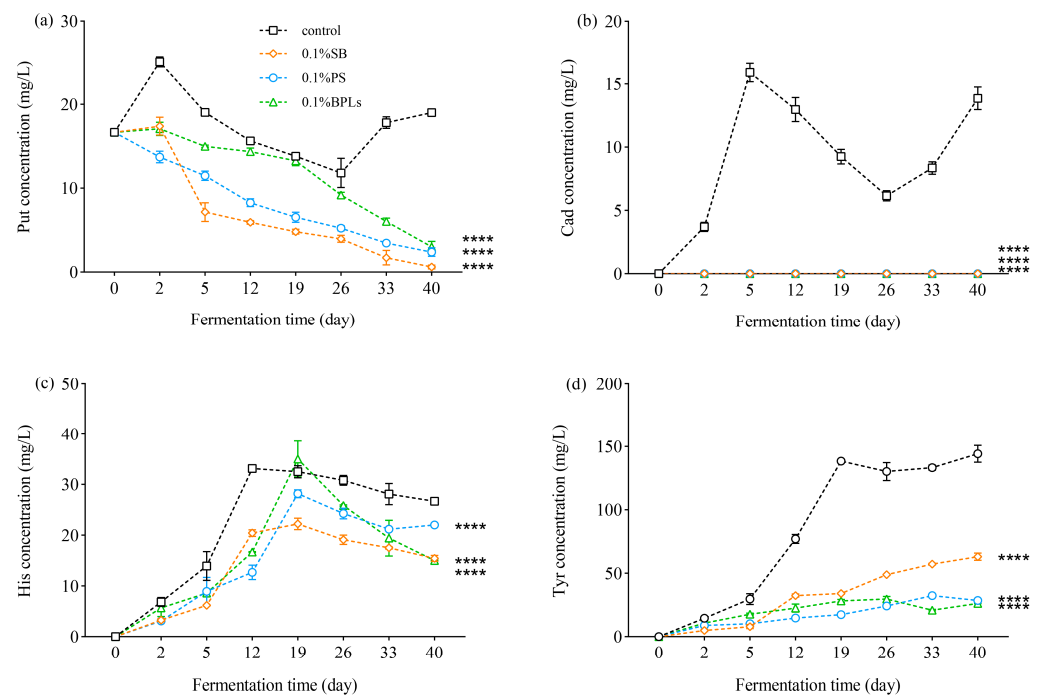


Figure 2. Cont.

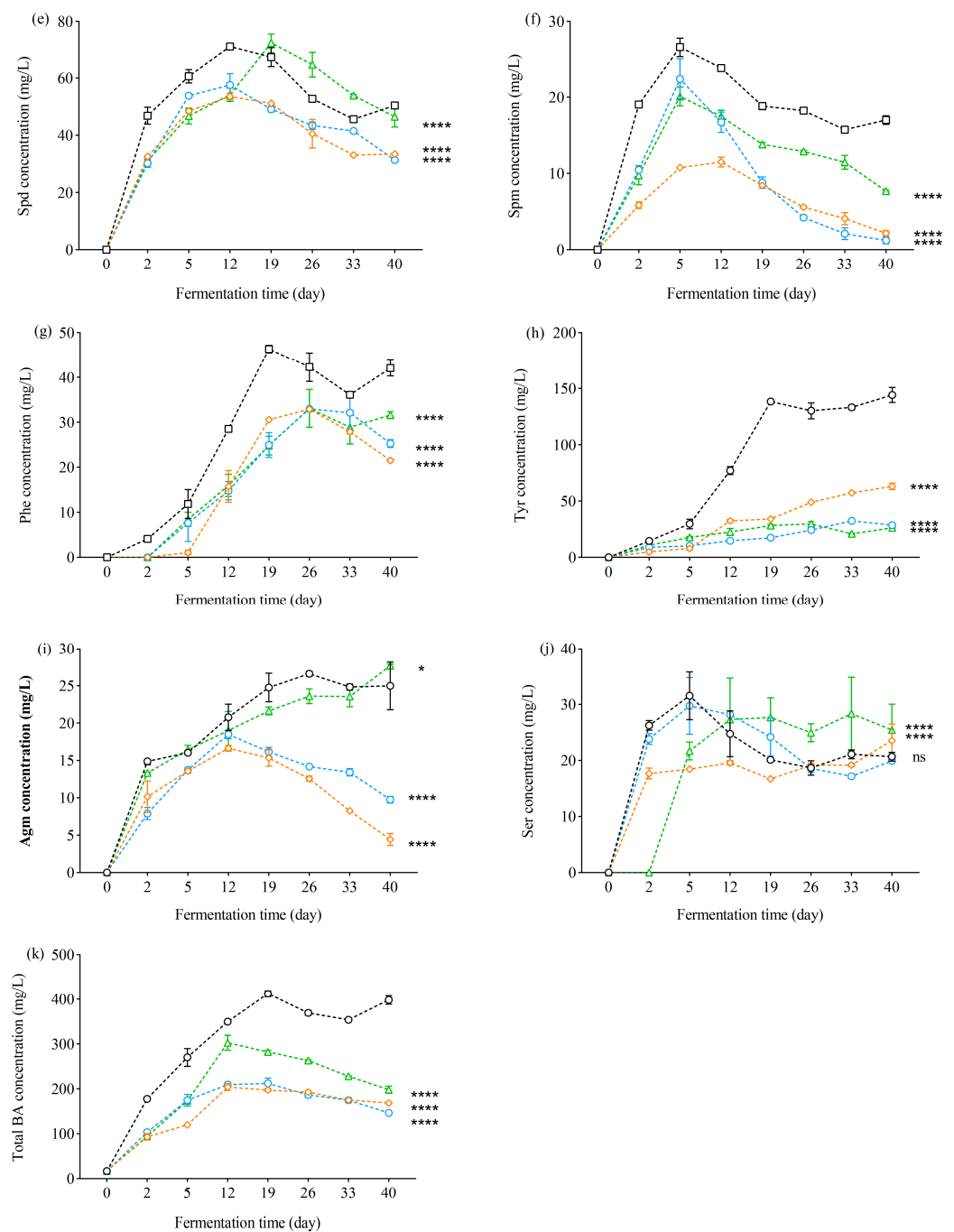


Figure 2. Changes in BA concentration of soy sauce with different additives (Control, $\square$, 1% SB, $\diamond$, 1% PS, $\circ$, 1% BPLs, $\triangle$) during the fermentation period. (a) putrescine (Put), (b) cadaverine (Cad), (c) histamine (His), (d) tyramine (Tyr), (e) spermidine (Spd), (f) spermine (Spm), (g) phenylethylamine (Phe), (h) tryptamine (Try), (i) agmatine (Agm), (j) serotonin (Ser), and (k) total BAs. * indicates a significant difference ($p < 0.05$), and **** ($p < 0.0001$) indicates an extremely significant difference. ns indicates no significant difference.

Figure 2b shows the changes in the Cad content during the fermentation of the soy sauce. Without adding food additives, the concentration of Cad increases to 15.90 ± 0.73 mg/L during the first 5 days, followed by a decrease to 6.16 ± 0.40 mg/L at the 26th day. At the completion of fermentation, its concentration increases to 13.86 ± 0.89 mg/L. In contrast, Cad was not detected in any of the experimental groups, indicating that SB, PS, and BPLs completely prevented the Cad production during the soy sauce fermentation. The main

reason is probably because the presence of SB, PS, and BPLs can affect undesirable microbial communities, such as *Caproiciproducens*, *Stenotrophomonas*, *Herbinix*, and *Enterobacter* genera, thus lowering the Put, Cad, and Tyr contents [27].

In terms of His, Figure 2c shows that the contents of the samples with SB, PS, and BPLs were significantly lower than those of the control group. At the beginning of fermentation, no His was detected in either the experimental or control group. However, as the brewing time increased, the His concentrations in all samples showed a trend of first increasing and then decreasing, with all samples reaching a high level on the 18th day of fermentation. When the fermentation is over, the His concentration in the control group is 26.70 ± 0.42 mg/L while the samples supplemented with 1‰ SB, 1‰ PS, and 1‰ BPLs contain 15.45 ± 0.59 mg/L, 22.01 ± 0.27 mg/L and 15.04 ± 0.05 mg/L of His, respectively.

Similarly, as shown in Figure 2d, compared to the control, SB, PS, and BPLs noticeably decreased the Tyr content during fermentation. The Tyr content in the control group is 144.27 ± 6.65 mg/L after fermentation, while the contents in the test groups with 1‰ SB, 1‰ PS, and 1‰ BPLs are 63.10 ± 2.98 mg/L, 28.63 ± 1.28 mg/L and 26.29 ± 0.28 mg/L, respectively. Adding additives can lower the formation of His and Tyr. Still, the ability of the three additives to restrain His and Tyr differs significantly during the fermentation of soy sauce, which remains consistent with the result found by Qian et al. [28]. It was concluded that PS, *Lactobacillus*, and Curcumin could deplete the generation of His and Tyr in the cured dried fish to different degrees, which might be determined by the different inhibition categories of the additives.

As depicted in Figure 2e shows the effect of different additives on the Spd formation during the fermentation of soy sauce. Both SB and PS slightly reduced the formation of Spd during the fermentation process of soy sauce, whereas BPLs did not exert a significant inhibitory effect. The Spd content in all samples increased rapidly in the first 5 days of fermentation, but the soy sauces containing additives showed significantly lower Spd levels than the control. Afterward, the Spd concentrations in the samples with the addition of 1‰ SB and 1‰ PS remained at a low level, whereas a great fluctuation occurred in the sample with 1‰ BPLs, whose content continued to rise rapidly, surpassing the control group from 18th to 33rd day and then fell slightly below the control at the end of fermentation. This is consistent with the reported finding on the suppressive effect of different aromatic species on the Spd content during the fermentation of cured fish [28].

The dynamic trend of the Spm content is shown in Figure 2f. For the control sample, the Spm concentration increased to a maximum of 26.57 ± 1.27 mg/L on day 5, followed by a decrease to 17.00 ± 0.56 mg/L. Also, the other samples with SB, PS, and BPLs show a similar trend, but their corresponding Spm concentrations are 2.16 ± 0.34 mg/L, 1.22 ± 0.43 mg/L, and 7.68 ± 0.12 mg/L, respectively, when the fermentation is finished. This illustrates that all three additions can repress the production of Spm, with SB and PS being more effective than BPLs. Previous studies have shown that Spd and Spm tend to have positive effects on improving body health, such as antioxidant and anti-inflammatory activities, as well as modulating immune functionality [29]. Thus, maintaining Spd and Spm at a rather high level is expected in the food industry.

Figure 2g displays the dynamic changes in the Phe content during fermentation. Throughout the fermentation process, the levels of Phe in the samples with SB, PS, and BPLs were significantly lower than those in the control group at the same time. The Phe content in the SB, PS, and BPLs samples increased promptly at the beginning of fermentation, all reached a maximum value of approximately 33 mg/L on day 26 of fermentation and then slowly decreased to a range of 20–30 mg/L. Therefore, BPLs can be considered an alternative to SB and PS in terms of inhibiting the Phe formation.

As depicted in Figure 2h, SB, PS, and BPLs hindered the Try production during the soy sauce fermentation. The Try content in the control sample soared to 44.94 ± 0.47 mg/L on the 5th fermentation day and then decreased to 22.97 ± 2.65 mg/L on day 33, followed by a slight increase to 39.50 ± 0.84 mg/L. It is obvious that the Try content in the samples, including SB and PS, was lower than that in the control at the same time. However, the

Try level in the sample containing BPLs is markedly higher than that in the control sample from day 20 to day 33, and then decreases to 15.01 ± 0.85 mg/L at the end of fermentation, which is much lower than the control sample.

Figure 2i shows the effect of different additives on the Agm concentration during the soy sauce fermentation. It is shown that SB and PS played prominent inhibitory roles in Agm formation; however, BPLs had no significant influence on the end product. For the samples without additives, the Agm concentration increased from 0 to 26.61 ± 0.33 mg/L during the first 26 days and then maintained a relative equilibrium. However, for the soy sauce containing SB and PS, their Agm amounts reach a maximum value of approximately 15 mg/L during the first 12 days, after which their contents decrease to 4.43 ± 0.80 mg/L and 9.75 ± 0.43 mg/L, respectively. For the samples containing BPLs, the level of Agm continued to increase to 27.69 ± 0.21 mg/L throughout the fermentation, consequently reaching the same level as the control group, demonstrating that BPLs could not prevent the Agm formation.

Figure 2j shows the variation in the Ser amount during the brewing of soy sauce. Overall, the effect of the three additives on the Ser content of the soy sauce was not significant. The levels of Ser in both the control and SB-supplemented soy sauces showed similar trends, increasing from 0 to approximately 30 mg/L within 0 to 5 days and then decreasing to approximately 20 mg/L. However, the concentration of Ser in the PS- and BPLs-added samples increased to 18.58 ± 0.75 mg/L and 24.94 ± 25.46 mg/L, respectively, and then remained level off. It can be seen that the Ser levels in all soy sauces are at the same level at the end of fermentation, indicating that the three additives are unable to inhibit the Ser formation. Furthermore, this suggests that there are significant differences in the production pathways of different BAs [5,30].

The effect of SB, PS, and BPLs on the total BA content during fermentation is shown in Figure 2k. It is clear that the three additives have a significant inhibitory effect on the total BA content. For the control sample, the total BA concentration increased from 16.67 ± 0.21 mg/L to 398.48 ± 9.32 mg/L, except for a slight decrease from day 20 to day 33 of the fermentation. For the other three groups, there was an increasing trend for the first 12 days, followed by a decrease in the total BA content. At the end of the fermentation, the levels of total BAs in the soy sauce with SB, PS, and BPLs were 168.47 ± 2.07 mg/L, 146.42 ± 0.57 mg/L, and 198.26 ± 7.88 mg/L, respectively, indicating that PS had the best inhibitory effect on the formation of the total BAs, followed by SB and BPLs.

3.2. Effect of Different Additives on the Saltless Soluble Solid (SSS)

Soluble saltless solids (SSS) are crucial factors that affect the quality of soy sauce. Figure 3 shows the changes in the SSS content during fermentation. It can be easily found that the SSS content in soy sauce added with SB and PS are both lower than that in the control group, and their corresponding SSS contents are 9.05 ± 0.04 g/100 mL and 11.52 ± 0.23 g/100 mL after 40-day fermentation, respectively, compared with 13.63 ± 0.38 g/100 mL in the blank group. However, the concentration of the SSS in the BPL-supplemented soy sauce samples was significantly higher than that in the control group. As the fermentation proceeds, its content increases from 9.05 g/100 mL to 15.40 ± 0.04 g/100 mL, which meets the extra standard for high-salt liquid-state fermented soy sauce [31]. These results demonstrated that the addition of SB or PS prevents the SSS from forming, whereas BPLs can facilitate this process. In addition, the SSS content of all the control and experimental groups decreased slowly in the early stages. Then, it increased to maximum levels at the end of fermentation, which is consistent with the changing pattern of the SSS content during both the traditional and optimized brewing processes of soy sauce [14,23].

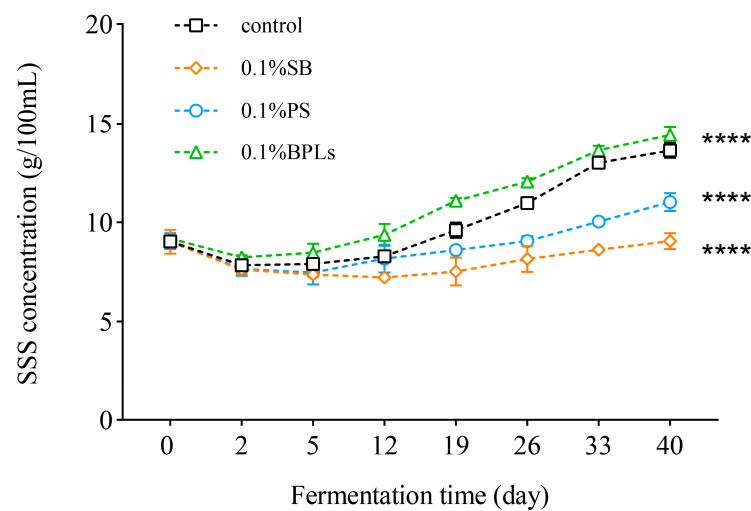


Figure 3. The SSS content during the brewing process of soy sauces. **** indicates a significant different ($p < 0.05$) compared to the control group.

3.3. Effect of Different Additives on Total Nitrogen (TN)

Total nitrogen (TN) is one of the most important quality indices of soy sauce products and contributes to its nutritional value and sensory characteristics [32]. The TN formation during soybean brewing can be caused by the activity of proteases and peptidases that hydrolyze proteins into small fragments of peptides, amino acids, and ammonia [33]. Figure 4 illustrates the variation in total nitrogen (TN) content over the fermentation time, with all samples exhibiting an increasing trend during this period. The TN levels in the soy sauces with 1‰ SB and 1‰ PS were significantly lower throughout the fermentation process than those in the control sample. Following the 40-day brewing, the TN concentrations of the samples containing SB and PS are 1.14 ± 0.09 g/100 mL and 1.39 ± 0.06 g/100 mL, respectively, compared to 2.17 ± 0.04 g/100 mL in the control group, the reason of which is that SB and PS would suppress the proteases and peptidases activities. Interestingly, the variation potential of TN in the BPLs group almost coincides with that of the control, which increases from 0.58 ± 0.07 g/100 mL to 2.25 ± 0.03 g/100 mL, reaching the extra standard for high-salt liquid-state fermented soy sauce [34]. Here, we can conclude that both 1‰ SB and 1‰ PS inhibit TN formation, whereas BPLs have no effect on the TN production during the fermentation of soy sauce.

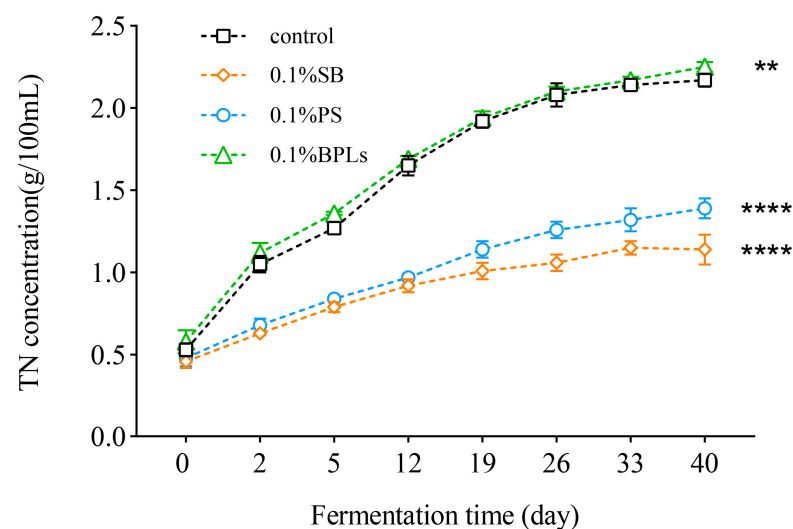


Figure 4. The TN content during the brewing process of soy sauces. ** represents a significant difference ($p < 0.01$) and **** indicates a significant different ($p < 0.001$) compared to the control group.

3.4. Effect of Different Additives on the Amino Acid Nitrogen (AAN)

The amino acid nitrogen (AAN) content can be considered a primary factor in grading the quality of soy sauce products, as it can reduce the nutritious protein level and enhance food sensory [2]. The changes in the AAN content with fermentation time are shown in Figure 5. It can be found that the AAN content in all samples maintained an increasing trend during 40 days of fermentation, which is in agreement with the TN change. Among them, SB and PS could reduce the AAN content throughout the fermentation of the sauces, corresponding to 0.79 ± 0.04 g/100 mL and 0.85 ± 0.02 g/100 mL at the end of fermentation, respectively, which is much than the content of the control group (1.30 ± 0.04 g/100 mL). However, the AAN content in the soy sauce with 1‰ BPLs is basically the same as that in the control group, and the AAN content at day 40 is 1.32 ± 0.06 g/100 mL, which meets the extra standard for high-salt liquid-state fermented soy sauce [3]. Thus, both SB and PS exert a crucial influence on reducing the content of the AAN, while the addition of 1‰ BPLs can slightly promote the ANN generation.

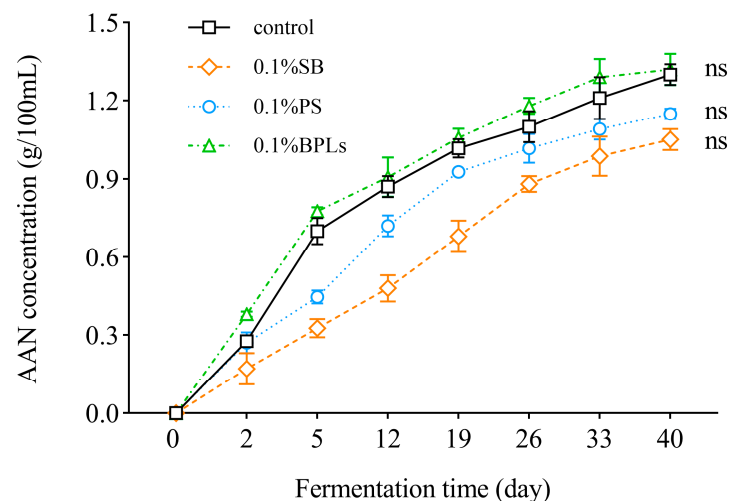


Figure 5. The AAN content during the brewing process of soy sauces. ns indicates no significant difference.

3.5. Effect of Different Additives on the Ammonium Salt (AS)

Some studies have reported that ammonium salt (AS) increases the measured value of AAN content in soy sauce [35]. The influence of different additives on the AS content of the fermented sauce is shown in Figure 6. Clearly, additives play a significant role in the AS generation. The AS content in all the samples increased throughout, but the increment rate was fast at the beginning of the fermentation and then decreased. On the 18th day, the AS content in the sample without any additive is 0.37 ± 0.02 g/100 mL, while the samples added with SB, PS, and BPLs are 0.17 ± 0.01 g/100 mL, 0.22 ± 0.01 g/100 mL, 0.25 ± 0.01 g/100 mL, respectively. After the 40-day fermentation, their contents increase to 0.42 ± 0.04 g/100 mL, 0.30 ± 0.05 g/100 mL, 0.26 ± 0.03 g/100 mL and 0.21 ± 0.04 g/100 mL, respectively. Thus, we can deduce that 1‰ SB, 1‰ PS, and 1‰ BPLs inhibited AS production, and the inhibitory effect was in the order of 1‰ SB > 1‰ PS > 1‰ BPLs.

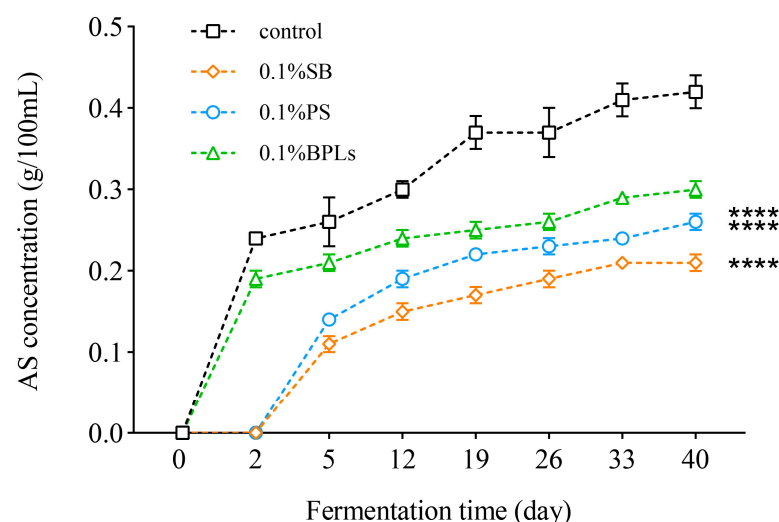


Figure 6. The AS content during the brewing process of soy sauces. **** indicates a significant different ($p < 0.05$) compared to the control group.

4. Conclusions

In this study, three different additives –commercial SB, PS, and natural BPLs –were used to explore their effects on the generation of Bas and their physicochemical properties, thus improving the safety and quality of soy sauce for industrial applications. The results illustrate that adding 1‰ SB, 1‰ PS, and 1‰ BPLs significantly increased the generation of Cad, Put, His, Tyr, Phe, Agm, and total BAs during the fermentation of soy sauce. However, the BPLs did not influence the contents of Spd, Spm, and Try. In addition to decreasing the BA content, it was found that both SB and PS obviously reduced characteristic indicators related to quality improvement, such as SSS, TN, AAN, and AS, whereas BPLs only inhibited AS from decreasing salt consumption. Thus, it was concluded that adding SB and PS reduced the BA formation along with the characteristic indicators in the soy sauce. However, BPLs, while inhibiting BA and AS production, can contribute to the development of characteristic indicators that further enhance the nutritional quality. Taken together, BPLs can be considered natural additives for considerably improving the quality of the traditional brewing soy sauce. Although a positive conclusion was obtained, a detailed application of the BPL content remains a topic for further study.

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Abbreviations

BAs	biogenic amines
AAN	amino acid nitrogen
Agm	agmatine
AS	ammonium salt
BPLs	proanthocyanidins extracted from Chinese bayberry leaves
Cad	cadaverine
His	histamine
PAs	polyamines
Phe	phenylethylamine
PS	potassium sorbate
Put	putrescine
Put	putrescine
SB	sodium benzoate
SD	standard deviation
Ser	serotonin
Spd	spermidine
Spm	spermine
SSS	soluble saltless solids
TN	total nitrogen
Try	tryptamine
Tyr	tyramine

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