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EFFECTS OF INCUBATION CONDITIONS ON THE NUTRIENT COMPOSITION AND ANTIOXIDATIVE ACTIVITY OF FERMENTED TOFU SUPPLEMENTED WITH PURPLE SWEET POTATO BY *ACTINOMUCOR ELEGANS*

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ABSTRACT

Background. Fermented tofu, a traditional product of Asian countries, is produced using *Actinomucor elegans*. The product is popularly consumed because of its unique nutrients, taste, and flavor. The fortification of tofu with purple sweet potato (PSP) can provide many health benefits, especially antioxidative properties. However, incubation conditions including temperature and relative humidity affect the growth of *Actinomucor elegans*, leading to changes in product quality.

Materials and methods. In this study, the effects of various temperatures (25, 30, and 35°C) and relative humidities (75, 85, and 95%) on the fermentation of tofu supplemented with PSP were carried out. The nutrient composition, including moisture, protein, lipid, ammonia, glucose, and free amino acid contents, bioactive compounds (total phenolic-TPC, total flavonoid-TFC, and anthocyanin contents), antioxidant activity in terms of DPPH and ABTS radical scavenging activities, and inhibitory activity (IC_{50}) were monitored.

Results. The results indicate that tofu supplemented with PSP can be successfully catalyzed by *Actinomucor elegans* at a suitable temperature and relative humidity of 30°C and 95%, respectively. The pehtze contained 63.8% moisture, 15.36% protein, 9.43% lipid, 12.49% free amino acid, 0.05% ammonia, and 125.41 mg/g glucose. The TPC, TFC, and anthocyanin contents were 62.91 mg GAE/g d.w., 17.32 mg QE/g d.w., and 309.54 µg/g d.w., respectively. The DPPH and ABTS radical scavenging activities, and the IC_{50} value were 85.50 µmol TE/g d.w., 120.79 µmol TEAC/mg, and 6.61 mg/mL, respectively.

Conclusions. Fermentation at 30°C and a relative humidity of 95% produced fermented PSP tofu with high nutrition, bioactive compounds, and antioxidant activity.

Keywords: *Actinomucor elegans,* antioxidant, purple sweet potato, fermented tofu

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INTRODUCTION

Fermented foods are produced as a result of the activity of microorganisms, principally yeasts, molds, and bacteria (Aidoo and Nout, 2010). Molds play a major role in traditional fermented foods, which have a long history of improving nutritional value (Soccol et al., 2017). Previous studies have shown that bioactive compounds and antioxidant activities can be increased in fermented wheat using *Actinomucor awamori*, *Aspergillus oryzae* (Bhanja et al., 2009), and *Monascus anka* (Bei et al., 2018). Mold fermentation is commonly used in soy processing to create enzymes, isoflavones, food bio-colorants, low-phytic acid, and high amino acid content (Handa et al., 2019). In several Asian countries, tofu is commonly fermented using *Actinomucor elegans* to make products (Huang et al., 2021) with various names, such as Sufu or Furu (China), Tempeh (Indonesia), Mao-tofu (Thailand), or Chao (Vietnam). It contains antioxidants such as phenolic, flavonoid, and isoflavone compounds (Yin et al., 2020). Previous results have revealed that fermented tofu's antioxidative activity is higher than that of non-fermented tofu (Liu and Zhao, 2017; Yan and Dong, 2019). Fermented tofu (Chao) is a traditional Vietnamese product produced by tofu incubation. *Actinomucor elegans* is a common mold strain used for tofu fermentation. As a result of enzymatic hydrolysis processes, enzymes released from molds hydrolyze the macromolecules in tofu, including carbohydrates, proteins, and lipids, into smaller molecules and elements, which are more rapidly digested and absorbed and produce the unique taste of the product (Xie et al., 2019).

Purple sweet potato (*Ipomoea batatas* L.) (PSP) is a unique agricultural resource with nutritious and antioxidative ingredients, mainly because it is rich in anthocyanin (Ngcobo et al., 2024). The antioxidants exist in all parts of PSP, including the roots, stems, leaves, and bulbs (Fu et al., 2016). Some previous studies have demonstrated that anthocyanin in PSP is a complex mixture of several forms that can resist high temperatures, enhance antioxidant, anti-inflammatory, and anti-tumor properties (Zhao et al., 2013), prevent acute and subacute alcoholic liver damage, and neutralize alcohol (Sun et al., 2014). PSP is also used as a material for several foods, such as bakery products, pasta, noodles, flakes, snacks, and alcoholic beverages (Bach et al., 2021). Tofu supplemented with PSP is a newly developed product based on traditional tofu production. The addition of PSP, a rich source of bioactive compounds and antioxidant activities, can improve the functional properties and nutrition of the resulting tofu, thus providing health benefits for consumers, such as the prevention of the development of harmful free radicals in the human body. Han et al. (2003) have documented that *Actinomucor elegans* can grow properly at temperatures of 25–32°C and relative humidity values of 87–95%. However, the process of PSP tofu fermentation by *Actinomucor elegans* for Chao's production is affected by environmental conditions such as temperature and relative humidity. In particular, the nature of changes in bioactive compounds and antioxidant activity in the incubated product (pehtze) is still unclear. Thus, this study aimed to elucidate the effects of incubation conditions on the nutritional properties and antioxidative activity of PSP-enriched fermented tofu during the fermentation process.

MATERIALS AND METHODS

Materials

Actinomucor elegans strain NCPF 7543 isolated from sufu was provided by the Institute of Food and Biotechnology, Can Tho University. HLDN 910 soybeans were purchased at Hung Loc Agricultural Research Center (Dong Nai Province, Viet Nam). Purple sweet potato (PSP) was a product of Thanh Binh Tan Limited Company (Vinh Long province, Viet Nam). All chemicals were analytical grades purchased from Merck KGAA Co. (Darmstadt, Germany).

Preparation of molds

Actinomucor elegans was incubated at 30°C for 36 hours in potato dextrose agar (PDA). Mold spores were separated from plate count by scraping the sporangia off the agar and then suspended in sterile distilled water with 0.85% salt, 0.1% peptone, and 0.05% Tween 80 (Han et al., 2003).

Preparation of purple sweet potato tofu

The tofu production was conducted following the procedure of Joo et al. (2023) with slight modifications. The soybeans (*Glycine max* L.) were soaked in overflowing water at 20°C for 6 hours and milled in water with a ratio of 1:8 (bean:water). Then, the soya milk

solution was collected, heated at 100°C for 15 minutes, and mixed with PSP solution.

To make the PSP solution, the PSP was cut into 1–2 mm thick slices, steamed at 100°C for 5 minutes, ground, and added to the water at a ratio of 1:2 (potato:water). Then, the PSP solution was mixed with the soya milk solution at a ratio of 0.75:1 (PSP: soya milk) (v/v). The mixture was then coagulated by adding nigari to the final concentration of 0.2% v/w at 90°C for 15 minutes. Then, it was put into a 12 × 10 × 7 cm tray and dehydrated using the compression molding method for 15 minutes. The coagulated tofu was pressed with fixed-distance spiral shafts. Then, PSP--enriched tofu was collected.

Preparation of pehtze

The PSP tofu was cut into cubes of 2 × 2 × 2 cm^3 and sterilized with a UV lamp for 10 minutes. The tofu was evenly spaced in plastic trays and incubated with a mold population of 10^6 cfu/mL at different temperatures (25, 30, and 35°C) and relative humidities (75, 85, and 95%) for 30 hours using a climate incubator (ICB-175C, Scitek, USA). The fermented PSP tofu (pehtze) was subjected to analysis.

Determination of the nutrient composition of pehtze

The moisture content (%) of pehtze was determined using the oven method (AOAC, 2000). The protein and ammonia contents (%) of pehtze were determined through total nitrogen content using the Kjeldahl method with a standard method number of ISO 20483:2013 (ISO, 2013). The fat content (%) of pehtze was determined using the Soxhlet method (AOAC, 2000). The free amino acid content (%) of pehtze was analyzed by conducting hydrolysis and quantitatively determined using HPLC equipment (1200 series, USA) with a standard method number of ISO 13903:2005 (ISO, 2005). The glucose content (mg/g) of pehtze was calculated according to the Acid Dinitrosalicylic Method (Mao et al., 2013) using glucose as a standard.

Determination of the total phenolic, flavonoid, and anthocyanin contents, and the antioxidant activity of pehtze

The total phenolic content (TPC) of pehtze was measured using the Folin-Ciocalteu method and expressed as gallic acid equivalent/g dry weight (mg GAE/g d.w.) (Jiang et al., 2013). Briefly, the sample was freeze dried (HRFD-P-L, Harvest Right, USA) for 27 hours. The dried sample was then defatted with petroleum ether using a solvent extractor (Ser 148/6, VELP Scientifica, Italy). 5 g of freeze-drying and fatty-free Pehtze was extracted with 12 mL of 70% acetone. 0.1 mL of the pehtze extract was diluted with 3.5 mL of distilled water. Then, 0.4 mL of Folin-Ciocalteu solution and 1 mL of 7.5% Na_2CO_3 were added to the mixture and kept 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 content (TFC) of pehtze was determined using the aluminum chloride colorimetric method and expressed as mg quercetin equivalent (QE) per gram dry weight of the sample (mg QE/g d.w.), as described by Khan et al. (2012). 0.5 mL of the pehtze extract was added to 3 mL of distilled water, 0.15 mL of 5% $NaNO_2$, and 0.3 mL of 10% $AlCl_3$. The mixture was incubated at room temperature for 30 minutes and measured at a wavelength of 415 nm using the spectrophotometer.

The anthocyanin content (μg/g) of pehtze was determined using the method of Wrolstad and Culver (2012). The absorbance of anthocyanin in different buffer solutions (pH 1.0 and 4.5) was measured at wavelengths of 520 and 700 nm, respectively, using a spectrophotometer. The anthocyanin content was calculated from a cyanidin-3-glucoside standard curve.

For DPPH radical scavenging capacity, 800 μL of the pehtze extract was mixed with 800 μl of 0.008% DPPH, shaken well, and incubated at room temperature for 30 minutes. Then, the mixture was measured at a wavelength of 517 nm. DPPH activity was expressed as μmol Trolox equivalent (μmol TE/g d.w.) (Sakshy and Paras, 2009).

The ABTS radical scavenging capacity of pehtze was measured by reacting the potassium persulfate reagent with the $ABTS^+$ radical [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate)]. Potassium persulfate (2.6 mM) and ABTS (7.4 mM) were mixed at a ratio of 1:1 (v:v) for 12 hours in the dark and at room temperature to generate ABTS free radicals. Thereafter, ABTS radical solution ($ABTS^{\bullet+}$) (0.5 mL) was mixed with distilled water (25 mL) to reach an absorbance of

1.1 ±0.02 units at 734 nm. To 0.15 mL of the pehtze extract, 2.85 mL of $ABTS^{\bullet+}$ were added and thoroughly vortexed. The mixture was then incubated in the dark for 45 min at room temperature, and its absorbance at 734 nm was read. The activity was recorded in µmol Trolox equivalent/mL (µmol TEAC/g d.w.) (Alam et al., 2013).

Sensory evaluation of pehtze

After incubation, pehtze samples were subjected to sensory evaluation using the quantitative descriptive analysis (QDA) method to assess product attributes with 40 panelists including students and lecturers aged 20–35 who were familiar with fermented tofu. GraphPad Prism software (GraphPad Software, LLC, US) was utilized for principal component analysis (Torres-Penaranda and Reitmeier, 2001).

Data analysis

Analysis of variance (ANOVA) was used to assess the significant differences among multiple groups under various treatments, followed by Tukey's test using Statgraphics (Virginia, US). The data are presented as triplicate experimental mean ±standard deviation (±SD), with a statistical significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Effect of temperature and relative humidity on the nutrient composition of PSP tofu and pehtze

The effects of relative humidity (Rh, %) and temperature (°C) on the nutrient composition of PSP tofu and pehtze are presented in Figure 1. The moisture content of PSP pehtze was significantly influenced by temperature and Rh during incubation (Figure 1A).

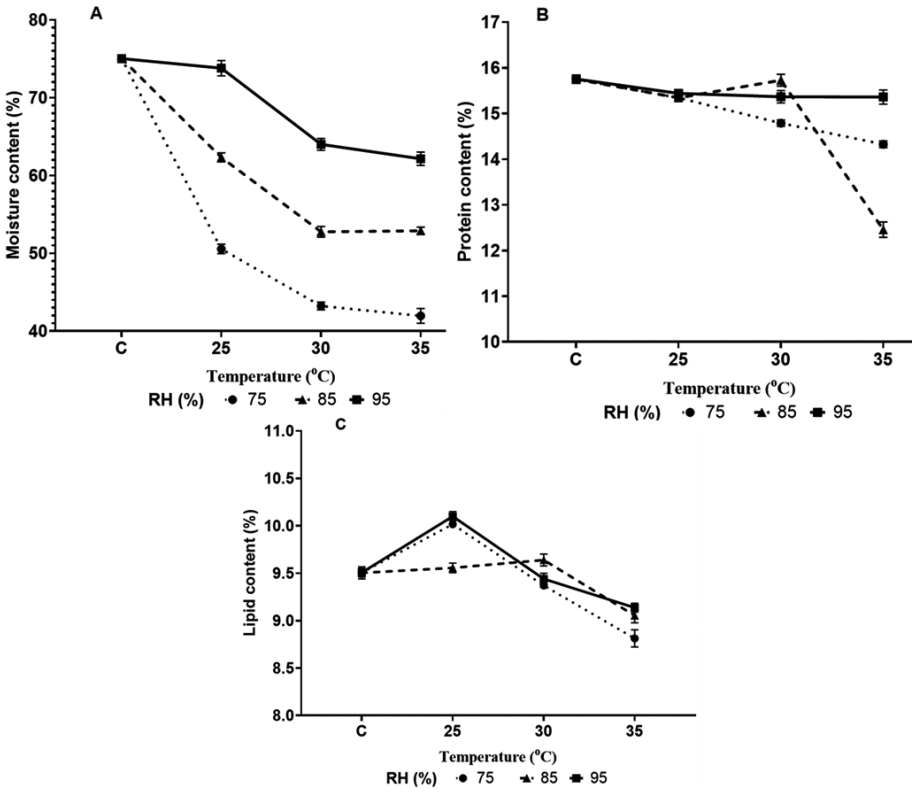

Fig. 1. Moisture (A), protein (B), and lipid (C) contents of PSP tofu and pehtze affected by temperature and relative humidity. C: Tofu before fermentation. Bars represent the standard deviation (n = 3)

Before fermentation, the moisture content of PSP tofu was 75.03 ±0.35 %. Under various fermentation conditions, the moisture content of PSP pehtze decreased as the temperature increased ($p < 0.05$). However, the incubation at higher Rh resulted in a higher moisture content ($p < 0.05$). The highest moisture content (73.80 ±0.26%) was observed at 25°C and 95% Rh, while the lowest value (41.97 ±1.70%) was observed at 35°C and 75% Rh. Generally, water transfer occurs from the 'wet' component or higher Rh to the 'dry' component or lower Rh in all heterogeneous products (Roudaut and Debeaufort, 2010). Abbasi et al. (2009) explained that an increase in temperature could accelerate the diffusion of moisture from the surface of materials to the environment. Besides, Rh and ventilation speed also increased the moisture emissions of materials, yielding a reduction in the moisture content of the pehtze. The current result is in line with that of Yin et al. (2005), who reported that water leached out from pehtze during fermentation, as was observed from a decrease in the moisture in pehtze.

The data presented in Figure 1B indicate the changes in the protein content of PSP tofu and pehtze as a function of temperature and Rh. The study highlighted significant differences between the protein content in the PSP tofu and pehtze incubated at various Rh and high temperatures. The fermentation process lowered the protein content in the PSP tofu. However, there was no significant difference in the protein content of the PSP tofu and the PSP pehtze incubated at a high Rh of 95%. The lowest protein content was 12.34 ±0.49 % recorded in PSP pehtze incubated at a Rh of 85% and a temperature of 35°C. Other than water, protein is the largest component of tofu. It is generally accepted that fungal proteases catalyze the degradation of protein into smaller molecular elements, which contributes to the particular characteristics of fermented tofu (Ma et al., 2013). In addition, Cheng et al. (2009) argued that the fermentation process by microorganisms deconstructs the protein in pehtze, leads to changes in protein structure, and generates other compounds during incubation that could reduce the total protein in tofu. Han et al. (2003) reported that the highest protease activity (108 U/g of pehtze d.m.) of *A. elegans* was found after incubation for 48 h at 25°C and Rh 95–97%. The biomass formation rate of *A. elegans* decreased at temperatures higher than 30°C. Therefore, *A. elegans* could not grow well and form biomass at 85% Rh and 35°C, leading to microbial contamination, mucus, and a bad smell. The protein was destroyed by microbial contamination, yielding lower protein content. These results suggested that a Rh of 95% and an incubation temperature of 30°C are appropriate for the growth of *Actinomucor elegans* and maintain the protein content of PSP pehtze.

The change in total lipid content (%) of PSP tofu and pehtze affected by different temperatures and relative humidities during the incubation was also evaluated (Fig. 1C). In general, the total lipid content in PSP tofu was 9.51 ±0.04%, and this value was slightly decreased after fermentation ($p < 0.05$). The results show that PSP tofu incubated at 35°C and a Rh of 75% yielded the lowest total lipid content (8.81 ±0.09%). *Actinomucor elegans* possesses the enzyme lipase, which could hydrolyze lipids in tofu into free fatty acid (Bei et al., 2018). Yin et al. (2020) reported that lipase was liberated from *A. elegans* during tofu fermentation, which causes a decrease in the fat content of fermented tofu. In addition, Han et al. (2003) proved that the highest lipase activity (172 U/g pehtze d.m.) produced by *A. elegans* was found after 48 h incubation at 25°C and Rh 95–97%. However, high temperatures (35°C) and low Rh (75%) are suboptimal conditions for the growth of *A. elegans*. Thus, contamination could be observed, which yielded a lower lipid content.

The ammonia (NH_3) content (%) of PSP tofu and pehtze affected by temperature and relative humidity is documented in Figure 2A. PSP tofu contained 0.02 ±0.01% ammonia. The ammonia content of PSP pehtze was significantly increased during incubation ($p < 0.05$). A higher amount of ammonia was recorded in PSP tofu incubated at high temperatures. This component increased almost five times (0.11 ±0.00%) after incubating PSP tofu at 35°C and Rh of 85% or 95%. Nevertheless, the higher Rh caused a lower ammonia content in PSP tofu. The ammonia content of PSP tofu incubated at 30°C and Rh of 95% was 0.05%. This value increased to 0.06% and 0.07%, when PSP tofu was incubated at a Rh of 85% and 75%, respectively. The ammonia compounds generated were also a result of protein hydrolysis (Aidoo and Nout, 2010). Han et al. (2004) found that the NH_3 content of tofu was low (0.13 mg/g), and owing to the protease generated during fungal growth, a significant amount of NH_3 was

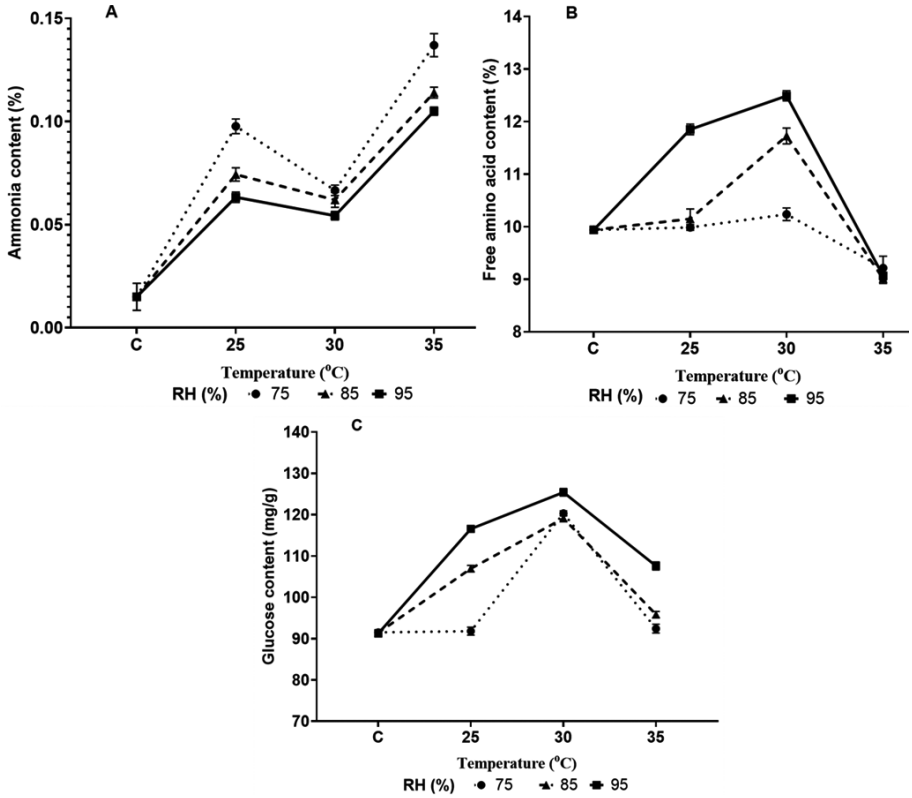

Fig. 2. Ammonia (A), free amino acid (B), and glucose (C) contents of PSP tofu and pehtze affected by temperature and relative humidity. C: Tofu before fermentation. Bars represent the standard deviation (n = 3)

measured in pehtze (3.6 mg/g). It was noted that the lowest ammonia content was found in PSP tofu incubated at 30°C for all ranges of Rh. The growth rate of *Actinomucor elegans* was optimum at 30°C (Han et al., 2003). Therefore, at temperatures higher or lower than 30°C, microbial contamination occurred. As a result, more products of protein degradation were produced, and higher ammonia content was generated. It is postulated that a temperature of 30°C and an Rh of 95% could promote the growth of fungi that produces hydrolytic enzymes in PSP tofu. This condition facilitated optimal hydrolysis and minimal protein degradation during the incubation process.

The free amino acid content (FAA, %) of PSP tofu and pehtze incubated at different Rh and temperatures is shown in Figure 2B. The FAA of PSP tofu tended to increase after incubation ($p < 0.05$). The FAA of PSP tofu was 9.94%, which increased to 12.49% for incubation at 95% Rh and 30°C. However, PSP tofu incubated at 35°C contained the lowest FAA values ($p < 0.05$), which was mainly due to the activation of proteolytic and lipolytic enzymes produced by *Actinomucor elegans.* In general, the optimum conditions for the growth rate of *Actinomucor elegans* were 25–30°C and Rh 95–97%. *A. elegans* produced the highest lipase activity (172 U/g pehtze d.m.) after 48 h of incubation at 25°C and RH 95–97% (Han et al., 2003). Thus, the enzyme activity is reduced at incubating temperatures above 30°C. The FAA of PSP tofu was also significantly affected by the Rh (%) ($p < 0.05$). PSP tofu obtained the highest FAA value (10.84%) when incubated at Rh 95% compared to samples incubated at Rh 85% and 75%, which had FAA values of 10.21% and 9.84%, respectively. The determination of free amino acids can accurately reflect hydrolysis (Ma et al., 2013). Studies on the modification of soy

protein before and during maturation have shown that at an early stage, the large protein molecules are degraded to oligopeptides, followed by the gradual release of peptides, free amino acids, and nitrogenous degradation products such as NH_3 (Aidoo and Nout, 2010). Zhao et al. (2017) documented that surimi incubation by *Actinomucor elegans* increased the FAA content by 10 times compared to initial samples after 30 hours of incubation. This result was similar to that of Ma et al. (2013), who reported that total free amino acids and most of the individual amino acids generally increased during sufu manufacturing.

Figure 2C shows the glucose content in PSP tofu and pehtze affected by the different incubating conditions. Generally, glucose content (mg/g) increased after fermentation ($p < 0.05$). The PSP tofu contained 91.41 mg/g glucose, which increased to the highest amount (125.40 ±0.92 mg/g) in PSP pehtze fermented at 30°C and 95% Rh. The total carbohydrate content of soybeans is about 25–30% and mainly includes starch, oligosaccharides, and dietary fiber, of which the oligosaccharides account for 10% of the total carbohydrates (Yan and Dong, 2019). During incubation, *Actinomucor elegans* efficiently produces enzymes such as α-amylase, glucoamylase, and α-galactosidase. These enzymes help to break down large molecules (polysaccharides, dextrin, and oligosaccharides) in tofu and purple sweet potato into glucose and other easily utilized sugars (Zhang et al., 2015; Zhao et al., 2017). Therefore, fermentation conditions of 30°C and 95% Rh could generate a richer nutrient composition, including protein, lipids, free amino acids, and glucose, in PSP tofu, which would be beneficial for the immune systems of consumers.

Effects of temperature and relative humidity on the bioactive compounds of PSP tofu and pehtze

The total phenolic (TPC, mg GAE/g), total flavonoid (TFC, mg QE/g), and anthocyanin (μg/g) contents of PSP tofu and pehtze affected by different temperatures and Rh are reported in Figure 3. The TPC in PSP tofu was 44.33 mg GAE/g, which was significantly increased after incubation ($p < 0.05$) (Fig. 3A). The highest TPC (62.18 mg GAE/g) was found in the PSP pehtze incubated at 30 °C and 95% Rh. Under incubation conditions of 35°C and 75% Rh, the TPC of the PSP pehtze had the lowest value (47.03 mg GAE/g). In plants, phenolics are usually found in conjugated forms, through hydroxyl groups with sugars, as glycosides. The increased total phenolic content in soybean after fermentation may be due to the release of aglycones from the soybean substrate by β-glucosidase produced by fungi (Oh et al., 2012). This result was in agreement with that of Yin et al. (2020), who recorded a significant increase in the TPC in tofu after fermentation by *Actinomucor elegans*. The increased phenolic content in tofu is closely related to the increased activity of β-glucosidase, α-amylase, protease, esterase, exoglucanase, and endoglucanase released by *A. elegans* during fermentation (Yin et al., 2020).

The TFC of PSP tofu increased significantly after fermentation at 25–30°C and an Rh of 95% ($p < 0.05$) (Fig. 3B). However, there was no significant difference in TFC between PSP tofu and pehtze incubated at other temperatures and Rh ($p > 0.05$). Fermented tofu is known as a good source of isoflavones, which belong to the flavonoid family of polyphenols (Yin et al., 2020). PSP solution formulated into tofu was rich in TPC (78.89 mg GAE/g), TFC (33.95 mg QE/g), and anthocyanin (1973.87 μg/g) (data not shown). These components contribute to the high bioactive compound content in PSP tofu. Thus, the increase in TPC observed in PSP pehtze coincided with an increase in the TFC of PSP tofu after fermentation. The current result is in line with that of Cai et al. (2016), who documented that the isoflavone aglycone content of daidzein and genistein in tofu was 0.064 and 0.020 g/100 g, respectively, which increased 0.26- and 0.91-fold during fermentation. By contrast with TPC and TFC, anthocyanin content (μg/g) in PSP tofu significantly decreased after fermentation by *Actinomucor elegans* (Fig. 3C). Before incubation, the anthocyanin content of PSP tofu was 323.93 μg/g. Nevertheless, the anthocyanin content in PSP pehtze was 296.17 μg/g when the PSP tofu was incubated at 35°C and 95% Rh. The higher temperature and Rh caused the greater loss of anthocyanin content. Temperature and Rh are the most important environmental parameters affecting food fermentation. They are closely related to microbial growth and the structural changes in phytochemicals during fermentation. For example, anthocyanin degradation depends on atmospheric conditions such as high temperature and Rh and the presence of oxygen. It is also directly related to the level of pseudobase and

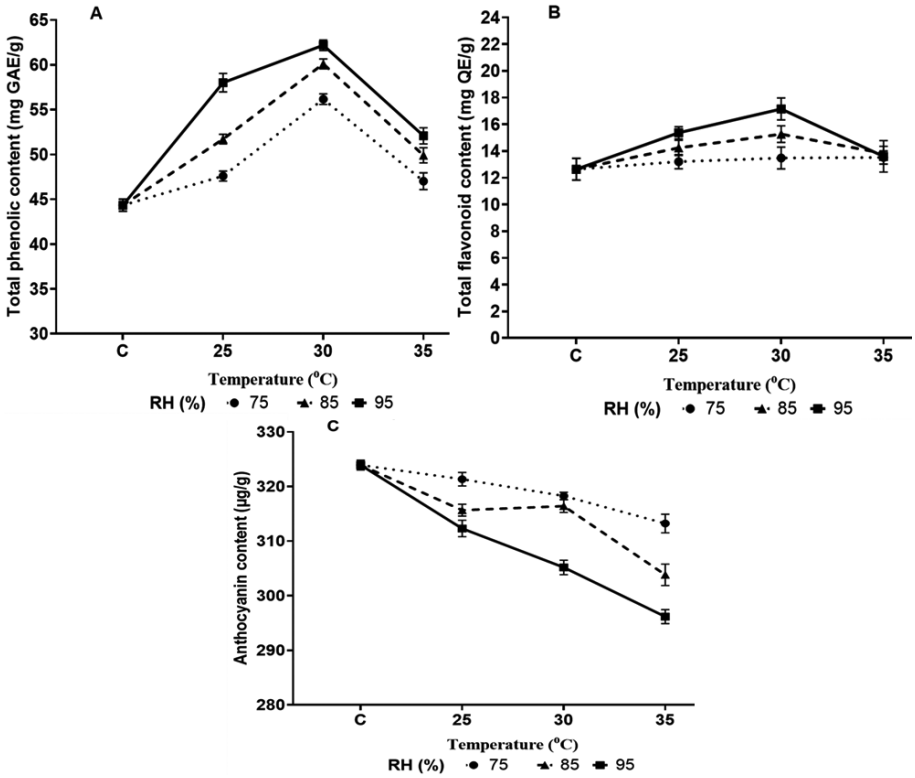

Fig. 3. Total phenolic (A), total favonoid (B), and anthocyanin (C) contents of PSP tofu and pehtze affected by temperature and relative humidity. C: Tofu before fermentation. Bars represent the standard deviation (n = 3)

is inversely related to the cation concentration (Hur et al., 2014). Thus, incubation at 30°C and 95% Rh could maintain bioactive components (phenolic, flavonoid, and anthocyanin compounds) in PSP tofu, which provide many health benefits for humans.

Effects of temperature and relative humidity on the antioxidant activity of PSP tofu and pehtze

Different bioactive compounds may be produced to counteract oxidation through different mechanisms and show different antioxidant activities (Hur et al., 2014). The DPPH radical scavenging capacity (µmol TE/g), ABTS radical scavenging capacity (µmol TEAC/g), and inhibitory activity IC_{50} (mg/mL) of PSP tofu and pehtze affected by temperature and relative humidity are presented in Figure 4. Tofu enriched with PSP solution possessed high antioxidative activity in terms of DPPH radical scavenging capacity and IC_{50} values, which were 84.52 µmolTE/g and 3.76 mg/ mL, respectively (data not shown). Therefore, PSP tofu before fermentation exhibits high DPPH (73.93 µmol TE/g) and ABTS (98.37 µmol TEAC/g) radical scavenging activities, and IC_{50} values (6.87 mg/mL) (Fig. 4). The DPPH and ABTS radical scavenging capacity tended to increase when PSP tofu was fermented by *Actinomucor elegans* (Fig. 4A and 4B, respectively). In particular, PSP pehtze incubated at 30°C and 95% Rh showed the highest DPPH and ABTS radical scavenging activity, at 81.75 µmol TE/g and 120.79 µmol TEAC/g, respectively. Other incubation conditions did not significantly affect the antioxidant activity of the PSP pehtze ($p > 0.05$). Furthermore, the IC_{50} value of the PSP tofu was higher than that of pehtze (Fig. 4C). The lowest IC_{50} value was recorded in PSP pehtze fermented at 30°C and 95% Rh. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of the target radical.

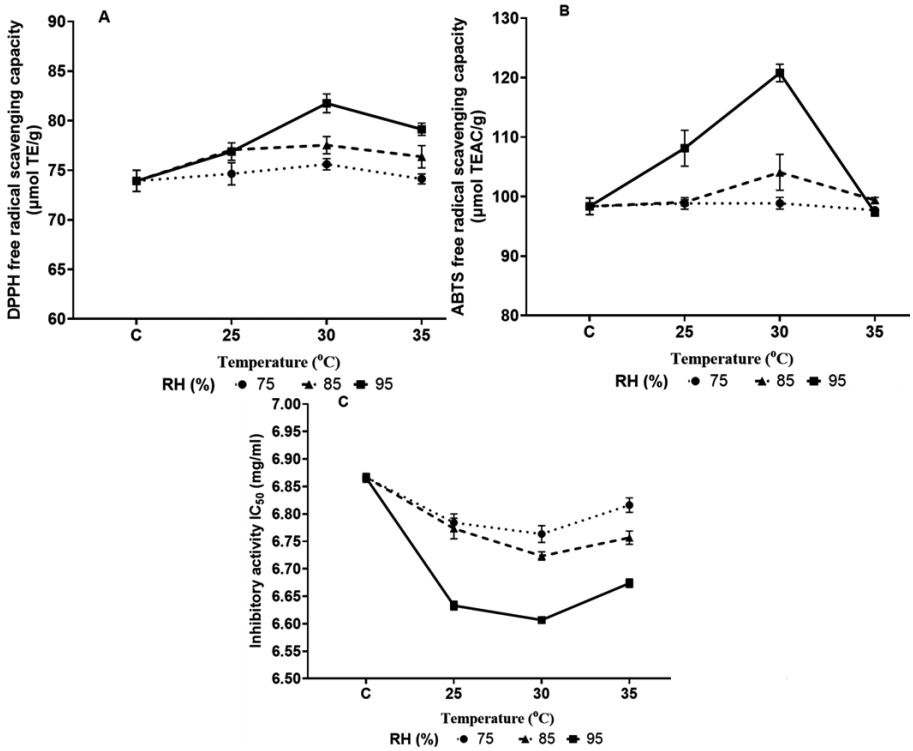

Fig. 4. DPPH radical scavenging capacity (A), ABTS radical scavenging capacity (B), and inhibitory activity IC_{50} (C) of PSP tofu and pehtze affected by temperature and relative humidity. C: Tofu before fermentation. Bars represent the standard deviation (n=3)

The smaller the IC_{50} value, the higher the free radical reduction activity (Li et al., 2009). The increases in antioxidant activity were closely related to antioxidative compounds generated during fermentation such as TPC, TFC, peptides, and amino acids. Several results revealed that the partial protein hydrolysates possessed high antioxidant capacity. Proteolysis exposed hydrophilic amino residue side chain groups for small peptides, resulting in more opportunity to chelate the water-soluble ABTS radical cation than large molecular peptides with fewer hydrophilic residue side chains (Cai et al., 2016; Sanjukta and Rai, 2016). Yin et al. (2020) found similar results when tofu was fermented with *Actinomucor elegans*. These results are in line with the higher amount of bioactive compounds generated under the optimum conditions of 30°C and 95% Rh. The results indicate that fermenting PSP tofu (pehtze) at 30°C and 95% Rh could enhance its antioxidant activity and contribute to various therapeutic properties.

Effects of temperature and relative humidity on the sensory properties of PSP tofu and pehtze

The quantitative testing data obtained were analyzed using Principal Component Analysis (PCA). PCA was used to determine the pattern or grouping of pehtze samples based on appearance, texture, and flavor attributes. These include ten attributes consisting of six appearance attributes (cracking, surface puffing, silk grown on the surface, yellow, darkening, and purple), two texture attributes (hardness and mushy), and two flavor attributes (strong musty smell and stink). In addition, the "general quality" of the samples was evaluated to serve as a basis for determining good treatment.

Figure 5 shows that the data variance is approximately 40.56% from F1 and 25.56% from F2. The "general quality" was located in quadrant 1, which contained the best samples of (30; 95) and (25; 95), which had purple color and hardness attributes. The "general quality" displayed the most significance on the first principal component axis and is located in

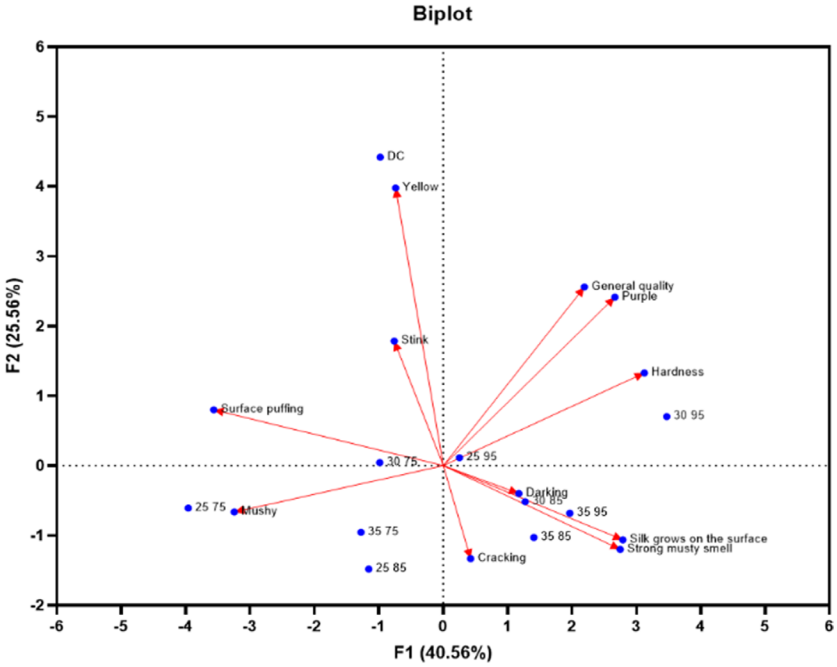

Fig. 5. Correlation between sensory properties of pehtze according to temperature and relative humidity

the positive direction of this axis, so it also represented other attributes belonging to quadrant 2, such as darkening, silk grown on the surface, and cracking. Samples in the region (30; 85), (35; 95), and (35; 85) showed relatively good quality. The other samples, located in quadrants 3 and 4, showed poor quality attributes such as surface puffing, mushy, stinky, and yellow color. The control sample was also in this region.

CONCLUSIONS

The nutrient composition, antioxidative activity, and sensory properties of fermented PSP tofu were affected by temperature and Rh. After fermentation, the moisture, protein, lipid, and anthocyanin contents decreased, while TPC, TFC, FAA, glucose content, and antioxidant activity (DPPH radical scavenging capacity, ABTS radical scavenging capacity, and inhibitory activity IC_{50}) increased. PSP tofu fermented with *A. elegans* at 30°C and a Rh of 95% maintains its nutrient composition and yields the most bioactive compounds and highest antioxidant activity, which could contribute to health benefits for customers.

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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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REFERENCES

Abbasi, S., Mousavi, S., Mohebbi, M., Kiani, S. (2009). Effect of time and temperature on moisture content, shrinkage, and rehydration of dried onion. Iran. J. Chem. Chem. Eng., 6(3), 57–70.

Aidoo, K.E., Nout, R. (2010). Functional yeasts and molds in fermented food and beverages. Fermented foods and beverages of the world. Boca Raton: CRC Press/Taylor & Francis.

Alam, M. N., Bristi, N. J., Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi. Pharm. J., 21(2), 143–152. https://doi.org/10.1016/j.jsps.2012.05.002

AOAC (2000). Official Methods of Analysis 17th ed. Washington DC: J. Assoc. Off. Anal. Chem.

Bach, D., Bedin, A. C., Lacerda, L. G., Nogueira, A., Demiate, I. M. (2021). Sweet potato (*Ipomoea batatas* L.): a versatile raw material for the food industry. Braz. Arch. Biol. Techn., 64, 1–14. https://doi.org/10.1590/1678-4324-2021200568

Bei, Q., Chen, G., Lu, F., Wu, S., Wu, Z. (2018). Enzymatic action mechanism of phenolic mobilization in oats (*Avena sativa* L.) during solid-state fermentation with *Monascus anka*. Food Chem., 245, 297–304. https://doi.org/10.1016/j.foodchem.2017.10.086

Bhanja, T., Kumari, A., Banerjee, R. (2009). Enrichment of phenolics and free radical scavenging property of wheat koji prepared with two filamentous fungi. Bioresour. Technol., 100(11), 2861–2866. https://doi.org/10.1016/j.biortech.2008.12.055

Cai, R. C., Li, L., Yang, M., Cheung, H. Y., Fu, L. (2016). Changes in bioactive compounds and their relationship to antioxidant activity in white sufu during manufacturing. Int. J. Food Sci. Tech., 51(7), 1721–1730. https://doi.org/10.1111/ijfs.13149

Cheng, Y. Q., Hu, Q., Li, L. Te, Saito, M., Yin, L. J. (2009). Production of sufu, a traditional Chinese fermented soybean food, by fermentation with *Mucor flavus* at low temperature. Food Sci. Technol. Res., 15(4), 347–352. https://doi.org/10.3136/fstr.15.347

Fu, Z. F., Tu, Z. C., Zhang, L., Wang, H., Wen, Q. H., Huang, T. (2016). Antioxidant activities and polyphenols of sweet potato (*Ipomoea batatas* L.) leaves extracted with solvents of various polarities. Food Biosci., 15, 11–18. https://doi.org/10.1016/j.fbio.2016.04.004

Han, B. Z., Ma, Y., Rombouts, F. M., Nout, M. J. R. (2003). Effects of temperature and relative humidity on growth and enzyme production by *Actinomucor elegans* and *Rhizopus oligosporus* during sufu pehtze preparation. Food Chem., 81(1), 27–34. https://doi.org/10.1016/S0308-8146(02)00347-3

Han, B. Z., Rombouts, F. M., Nout, M. J. R. (2004). Amino acid profiles of sufu, a Chinese fermented soybean food. J. Food Compos. Anal., 17(6), 689–698. https://doi.org/10.1016/j.jfca.2003.09.012

Handa, C. L., de Lima, F. S., Guelfi, M. F. G., Fernandes, M. da S., Georgetti, S. R., Ida, E. I. (2019). Parameters of the fermentation of soybean flour by *Monascus purpureus* or *Aspergillus oryzae* on the production of bioactive compounds and antioxidant activity. Food Chem., 271, 274–283. https://doi.org/10.1016/j.foodchem.2018.07.188

Huang, Z., He, W., Zhao, L., Liu, H., Zhou, X. (2021). Processing technology optimization for tofu curded by fermented yellow whey using response surface methodology. Food Sci. Nutr., 9(7), 3701–3711. https://doi.org/10.1002/fsn3.2331

Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I., Kim, G. B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. Food Chem., 160, 346–356. https://doi.org/10.1016/j.foodchem.2014.03.112

ISO (2005). Determination of amino acids content. In: Animal Feeding Stuffs. Geneva, Switzerland: The International Organization for Standardization, 13903.

ISO (2013). Determination of the nitrogen content and calculation of the crude protein content Kjeldahl method. In: Cereals and Pulses. Geneva, Switzerland: The International Organization for Standardization, 20483.

Jiang, S., Cai, W., Xu, B. (2013). Food quality improvement of soy milk made from short-time germinated soybeans. Foods, 2(2), 198–212. https://doi.org/10.3390/foods2020198

Joo, K. H., Kerr, W. L., Cavender, G. A. (2023). The Effects of Okara ratio and particle size on the physical properties

and consumer acceptance of tofu. Foods, 12(16), 3004. https://doi.org/10.3390/foods12163004

Khan, H., Farid Khan, M., Ali Khan, B., Wahab, A., Umer Jan, S., …, Khan, D. I. (2012). Oxidation of glutathione (GSH) in blood plasma due to oxidative stressors: A case study of silver. Afr. J. Pharm. Pharmacol., 6(21), 1502–1507. https://doi.org/10.5897/ajpp11.790

Li, X., Wu, X., Huang, L. (2009). Correlation between antioxidant activities and phenolic contents of radix *Angelicae sinensis* (Danggui). Molecules, 14(12), 5349–5361. https://doi.org/10.3390/molecules14125349

Liu, X., Zhao, X. H. (2017). Immune potentials of the *Mucor*-fermented Mao-tofu and especially its soluble extracts for the normal mice. Food Agr. Immunol., 28(5), 859–875. https://doi.org/10.1080/09540105.2017.1318834

Ma, Y. L., Wang, J. H., Cheng, Y. Q., Yin, L. J., Li, L. Te. (2013). Some biochemical and physical changes during manufacturing of grey sufu, a traditional Chinese fermented soybean curd. Int. J. Food Eng., 9(1), 45–54. https://doi.org/10.1515/ijfe-2012-0204

Mao, C., He, G., Du, X., Cui, M., Gao, S. (2013). Biochemical changes in the fermentation of the soy sauce prepared with bittern. Adv. J. Food Sci. Technol., 5(2), 144–147. https://doi.org/10.19026/AJFST.5.3234

Ngcobo, A., Mianda, S. M., Seke, F., Sunette, L. M., Sivakumar, D. (2024). Phytonutritional composition and antioxidant properties of southern African, purple-fleshed sweet potato (Ipomoea batatas L.) storage roots. Antioxidants, 13(3), 338. https://doi.org/10.3390/antiox13030338

Oh, Y. S., Hwang, J. H., Lim, S. Bin. (2012). Physiological activity of tofu fermented with mushroom mycelia. Food Chem., 133(3), 728–734. https://doi.org/10.1016/j.foodchem.2012.01.083

Roudaut, G., Debeaufort, F. (2010). Moisture loss, gain and migration in foods and its impact on food quality. In: L.H. Skibsted, J. Risbo and M. L. Andersen (Eds), Chemical deterioration and physical instability of food and beverages (pp. 143–185). Sawston, UK: Woodhead Publishing.

Sakshy, S., Paras, S. (2009). Comparative study of *cuscuta reflexa* and *cassytha filiformis* for diuretic activity. Phcog. Res., 1, 327–330.

Sanjukta, S., Rai, A. K. (2016). Production of bioactive peptides during soybean fermentation and their potential health benefits. Trends Food Sci. Technol., 50, 1–10. https://doi.org/10.1016/j.tifs.2016.01.010

Soccol, C. R., Costa, E. S. F. da, Letti, L. A. J., Karp, S. G., Woiciechowski, A. L., Vandenberghe, L. P. de S. (2017). Recent developments and innovations in solid state fermentation. Biotechnol. Res. Innov., 1(1), 52–71. https://doi.org/10.1016/j.biori.2017.01.002

Sun, H., Mu, T., Liu, X., Zhang, M., Chen, J. (2014). Purple sweet potato (*Ipomoea batatas* L.) anthocyanins: Preventive effect on acute and subacute alcoholic liver damage and dealcoholic effect. J. Agric. Food Chem., 62(11), 2364–2373. https://doi.org/10.1021/JF405032F

Torres-Penaranda, A. V., Reitmeier, C. A. (2001). Sensory descriptive analysis of soymilk. J. Food Sci., 66(2), 352–356. https://doi.org/10.1111/J.1365-2621.2001.tb11345.x

Wrolstad, R. E., Culver, C. A. (2012). Alternatives to those artificial FD & C food colorants. Annu. Rev. Food Sci. Technol., 3(1), 59–77. https://doi.org/10.1146/annurev-food-022811-101118

Xie, C., Zeng, H., Li, J., Qin, L. (2019). Comprehensive explorations of nutritional, functional and potential tasty components of various types of sufu, a Chinese fermented soybean appetizer. Food Sci. Technol. (Brazil), 39, 105–114. https://doi.org/10.1590/fst.37917

Yan, S., Dong, D. (2019). Main parameters and the dynamics of volatile compounds during the fermentation of Chinese *Mao-tofu* from Huangshan region. Food Sci. Biotechnol., 28(5), 1315–1325. https://doi.org/10.1007/S10068-019-00581-0

Yin, L. J., Li, L. Te, Liu, H., Saito, M., Tatsumi, E. (2005). Effects of fermentation temperature on the content and composition of isoflavones and beta-glucosidase activity in sufu. Biosci. Biotechnol. Biochem., 69(2), 267–272. https://doi.org/10.1271/bbb.69.267

Yin, L., Zhang, Y., Wu, H., Wang, Z., Dai, Y., Zhou, J., Liu, X., Dong, M., Xia, X. (2020). Improvement of the phenolic content, antioxidant activity, and nutritional quality of tofu fermented with *Actinomucor elegans*. Food Sci. Technol., 133, 110087. https://doi.org/10.1016/j.lwt.2020.110087

Zhang, Z., Lei, M., Liu, R., Gao, Y., Xu, M., Zhang, M. (2015). Evaluation of alliin, saccharide contents and antioxidant activities of black garlic during thermal processing. J. Food Biochem., 39(1), 39–47. https://doi.org/10.1111/jfbc.12102

Zhao, D., Lu, F., Gu, S., Ding, Y., Zhou, X. (2017). Physicochemical characteristics, protein hydrolysis, and textual properties of surimi during fermentation with Actinomucor elegans. Int. J. Food Prop., 20(3), 538–548. https://doi.org/10.1080/10942912.2016.1168834

Zhao, J. G., Yan, Q. Q., Lu, L. Z., Zhang, Y. Q. (2013). In vivo antioxidant, hypoglycemic, and anti-tumor activities of anthocyanin extracts from purple sweet potato. Nutr. Res. Pract., 7(5), 359–365. https://doi.org/10.4162/NRP.2013.7.5.359