

EFFECTS OF SALT AND ALCOHOL CONCENTRATIONS ON NUTRITIONAL COMPONENTS AND ANTIOXIDANT ACTIVITY DURING RIPENING OF PURPLE SWEET POTATO SUFU

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ABSTRACT

Background. Sufu (fermented tofu) is a traditional food in several Asian countries, produced through the fermentation of tofu with molds such as *Actinomucor elegans*. Enzymes from microorganisms metabolize tofu substrates, generating volatile compounds that contribute to the product's distinctive flavor when combined with alcohol and salt during ripening. This fermentation process also improves digestibility. Incorporating purple sweet potato (PSP) into tofu creates a novel product with enhanced nutritional value and antioxidant activity.

Materials and methods. Local raw materials and an *Actinomucor elegans* strain from the Institute of Food and Biotechnology, Can Tho University, were used. The effects of varying salt concentrations (8, 10, and 12% w/v) and alcohol concentrations (8, 10, 12, and 14% v/v) in the brine were examined during PSP sufu ripening (10, 20, and 30 days). Nutritional components, including soluble protein, free amino acids, ammonia content, and bioactive compounds, including total phenolic content (TPC), total flavonoid content (TFC), and anthocyanins, were analyzed. Antioxidant activity was assessed using DPPH radical scavenging, expressed as IC₅₀ values.

Results. Optimal brine parameters for PSP sufu production were identified. The nutrient composition included 18.5% free amino acids, 6.94% soluble protein, 1.55% NH₃, pH 5.66, and 0.36% total acid. TPC, TFC, and anthocyanin content were 82.7 mg GAE/g d.w., 22.6 mg QE/g d.w., and 117.86 μg/g d.w., respectively. DPPH radical scavenging activity was 97.5 μmol TE/g d.w., with an IC₅₀ value of 4.66 mg/ml.

Conclusions. PSP sufu demonstrates high nutritional and antioxidant quality when ripened for 30 days in brine containing 10% salt and 12% alcohol. These aging conditions promote favorable biochemical changes, enhancing both its functional properties and nutritional value.

Keywords: antioxidant, purple sweet potato, tofu ripening, salt, alcohol

INTRODUCTION

Sufu, also known by various names such as Mao-tofu (Thailand), Tempeh (Indonesia), or Chao (Vietnam) (Huang et al., 2021), is widely consumed as a condiment. Its flavor results from proteolysis and the accumulation of free amino acids, such as glutamic acid. As a natural flavor enhancer, sufu contributes to the umami

profile of soups, stir-fried dishes, and meat marinades, reducing the need for synthetic additives. Sufu is formed through microbial activity, primarily involving bacteria and molds. Common microbial strains in sufu production include *Mucor*, *Actinomucor*, *Bacillus*, *Aspergillus*, and *Rhizopus* (Li et al., 2021). Molds are especially

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important in soy-based fermentations because they produce strong enzymes, reduce phytic acid content, and increase essential amino acid levels (Handa et al., 2019).

The sufu production process can be divided into four main stages: tofu preparation, fermentation of tofu into pehtze, salting of pehtze, and ripening of sufu in a brine solution (Liu et al., 2018). The brining stage is critical, as it strongly influences the aroma and taste of the final product. Typical brine formulations contain salt (10–12%), alcohol (8–12%), sugar (5–10%), and other spices. Traditionally, salt content exceeded 14%, leading to aging periods longer than six months (Han et al., 2001). Modern production often reduces salt levels to shorten ripening time and lower sodium content in the final product (Liu et al., 2018).

Purple sweet potato (*Ipomoea batatas* L.) is a highly nutritious agricultural product with strong antioxidant properties, primarily due to its high anthocyanin content (Faramitha et al., 2024). With a global cultivation area of approximately 60 million hectares and a yield exceeding 860 million tons (FAO, 2021), PSP is widely used in both the food and pharmaceutical industries. It serves as a food additive, gelling agent, thickener, film-forming agent, or emulsifier. The purple anthocyanin pigments act as natural food colorants and possess antioxidant and anti-inflammatory properties, which are valuable in medicine (Santos-Sánchez et al., 2017). However, fresh PSP tubers have a short shelf life, leading to rapid spoilage and reduced economic value (Nguyen et al., 2023).

Incorporating PSP into tofu produces PSP-enriched sufu, creating a novel product with potential market value. The additives used during ripening, particularly salt and alcohol concentrations, influence the fermentation process and final product quality. This study therefore evaluated the effects of varying salt and alcohol levels in the brine during sufu ripening, to identify optimal conditions for achieving the highest nutritional content and antioxidant activity.

MATERIALS AND METHODS

Materials

The pure strain of *Actinomucor elegans* was obtained from the American Type Culture Collection (ATCC) and is currently stored at the Institute of Food and Biotechnology, Can Tho University.

The soybean variety HLDN 910 was purchased from the Hung Loc Agricultural Research Center (Dong Nai Province, Viet Nam). PSP was supplied by Thanh Binh Tan Limited Company (Vinh Long Province, Viet Nam). Refined sea salt (97% NaCl) was obtained from Bac Lieu Province, Viet Nam. Alcohol was sourced as Binh Tay rice wine (35% vol) from Binh Duong Province, Viet Nam.

All chemicals used were of analytical grade and purchased from Merck KGAA Co. (Darmstadt, Germany).

Methods

Preparation of PSP tofu

Tofu was prepared following the method of Joo et al. (2023) with some modifications. Soybeans (*Glycine max* L.) were soaked in running water at a flow rate of 2.5 liters per minute and a temperature of 20°C for 6 hours, then ground (Model: ACHI, China) with water at 70°C in a 1:8 ratio (soybeans:water). The resulting soy milk was collected, boiled at 100°C for 15 minutes, and then mixed with the PSP solution.

PSP were sliced and steamed at 100°C for 5 minutes, then ground (Model: SK-2000, Japan) with water at a 1:2 ratio (PSP:water) to prepare the PSP solution. The PSP solution was blended with soy milk at a ratio of 0.75:1 (PSP:soy:milk,v/v). Coagulation was achieved by adding 0.2% nigari ($MgCl_2$) at 90°C for 15 minutes. After coagulation, the mixture was molded into dimensions of 12 × 10 × 7 cm and pressed for 15 minutes using a screw press to remove excess water. The final product was PSP-enriched tofu (purple tofu).

Preparation of pehtze

Tofu cubes (2 × 2 × 2 cm) were exposed to UV rays for approximately 10 minutes and then inoculated with *Actinomucor elegans* mold. The cubes were incubated at 30°C and 95% relative humidity for 30 hours to produce purple pehtze blocks.

Preparation of ripening

After dry salting for 20 hours, the purple pehtze blocks were rinsed with clean water, drained, and arranged in glass jars, which were then sealed. The pehtze blocks were submerged in brine at a 1:1 ratio (pehtze:water). The brine contained varying concentrations of salt (8, 10, and 12% w/v) and alcohol (8, 10, 12, and 14% v/v). Ripening was conducted under ambient temperature

conditions. Quality parameters were evaluated after 10, 20, and 30 days, and compared with a control sample (C sample).

Determination of the nutrient composition of pehtze

Soluble protein content (%) was determined following the method of the Ministry of Science and Technology (2011). Free amino acids (%) were analyzed via hydrolysis according to Ye et al. (2020). Ammonia content (%) was measured based on total nitrogen content using the Kjeldahl method (ISO, 2013). The pH of sufu was measured with a pH meter (HI122-01 Hanna, Italy), and total acid content was determined by titration with 0.1 N NaOH to a pH of 8.2 using phenolphthalein as an indicator, with results expressed as % lactic acid (Liang et al., 2016). All analyses were performed in triplicate, and results are expressed as the average on a dry basis.

Determination of the total polyphenol, flavonoid, anthocyanin contents, and antioxidant activities of PSP sufu

The total phenolic content (TPC) of the PSP sufu was measured using the Folin-Ciocalteu method and expressed as gallic acid equivalents per gram of dry weight (mg GAE/g d.w.) (Jiang et al., 2013). Briefly, 5 g of freeze-dried, defatted sufu was extracted with 12 mL of 70% acetone. A 0.1 mL aliquot of the extract was diluted with 3.5 mL of distilled water, followed by the addition of 0.4 mL of Folin-Ciocalteu reagent and 1.0 mL of 7.5% (w/v) sodium carbonate solution. The mixture was thoroughly mixed and incubated at room temperature for 2 hours. The reaction solution was measured at a wavelength of 765 nm using a spectrophotometer (U-2800 Shimadzu, Japan).

The total flavonoid (TFC) content was determined using the aluminum chloride colorimetric method and expressed as mg quercetin equivalent (QE) per gram of dry weight (mg QE/g d.w.) (Khan et al., 2012). In this assay, 3 mL of distilled water, 0.15 mL of 5% NaNO₂, and 0.3 mL of 10% AlCl₃ were added to 0.5 mL of the sufu extract, incubated at room temperature for 30 minutes, and the reaction solution was measured at a wavelength of 415 nm using the spectrophotometer.

Anthocyanin content (µg/g) was determined according to the method of Wrolstad and Culver (2012).

The absorbance of anthocyanins in buffer solutions at pH 1.0 and 4.5 was recorded using a spectrophotometer at 520 nm and 700 nm wavelengths, respectively. The anthocyanin concentration was calculated using a standard curve of cyanidin-3-glucoside.

DPPH radical scavenging activity was measured by mixing 800 µl of the PSP sufu extract with 800 µL of 0.008% DPPH solution. The mixture was shaken and incubated at room temperature for 30 minutes. Absorbance was recorded at a wavelength of 517 nm, and results were expressed as µmol Trolox equivalents per gram of dry weight (µmol TE/g d.w.) (Sakshy and Paras, 2009).

Data analysis

Analysis of variance (ANOVA) was used to assess significant differences among treatment groups, followed by the LSD test using Statgraphics (Virginia, US). Data are presented as mean ± standard deviation (SD) of three independent replicates. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of salt and alcohol concentrations on the nutrients of PSP sufu

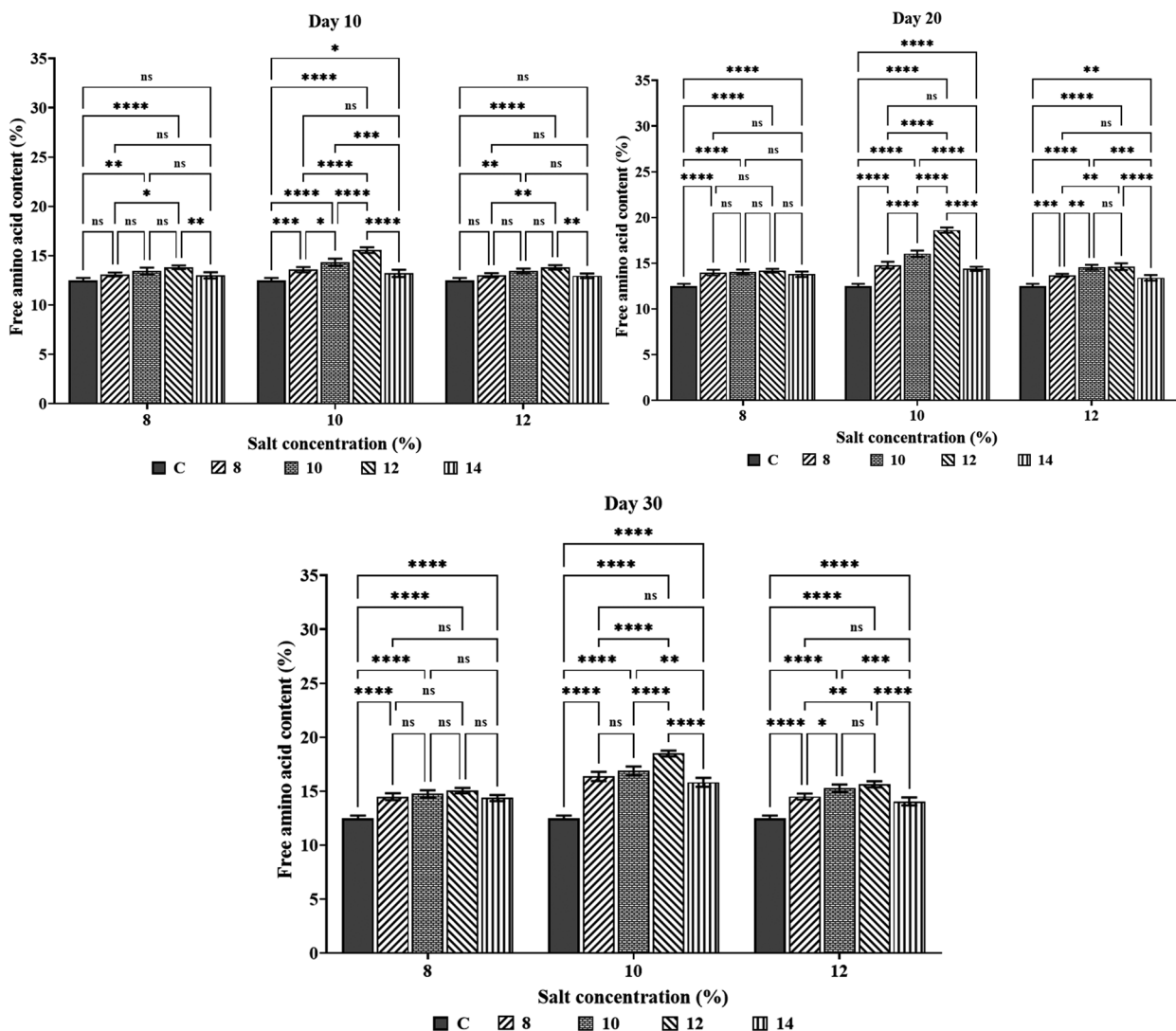
The results in Figure 1 show that the concentrations of salt and alcohol added to the brine influenced the free amino acid (FAA) content. Overall, FAA content tended to increase during the 30-day ripening of the PSP sufu samples.

Specifically, on day 10, a slight increase in FAA content was observed compared to sample C, but no significant statistical differences ($p > 0.05$) were found among samples with alcohol concentrations of 8, 10, and 12%, and salt concentrations of 8 and 12%. At this stage, the samples with 10% salt and 12% alcohol exhibited the highest FAA content. By days 20 and 30, FAA content had significantly increased compared to sample C. On day 30, the PSP sufu sample reached the highest FAA level during the entire ripening period, at 18.49%, which was nearly 1.48 times higher than sample C. Between days 20 and 30, samples with 8% salt showed no statistically significant differences in FAA content, and alcohol concentration had no effect ($p > 0.05$). This indicates that a salt concentration of 8% does not affect the ripening of PSP sufu.

Among individual amino acids, glutamic acid and aspartic acid increased notably, contributing to the umami flavor of the sufu (Xie et al., 2023). Han et al. (2004) also demonstrated that salt concentration influences FAA accumulation in different sufu types. In red sufu, FAA decreased as salt increased from 8% to 11% to 14%, with values 3.2, 2.2, and 1.6 times higher than the control sample, respectively. By contrast, white sufu showed an increase of 3.1, 3.4, and 4.0 times at

salt concentrations of 8%, 11%, and 14% (w/v), respectively. For grey sufu, no suitable model was identified (Han et al., 2004). Overall, FAA accumulation in sufu is influenced by multiple factors but is strongly associated with protein hydrolysis during ripening (Ivankin, 2023).

The results of the soluble protein content analysis are shown in Figure 2. After 10 days of ripening, the soluble protein content in the tested samples was higher than that of the control sample (C), although many



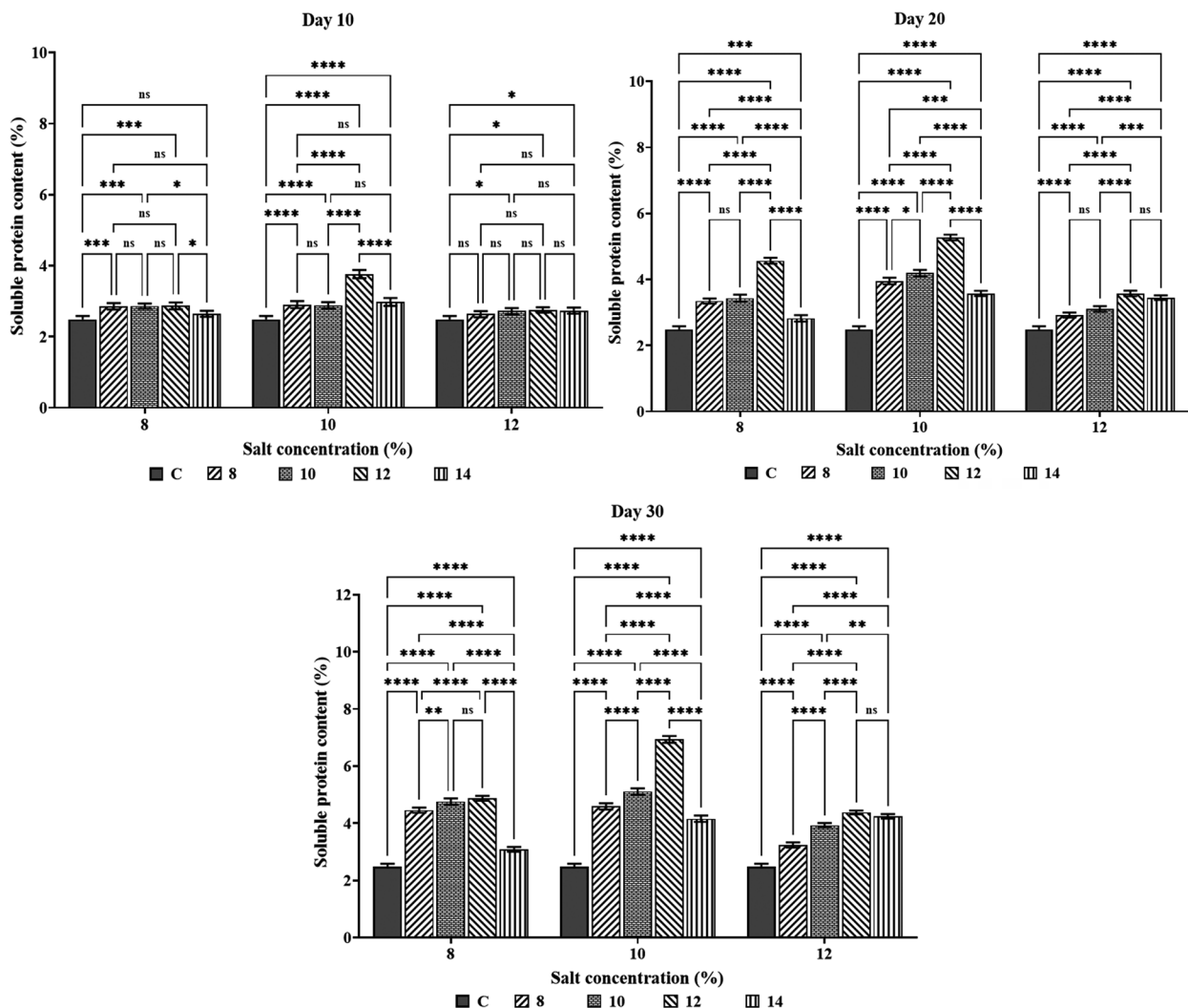
C – Pehtze before fermentation. Bars represent the standard deviation ($n = 3$). Significance levels: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$; ns: not significant ($p > 0.05$).

Fig. 1. Effect of salt and alcohol concentrations on free amino acids content of PSP sufu

differences were not statistically significant. This suggests that ripening for 10 days did not result in substantial progress. However, after 20 days of ripening, there was a significant increase in soluble protein content compared to the C sample.

During ripening, soluble protein increases as a result of protein hydrolysis. A similar trend was observed on day 30, when the highest soluble protein content (6.93%) was found in the sample with 10% salt and 12% alcohol. This increase is attributed to the

enzymatic activity of microorganisms, particularly proteases, which hydrolyze the protein in tofu and contribute to the structural formation of sufu (Xie et al., 2023). NaCl and alcohol concentrations have a significant impact on soluble protein content during sufu production (Ma et al., 2013). A 10% salt concentration resulted in better salinity compared with other levels. Salt not only imparts a salty taste but also regulates enzyme activity, influences biochemical changes, and inhibits the growth of harmful microorganisms.



C – Pehtze before fermentation. Bars represent the standard deviation ($n = 3$). Significance levels: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$; ns: not significant ($p > 0.05$).

Fig. 2. Effect of salt and alcohol concentrations on soluble protein content of PSP sufu

Table 1. Effect of salt and alcohol concentrations on pH and total acid content during the ripening of PSP sufu

Salt levels %	Alcohol levels %	Ripening time, days					
		10	20	30	10	20	30
		pH			Total acid content, %		
0	0	6.75 ±0.049 ^a	6.75 ±0.049 ^a	6.75 ±0.049 ^a	0.02 ±0.019 ^g	0.02 ±0.019 ^h	0.02 ±0.019 ⁱ
8	8	5.85 ±0.044 ^f	5.66 ±0.052 ^h	5.25 ±0.040 ^f	0.23 ±0.018 ^a	0.65 ±0.056 ^a	1.01 ±0.032 ^a
	10	5.90 ±0.070 ^f	5.76 ±0.040 ^{fg}	5.32 ±0.049 ^f	0.20 ±0.015 ^a	0.62 ±0.024 ^a	0.89 ±0.039 ^b
	12	6.07 ±0.061 ^e	5.82 ±0.046 ^f	5.60 ±0.038 ^{de}	0.19 ±0.019 ^b	0.61 ±0.054 ^a	0.75 ±0.046 ^c
	14	6.31 ±0.046 ^c	6.08 ±0.053 ^d	5.74 ±0.050 ^{bc}	0.07 ±0.022 ^b	0.40 ±0.039 ^b	0.66 ±0.023 ^d
10	8	5.85 ±0.053 ^f	5.66 ±0.052 ^{gh}	5.30 ±0.045 ^f	0.10 ±0.012 ^{cdef}	0.36 ±0.018 ^b	0.86 ±0.028 ^b
	10	6.37 ±0.047 ^{bc}	5.99 ±0.072 ^e	5.67 ±0.059 ^{cde}	0.08 ±0.012 ^c	0.21 ±0.020 ^c	0.58 ±0.016 ^c
	12	6.34 ±0.053 ^c	6.17 ±0.046 ^{bc}	5.66 ±0.040 ^{cde}	0.07 ±0.010 ^{cd}	0.13 ±0.013 ^{cf}	0.36 ±0.024 ^f
	14	6.33 ±0.044 ^c	6.19 ±0.060 ^b	5.65 ±0.047 ^{de}	0.05 ±0.013 ^{def}	0.09 ±0.014 ^{efg}	0.24 ±0.021 ^g
12	8	6.11 ±0.059 ^{de}	5.76 ±0.053 ^{fg}	5.60 ±0.049 ^c	0.08 ±0.009 ^{def}	0.18 ±0.009 ^{cd}	0.23 ±0.013 ^{gh}
	10	6.18 ±0.061 ^d	5.92 ±0.049 ^e	5.68 ±0.062 ^{cde}	0.06 ±0.011 ^{cde}	0.14 ±0.010 ^{de}	0.21 ±0.012 ^{ghi}
	12	6.37 ±0.045 ^{bc}	6.09 ±0.064 ^{cd}	5.69 ±0.079 ^{cd}	0.05 ±0.017 ^{def}	0.08 ±0.015 ^{fg}	0.19 ±0.016 ^{hi}
	14	6.45 ±0.049 ^b	6.16 ±0.040 ^{bcd}	5.78 ±0.072 ^b	0.05 ±0.012 ^{efg}	0.08 ±0.014 ^g	0.18 ±0.010 ⁱ

Note: different superscripts in the same column indicate significant differences in the data ($p < 0.05$).

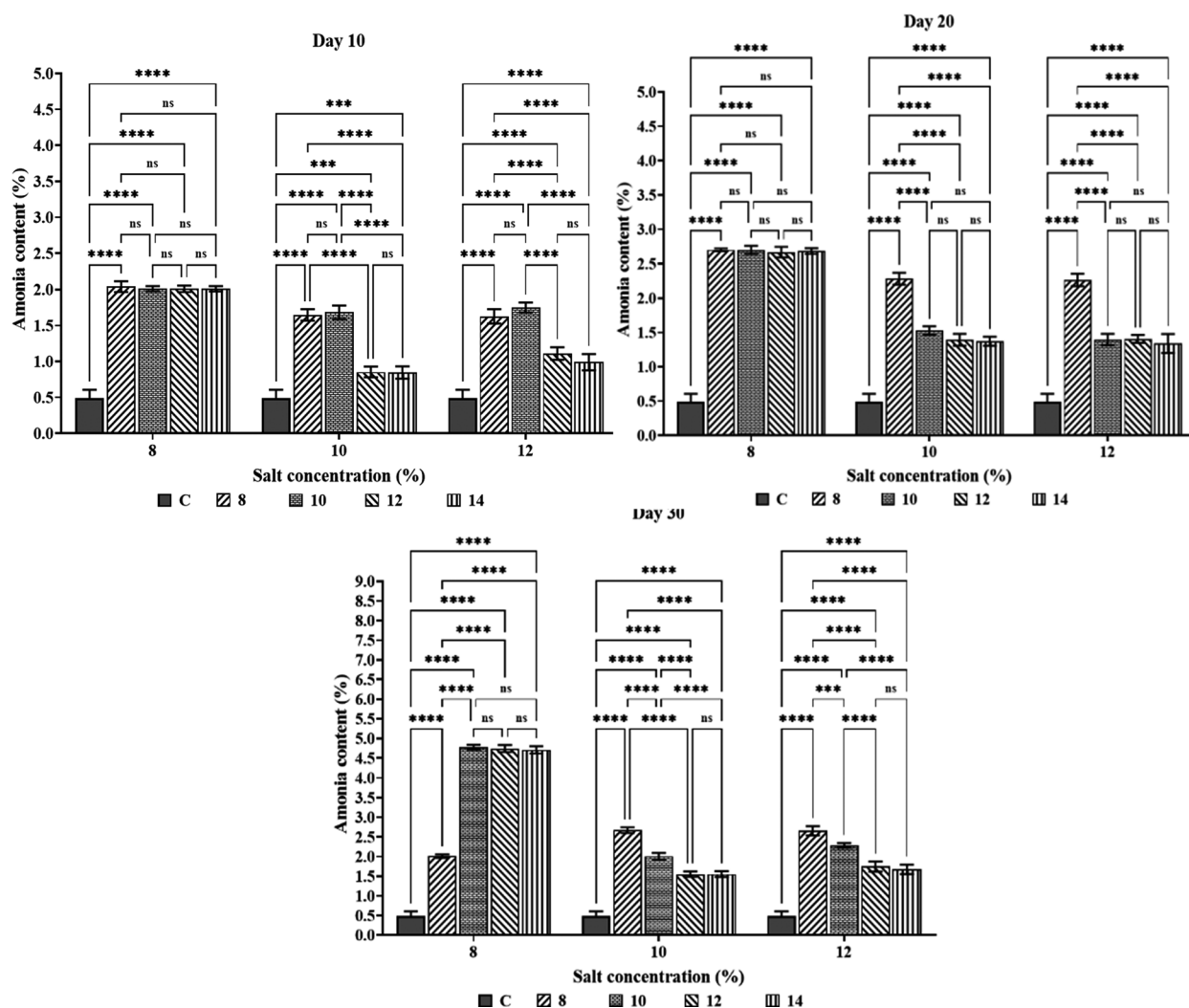
The concentrations of salt and alcohol in the brine affect the pH and total acid content during the ripening of PSP sufu, as shown in Table 1. For the C sample (0% salt and 0% alcohol), the initial pH was 6.75, and the total acid content was 0.02%. The pH of PSP sufu gradually decreased from day 10 to day 30 of ripening, with samples containing higher salt and alcohol concentrations showing a less pronounced pH reduction. This suggests lower salt and alcohol concentrations favor hydrolysis, promoting more vigorous bacterial growth and faster ripening.

Total acid content exhibited an inverse trend relative to pH, increasing over the ripening period. The observed pH values and total acid content in PSP sufu are consistent with previous studies on soy products and white sufu fermented with *Actinomucor elegans* and *Actinomucor taiwanensis*. These changes are attributed to the self-degradation of microbial cells during ripening, as well as the accumulation of free amino acids, fatty acids, and peptides containing carboxylic chains. These compounds, resulting from the hydrolysis of

tofu components and carbohydrate fermentation, reduce pH and increase acidity (Bao et al., 2020).

Ammonia (NH₃) content is a key indicator used to assess the effectiveness of the fermentation period (Makian et al., 2024). The NH₃ content, which varies according to salt and alcohol concentrations during the 30-day ripening of sufu, is shown in Figure 3. NH₃ content increases steadily from day 10 to day 30. At a salt concentration of 8%, NH₃ rises rapidly during the first 10 days and then slows after 20 days, suggesting that microorganisms and enzymatic activity are more intensive at this salt level, accelerating hydrolysis. Alcohol concentrations ranging from 8% to 14% did not show statistically significant differences.

By day 30, the lowest NH₃ content (~1.55%) was observed at a salt concentration of 10% combined with alcohol concentrations of 12–14%. Overall, NH₃ accumulation during ripening is primarily due to protein hydrolysis by microbial and enzymatic activity. Higher ripening temperatures promote NH₃ production, whereas lower temperatures inhibit it (Guo et al., 2025).



C – Pehtze before fermentation. Bars represent the standard deviation ($n = 3$). Significance levels: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$; ns: not significant ($p > 0.05$).

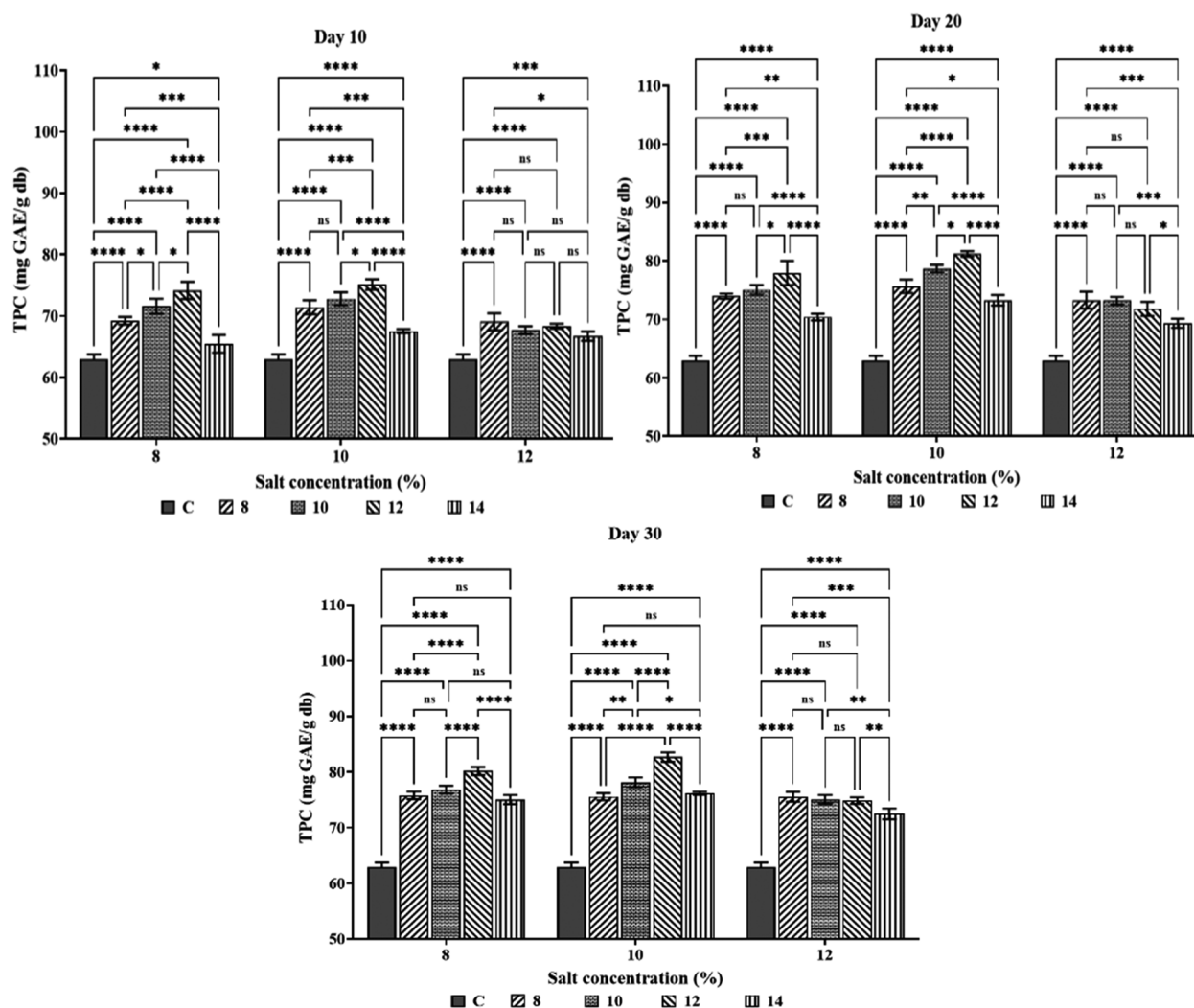
Fig. 3. Effect of salt and alcohol concentrations on ammonia content of PSP sufu

Salt concentration strongly affects protein hydrolysis and reduces biogenic amines, including NH_3 (Gardini et al., 2016). At 8% salt, the formation of biogenic amines is markedly inhibited (Shi et al., 2023). Alcohol also contributes to reducing biogenic amine formation (Shi et al., 2023). Specifically, adding 10% alcohol decreases biogenic amine content by 11% (Qiu et al., 2018), and using 15% alcohol significantly lowers the levels of biogenic amines, especially cadaverine (Liang et al., 2019). Alcohol inhibits biogenic amine formation by affecting microbial metabolic activity and

viability, reducing decarboxylase activity, and slowing microbial growth.

Effects of salt and alcohol concentrations on the bioactive compounds of PSP sufu

Figure 4 shows the effect of salt and alcohol concentrations on the total phenolic content (TPC) of PSP sufu during 30 days of ripening. The results indicate that TPC increases throughout ripening and is significantly higher than in the control sample ($p < 0.05$). After 10 days of ripening, samples with 8% and 10% salt



C – Pehtze before fermentation. Bars represent the standard deviation ($n = 3$). Significance levels: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$; ns: not significant ($p > 0.05$).

Fig. 4. Effect of salt and alcohol concentrations on total phenolic content of PSP sufu

showed noticeable changes in TPC with varying alcohol concentrations (8–14%), whereas in samples with 12% salt, alcohol concentrations did not affect TPC.

On days 20 and 30, TPC increased with both higher salt and alcohol concentrations. The highest TPC (82.66 mg GAE/g dry weight) was observed in the sample containing 10% salt and 12% alcohol on day 20.

The increase in TPC during fermentation is attributed to the activation of hydrolytic enzymes, such as β -glucosidase, β -xylosidase, and α -arabinofuranosidase.

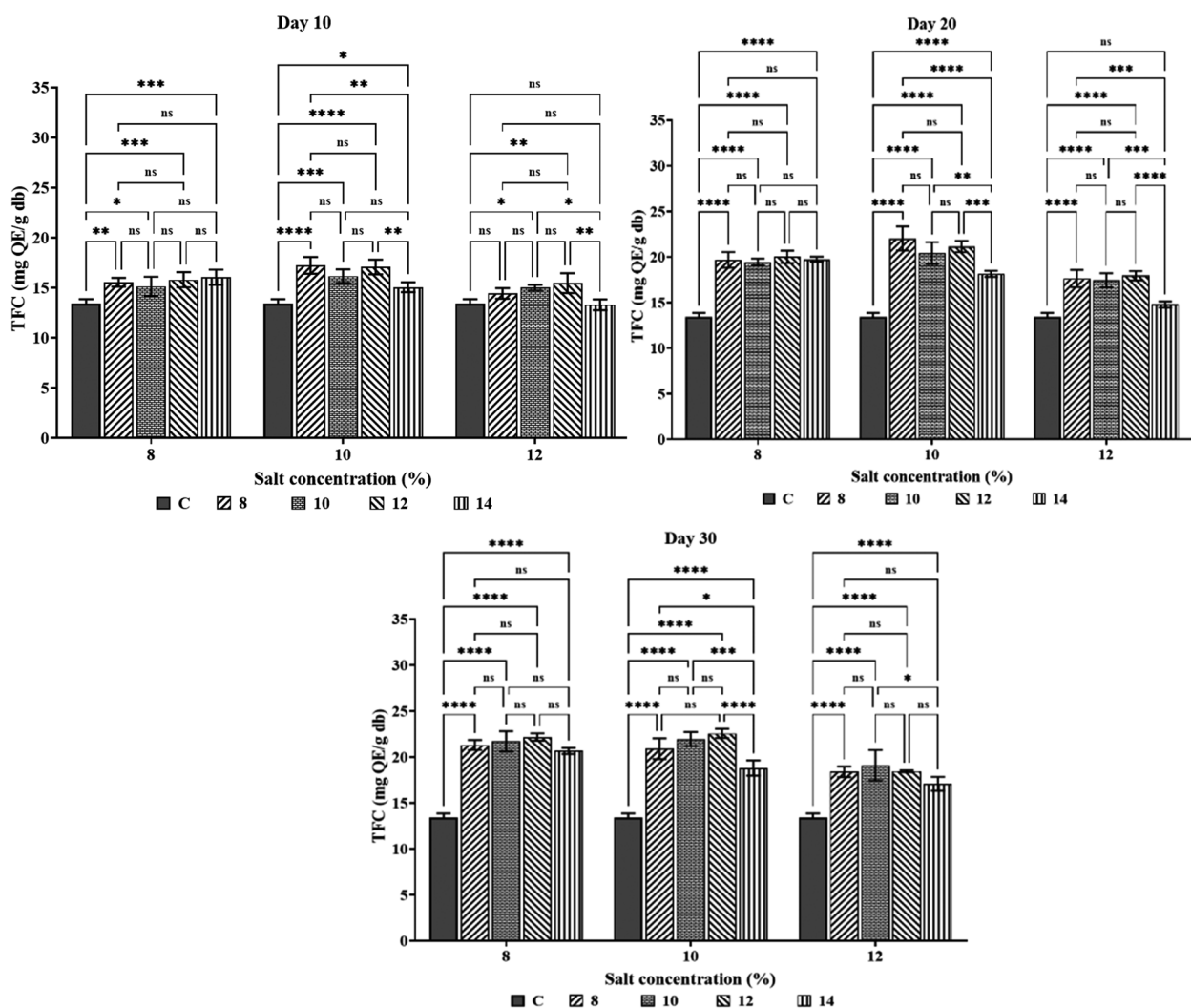
These enzymes cleave the bonds between phenolic compounds and glycosides, releasing biologically active phenolic monomers, modifying hydroxyl groups within the phenolic structure, and increasing the level of free phenolic radicals (Ajila et al., 2012). Additionally, fermentation can generate new compounds, some belonging to the phenolic group (Ayyash et al., 2018). However, prolonged fermentation may reduce TPC due to the oxidation of phenolic compounds, and certain phenolics, such as gallic acid, may degrade into aliphatic substances.

Dhull et al. (2020) also confirmed that fermentation significantly enhances phenolic compound content.

The changes in the total flavonoid content (TFC) of PSP sufu under varying salt and alcohol concentrations during ripening are shown in Figure 5. TFC gradually increased from day 10 to day 30, likely due to the hydrolysis of bound phenolic compounds or microbial biosynthesis of flavonoids during ripening (Ajila et al., 2012; Ayyash et al., 2018). Notably, at certain time points, especially on day 10, TFC did

not differ significantly among samples with different alcohol concentrations. However, on days 20 and 30, samples with 8% salt exhibited significantly higher TFC than those with 12% salt ($p < 0.05$). Across alcohol concentrations of 8 to 12%, TFC was consistently higher than in samples with 14% alcohol at all time points ($p < 0.001$). The highest TFC was observed in the sample with 10% salt and 12% alcohol on day 30.

Salt likely created a hypertonic environment that slightly disrupted plant cell membranes, facilitating



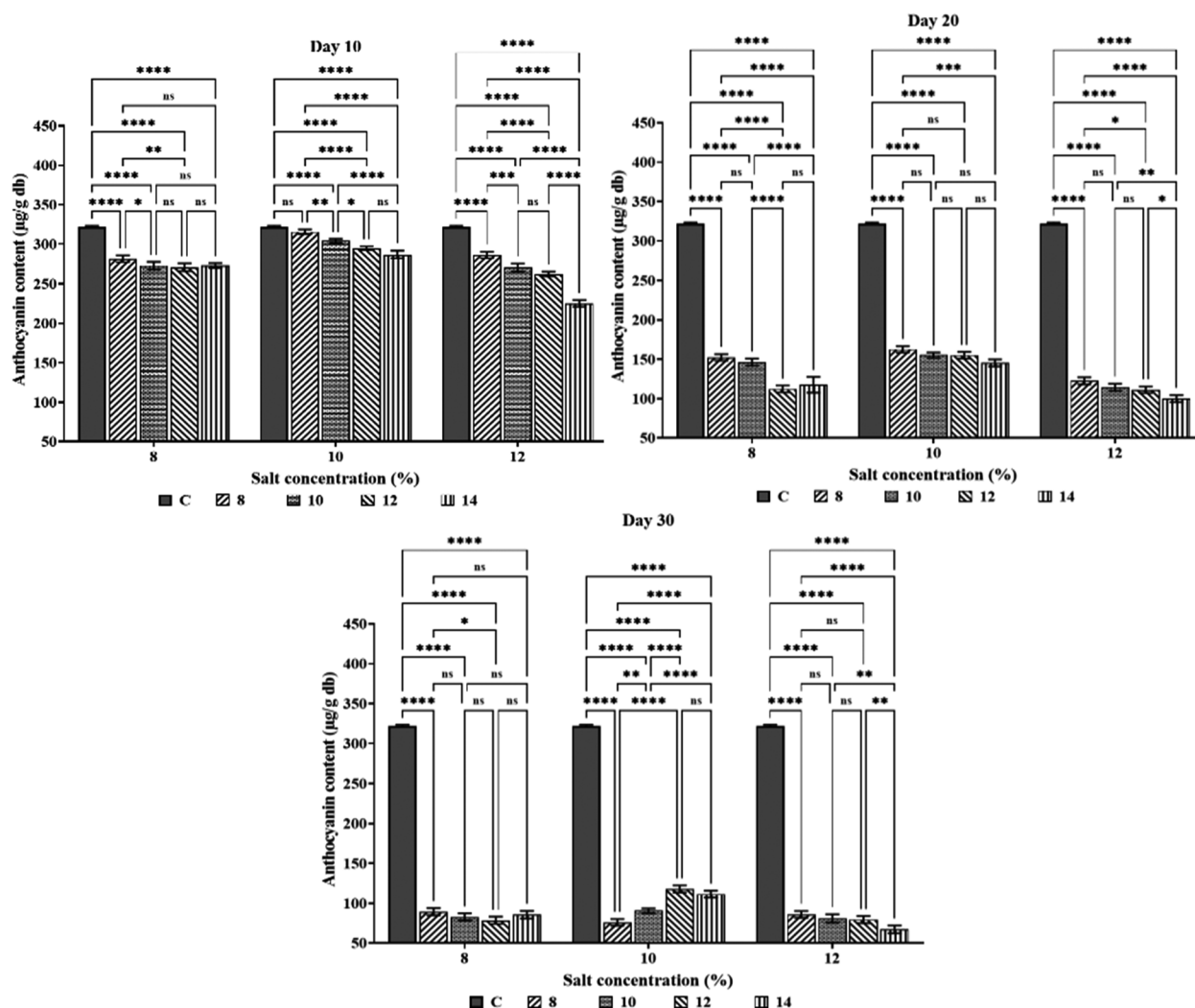
C – Pehtze before fermentation. Bars represent the standard deviation ($n = 3$). Significance levels: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$; ns: not significant ($p > 0.05$).

Fig. 5. Effect of salt and alcohol concentrations on total flavonoid content of PSP sufu

the release of flavonoids bound to proteins. It also inhibited undesirable microorganisms and promoted favorable conditions for both endogenous and exogenous enzymes, enhancing the hydrolysis of flavonoid glycosides into more bioavailable and biologically active aglycone forms. Additionally, salt limited oxidative processes, reducing flavonoid losses and potentially supporting TFC accumulation under suitable conditions (Chernane et al., 2015).

The concentrations of salt and alcohol significantly affected the anthocyanin content of PSP sufu during

ripening, as shown in Figure 6. Anthocyanin content decreased with increasing salt and alcohol concentrations over the ripening period compared to the control (C). At all three ripening time points, samples with 8% salt had significantly higher anthocyanin content than those with 10% or 12% salt ($p < 0.05$). Similarly, as alcohol concentration increased from 8 to 14%, samples with 8 and 10% alcohol maintained higher anthocyanin levels on day 10 than those with 12 or 14% alcohol, with significant differences ($p < 0.001$). This trend persisted on days 20 and 30.



C – Pehtze before fermentation. Bars represent the standard deviation ($n = 3$). Significance levels: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$; ns: not significant ($p > 0.05$).

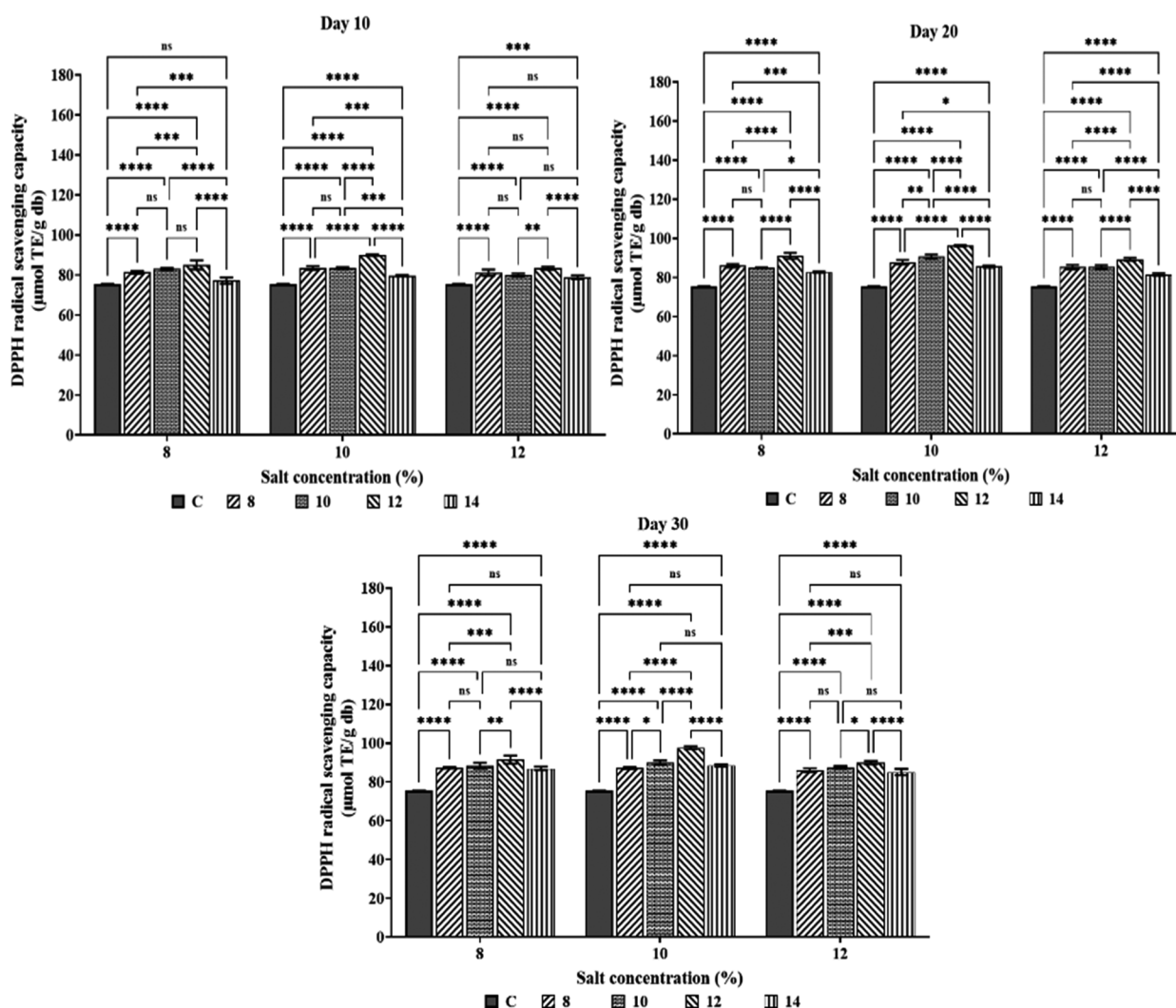
Fig. 6. Effect of salt and alcohol concentrations on anthocyanin content of PSP sufu

These findings align with Chen et al. (2019), who reported that increasing salt concentrations reduces anthocyanin content by promoting the dissociation of anthocyanin molecules into carbenium ions, which facilitates hydration reactions and leads to the loss of anthocyanin monomers. Additionally, Tseng et al. (2006) found that high alcohol concentrations destabilize anthocyanins through oxidation. At elevated alcohol levels, anthocyanins predominantly exist in the flavylium cation form, which is more susceptible to oxidative attack or conversion to carbinol base or

chalcone forms, resulting in color loss and anthocyanin degradation.

Effects of salt and alcohol concentrations on antioxidant activity of PSP sufu

The DPPH free radical scavenging activity of PSP sufu is shown in Figure 7. Salt concentration had a statistically significant effect on DPPH scavenging at 10, 20, and 30 days of ripening. At all three time points, DPPH scavenging increased as salt concentration rose from 8% to 12%. However, the difference

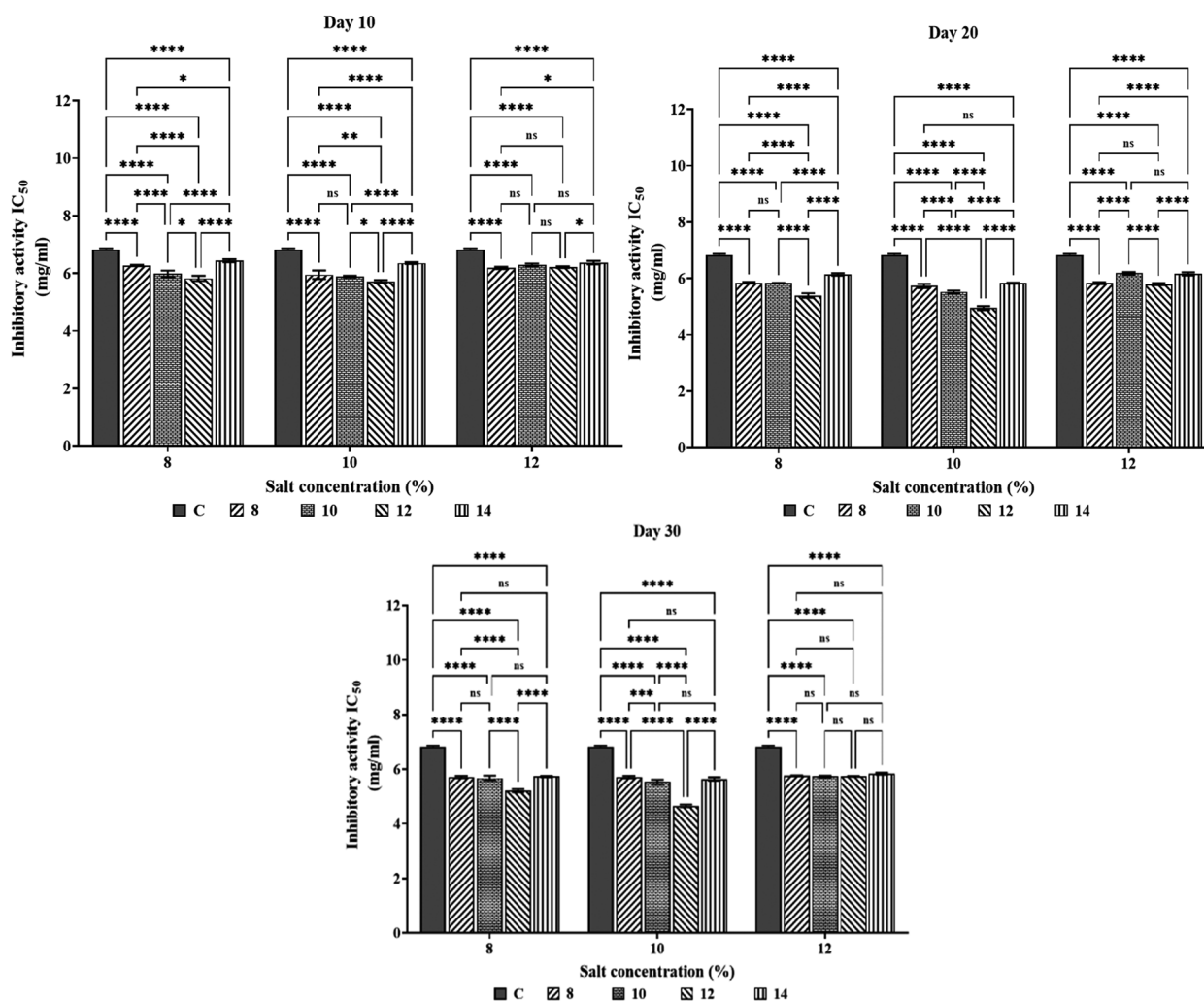


C – Pehtze before fermentation. Bars represent the standard deviation ($n = 3$). Significance levels: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$; ns: not significant ($p > 0.05$).

Fig. 7. Effect of salt and alcohol concentrations on DPPH radical scavenging of PSP sufu

between samples with 10 and 12% salt was not always statistically significant. This suggests that salt may facilitate the ripening process, promoting the formation of antioxidant compounds. Alcohol concentration also significantly affected DPPH scavenging. At higher alcohol levels (12% and 14%), DPPH scavenging slightly decreased compared to samples with 8% and 10% alcohol. These findings are consistent with Xie et al. (2022), who reported that salt concentrations around 10–12% optimize antioxidant activity in fermented soy flour by supporting beneficial microorganisms and enhancing the ripening process.

The effect of salt and alcohol concentrations on the half-maximal inhibitory concentration (IC_{50}) of PSP sufu after 10, 20, and 30 days of ripening is shown in Figure 8. The IC_{50} value of the control sample (C) was the highest, indicating the lowest inhibitory ability. In the experimental samples, IC_{50} decreased during ripening, demonstrating an increased ability to neutralize 50% of free radicals from 10 to 30 days. The sample with 10% salt and 12% alcohol exhibited the lowest IC_{50} value, indicating the highest free radical-scavenging capacity. These results suggest that using 10% salt and 12% alcohol may be the optimal condition for



C – Pehtze before fermentation. Bars represent the standard deviation ($n = 3$). Significance levels: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$; ns: not significant ($p > 0.05$).

Fig. 8. Effect of salt and alcohol concentrations on the inhibitory activity of PSP sufu

Table 2. Correlations between nutritional components, bioactive compounds, and free radical scavenging activity of PSP sufu during the ripening period

	Ammonia	Antho- cyanin	DPPH	FAA	IC ₅₀	pH	Soluble protein	TFC	Total acid	TPC
Ammonia	1									
Anthocyanin	-0.575 ****	1								
DPPH	0.412 ****	-0.665 ****	1							
FAA	0.203 *	-0.555 ****	0.869 ****	1						
IC ₅₀	-0.474 ****	0.646 ****	-0.958 ****	-0.855 ****	1					
pH	-0.699 ****	0.762 ****	-0.649 ****	-0.499 ****	0.680 ****	1				
Soluble protein	0.327 ***	-0.670 ****	0.827 ****	0.846 ****	-0.847 ***	-0.618 ****	1			
TFC	0.638 ****	-0.743 ****	0.822 ****	0.735 ****	-0.823 ****	-0.772 ****	0.783 ****	1		
Total acid	0.756 ****	-0.612 ****	0.453 ****	0.327 ***	-0.485 ****	-0.766 ****	0.532 ****	0.734 ****	1	
TPC	0.532 ****	-0.691 ****	0.948 ****	0.833 ****	-0.964 ****	-0.742 ****	0.812 ****	0.859 ****	0.568 ****	1

Note: $p^{****} \leq 0.0001$; $p^{***} \leq 0.001$; $p^{**} \leq 0.01$; $p^* \leq 0.05$.

PSP Sufu ripening, promoting the formation of phenolic compounds that enhance free radical scavenging. A lower IC₅₀ value corresponds to higher antioxidant activity. The increase in antioxidant activity is associated with compounds generated during fermentation, such as TPC, TFC, peptides, and amino acids. Several studies have shown that protein hydrolysis can produce peptides with antioxidant activity. Protein breakdown exposes hydrophilic, amino-containing chains, which enhances the ability to scavenge water-soluble free radicals compared with peptides that have not undergone hydrolysis (Sanjukta and Rai, 2016).

Correlation between nutritional components, bioactive compounds, and free radical scavenging activity of PSP sufu during the ripening period

The correlations between nutritional components, bioactive compounds, and free radical scavenging

activity are shown in Table 2. The analysis revealed a strong positive correlation between TPC and DPPH radical scavenging activity ($R^2 = 0.948$), indicating that TPC is the primary contributor to the free radical scavenging ability of PSP sufu. Accordingly, TPC exhibited a strong negative correlation with IC₅₀ ($R^2 = -0.964$). Free amino acids (FAA) were highly correlated with soluble protein content ($R^2 = 0.846$) and also contributed to the DPPH free radical scavenging activity ($R^2 = 0.869$), showing a negative correlation with IC₅₀ ($R^2 = -0.855$). Notably, anthocyanin content decreased significantly during ripening, resulting in a relatively low correlation with free radical scavenging activity ($R^2 = 0.64$).

CONCLUSIONS

The concentrations of salt and alcohol in the soaking solution played a crucial role in the ripening process

and significantly influenced the quality of PSP sufu. Nutritional indices, such as FAA, soluble protein, total acid, TPC, and TFC, increased with higher salt and alcohol concentration and prolonged ripening. In contrast, NH_3 and anthocyanin contents decreased under the same conditions. These compositional changes enhanced DPPH radical scavenging capacity and reduced IC_{50} values compared with the control samples. The optimal product quality was achieved with 10% salt, 12% alcohol, and a ripening period of 30 days. Nevertheless, further studies are required to investigate changes in the odor profile of PSP sufu during ripening and to compare these characteristics with commercial sufu products to optimize sensory quality.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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