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# COMPARATIVE ANALYSIS OF SENSORY PROFILES OF TEMPEH PREPARED WITH AMARANTH vs. SOY USING *Rhizopus oligosporus* AS INOCULUM

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## ABSTRACT

The study aimed to develop a *Rhizopus oligosporus* starter culture for using it in the preparation of amaranth-based and soy-based tempeh and to compare their resulting sensory profiles using the Rate-All-That-Apply (RATA) technique. In this study, the *Rhizopus oligosporus* inoculum for tempeh preparation was produced by Solid State Fermentation in rice. During inoculum production, the effects of initial pH (4.5, 5.0, 5.5, 6.0, and 6.5) and initial temperature (25, 30, 35, and 40 °C) were determined. Products generated during fungal metabolism were analyzed: organic acid content by titration, lactic acid production by spectroscopy, and the final pH of the medium. Once the appropriate conditions for inoculum production were determined, a comparative analysis of the sensory profiles of amaranth-based and soy-based tempeh was performed using the Rate That Apply (RATA) technique. The results showed that the highest lactic acid production was obtained when the initial pH of the medium was adjusted to pH 5.5 (1.75 g $L^{-1}$) and at 40 °C (1.85 g $L^{-1}$). Likewise, the highest organic acid production was observed at an initial pH of 5.0 (0.066 g $L^{-1}$), pH 6.0 (0.105 g $L^{-1}$), and at 35 °C (0.69 g $L^{-1}$). Different sensory profiles were observed. The soy-based tempeh differed from the amaranth-based tempeh in its fishy odor and flavor. Therefore, the lactic acid content, organic acids, and final pH of the medium are influenced by the initial pH and temperature of the medium. The sensory profile of tempeh is influenced by the raw materials used in its preparation.

**Keywords:** fermentation, inoculum, amaranth, tempeh, sensory profile.

## INTRODUCTION

The plant-based protein market is experiencing constant growth, driven by an increasing consumer demand for more sustainable, healthy, and innovative options (Das *et al*., 2021). According to Mintel's global database, the plant-based protein market is projected to reach $160 billion USD by 2030 (Mintel, 2025). Amaranth has emerged as a promising alternative within the plant-based protein market due to its high-quality protein content. This grain is recognized for its complete profile of essential amino acids, as well as its mineral and fiber content (Das *et al*., 2021). Studying the diversification of amaranth uses is a strategy to leverage the nutritional benefits of this grain. Currently, various strategies are being explored to promote the diversification of amaranth-based products with the aim of integrating it into the human diet (FAO, 2024). A new alternative for diversifying amaranth-based products is incorporating this grain into tempeh preparation. Amaranth's nutritional properties can significantly enhance the product, including calcium, iron, potassium, vitamins B1, B2, B3, and B6, 14–20 % fiber, 7–9 % fat and 14–18 % protein (Mătieș *et al*., 2024).

In 2023, the global tempeh market was valued at $5.17 billion USD, and sustained growth of 5.8 % annually is projected through 2030 (Grand View Research, 2023). This traditional Indonesian food is traditionally prepared from soybeans fermented with fungi of the genus *Rhizopus* and is consumed as an accessible and inexpensive source of high-quality protein (Do Prado *et al*., 2021). Tempeh has been recognized with multiple health benefits, such as glycemic control, antioxidant effects, antidiabetic effects, blood glucose reduction and cholesterol reduction (Do Prado *et al*., 2021; Prativi *et al*., 2023). The tempeh production process is considered an economical and sustainable technology that allows to produce protein-rich foods using various legumes or grains (Ahnan-Winarno *et al*., 2021).

Currently, it has been shown that the use of *Rhizopus oligosporus* in fermented products improves their sensory properties compared to other species of the same genus, such as *Rhizopus oryzae* and *Rhizopus delemar* (Wikandari *et al*., 2021). Therefore, commercial inoculants for tempeh production typically contain *Rhizopus oligosporus* spores mixed with rice flour, rice bran, or wheat bran (Codex Alimentarius, 2013). The interaction between the grain and the fungus results in the characteristics of tempeh, which are usually analyzed through sensory evaluation. In their study, Tan *et al*. (2024), used a 9-point hedonic scale, evaluated the liking and acceptability of soy tempeh, demonstrated that appearance, flavor, aroma, and texture impact consumer acceptability. Mahdi *et al*. (2023), using the Rate-All-That-Apply (RATA) technique, they identified the main attributes of tempeh, such as white color, mushroom odor, bean odor, umami flavor, and bitter flavor.

Tempeh production requires the availability of *Rhizopus oligosporus* inoculum. During fermentation for inoculum production, byproducts such as organic acids and $CO_2$ are generated. Studying fungal metabolic byproducts allows for the identification of suitable inoculum production conditions. Tempeh prepared from amaranth may have a different sensory profile than that prepared from soy, given the differences in their chemical composition. The present study aimed to develop a *Rhizopus oligosporus* starter culture for using it in the preparation of amaranth-based and soy-based tempeh and to compare their resulting sensory profiles using the Rate-All-That-Apply (RATA) technique.

## MATERIALS AND METHODS

### Material

Medium-grain rice, Cíes® brand (PROMEXA, Veracruz, Mexico), purchased at a local supermarket in downtown Córdoba, Veracruz was used as substrate for inoculum development. Amaranth grain was of the species *Amaranthus cruentus* cultivated in San Mateo Coatepec, Puebla, Mexico, during 2022. Soybeans were purchased at the local supermarket in Córdoba, Veracruz, Mexico.

### Microorganism

The fungal strains of *Rhizopus oligosporus* were obtained according to the method proposed by Kim *et al*. (2013): 200 g of rice were soaked for 24 h. After reaching 80 % moisture, grains were incubated for 7 days at 30 °C. The fermented substrate was dried at 40 °C in an incubator (model 21-250ER, Quincy Lab, USA) for 24 h and pulverized in a cyclonic mill (model 310-014, UDY, Rome Court, USA). For the cultivation stage, the powder was spread onto PDA plates and incubated for 4 days at 25 °C. Finally, the cultures were subcultured in 250 mL Pyrex® flasks containing 50 mL of PDA and incubated for 5 days at 25 °C to obtain a pure culture.

For the strain preservation the fungal cultures were inoculated into Pyrex® test tubes containing 8 mL of Potato Dextrose Agar (PDA) medium and incubated for 48 h at 30 °C. The strain was harvested by adding 5 mL of 0.1 % Tween 20 sterile solution to the fungal cultures, then the mixture was vortexed (UNICO, L-VM1000, USA). A 0.5 mL aliquot of this suspension was transferred to Pyrex® flasks containing 50 mL of PDA medium and incubated for 6 days at 25 °C (Wikandari, 2021). Spores were harvested using a 0.1 % Tween 20 sterile solution and transferred to 250 mL Pyrex® glass flasks containing 20 mL of previously sterilized 10 % Svelty® nonfat dry milk. The mixture was stirred on a magnetic stirrer (model PC-353 Stirrer, Corning, USA). The suspension was transferred to 50 mL Falcon® tubes and homogenized using a vortex mixer. The fungi suspensions were frozen at-70 °C in a deep freezer and then freeze-dried. The stock cultures were preserved in 2 mL cryovials at -20 °C until use.

### Inoculum

From the stock culture preserved in Svelty® skim milk, the inoculum for solid-state fermentation was produced at 30 °C for 4 days in 250 mL Pyrex® flasks containing 50 mL of PDA medium. Spores were harvested using 0.1 % Tween 20 and stored in 50 mL Corning® centrifuge tubes until used as inoculum for solid-state fermentation.

### Substrate

Rice was pre-conditioned for use as a substrate during solid-state fermentation to produce inoculum for tempeh preparation. It was washed and soaked (1:1 rice:water ratio) for 1 h, then cooked for 3 min in boiling water and centrifuged using a manual food centrifuge.

### Solid-State Fermentation

Solid-state fermentation (SSF) was carried out using rice as support/substrate in trays. The SSF conditions were adjusted at 1.5 cm bed height, 60 % moisture, and 1 % v/w inoculum ($1 \times 10^6$ spores $mL^{-1}$ concentration). Samples of the fermented substrate were taken and stored at –20 °C until needed for the determination of lactic acid, organic acids, and final pH. To produce the inoculum intended for tempeh preparation, the fermented substrate was freeze-dried for three days. Two grams of inoculum were packaged in vacuum-sealed bags and stored at –20 °C until needed for tempeh production.

### Determination of lactic acid, organic acids, and final pH analysis

Samples of rice fermented with *Rhizopus oligosporus* were ground in a mortar and suspended in distilled water at a 1:10 ratio (rice: water). The mixture was stirred and centrifuged at 1784 x g for 15 min. The supernatant was decanted and used for the quantification of lactic acid, organic acids, and final pH analysis.

### Lactic Acid

The lactic acid content was analyzed using the spectrophotometric method (Borrshchevskaya, 2016). To analyze the lactic acid content in the fermented substrate, 50 $\mu$L of the supernatant sample was added to 2 mL of ferric chloride ($FeCl_3$) solution. The lactic acid concentration was calculated using the lactic acid standard curve with a detection range of 0.078–10.0 g $L^{-1}$. The analysis was performed in triplicate.

### Organic acids

The determination of organic acids was performed according to the methodology proposed by Paul *et al*. (2010). The analysis was performed in triplicate.

### Final pH

The pH determination was carried out according to AOAC 943.02/90. 10 mL of the supernatant sample were used, and the reading was taken with a potentiometer (model HI98103, Hanna Instruments®, México). The analysis was performed in triplicate.

### Respiratory Analysis

The growth of *Rhizopus oligosporus* was monitored in two phases to identify when the exponential phase occurred. In the first phase, $CO_2$ levels were monitored for 35 h without adjusting the pH or temperature of the medium. In the second phase, monitoring was performed under appropriate pH and temperature conditions that allowed for a higher production of lactic acid and organic acids as determined in the first phase.

To measure $CO_2$ release an SCD40 $CO_2$ sensor, an I2C digital interface with a digital output signal, and an integrated temperature and humidity sensor were used. The sensor allowed monitoring of $CO_2$ release over time, avoiding costly procedures. The sensor was integrated with an Arduino UNO board and a data logger base (TZT, China). Temperature, relative humidity, and $CO_2$ levels were recorded every 20 min. The data was stored on a 64 GB MicroDrive® SD card.

### Effect of pH on the production of lactic acid, organic acids, and final pH

The effect of pH on the production organic acids was evaluated by adjusting the substrate to values of 4.5, 5.0, 5.5, 6.0, and 6.5.

### Effect of temperature on the production of lactic acid, organic acids, and final pH

Solid-state fermentation with rice was carried out at temperatures of 25, 30, 35, and 40 °C to evaluate its effect on the production of organic acids, and final pH of the substrate.

### Tempeh Production

For tempeh preparation, the inoculum used was produced by solid-state fermentation of rice with *Rhizopus oligosporus*. The substrates were pre-conditioned for use in tempeh preparation.

### Soybeans preconditioning

The soybeans were soaked for 24 h. The hulls were removed and the beans were cooked for 40 min and then cooled to room temperature. The thickness of their hulls is between 70-230 $\mu$m, being a thick layer, the soaking and cooking process facilitates its removal (Lemes & Catão, 2024). The hull is usually removed to facilitate the penetration of *Rhizopus oligosporus* and to allow it to obtain the necessary nutrients for its growth.

### Amaranth

Amaranth grains were soaked for 24 h. They were then cooked for 5 min without removing the hull and allowed to cool to room temperature. The amaranth hull was not removed because the amaranth grain is very small, the thickness of its hull is between 3.7–50 $\mu$m. Unlike soybeans, the amaranth hull is thinner and is attached to the endosperm, which prevents its detachment during soaking or cooking (Ninfali *et al*., 2020).

### Preparation of Soy and Amaranth Tempeh

For tempeh preparation, 400 g of conditioned amaranth and 400 g of conditioned soybeans batches were used, inoculated with 2 g of *Rhizopus oligosporus* inoculum and incubated for 12 h at 30 °C. Finally, the amaranth-based tempeh and the soy-based tempeh (Figure 1) were stored at –20 °C until used for sensory evaluation.

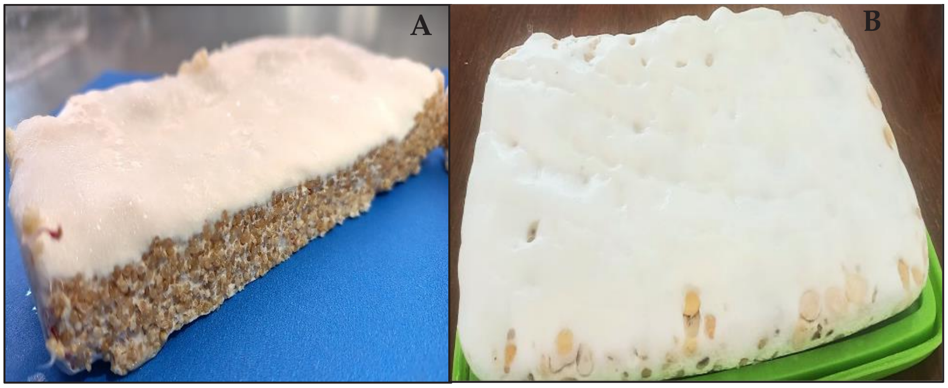

**Figure 1.** Tempeh prepared with amaranth and soy. A: amaranth tempeh; B: soy tempeh.

### Protein Determination

The protein content was determined for soybeans and for raw, unconditioned soybeans. It was also determined for amaranth-based tempeh and soy-based tempeh. The analysis was performed on a dry basis using the Kjeldahl method according to AOAC 955.04. The tests were performed in triplicate.

### Sensory testing

### Sample Preparation

Sample size used for sensory testing were 2.5 x 2.5 x 2 cm tempeh cubes for soy (15 g) and amaranth (12 g). Cubes were baked for 50 min at 160 °C. Subsequently, each cube was placed in 60 mL souffle disposable containers and kept at room temperature prior to consumer testing.

### Obtaining Attributes

For soy tempeh, 12 attributes were obtained for sight, 20 for smell, 18 for taste, and 10 for touch. For amaranth tempeh, 20 attributes were obtained for sight, 13 for smell, 12 for taste, and 9 for touch. To reduce the number of attributes, a refinement process was carried out to eliminate synonyms or words irrelevant to the product.

To select the most representative attributes of soy and amaranth tempeh, the CATA method was used. Participants were presented with a Google® Forms questionnaire containing the list of attributes and instructed to select all those that applied to the product. The selection of representative attributes for amaranth and soy tempeh was based on attribute frequencies. A binomial test was performed to determine the attributes with frequencies significantly different from 0 (H0: frequency = 0; Ha: frequency ≠ 0). The attributes that were significant ($p < 0.05$) were selected, included in the attribute list and used to perform the following evaluation of amaranth-based and soy-based tempeh.

### Rate-All-That-Apply (RATA)

The Rate-All-That-Apply (RATA) technique was used to obtain the sensory profiles of soy-based and amaranth-based tempeh. The evaluation was carried out at the Technological University of Central Veracruz. 75 consumers participated, including students and academics from the municipalities of Amatlán de los Reyes, Atoyac, Carrillo Puerto, Córdoba, Coscomatepec, Cosolapa, Cuichapa, Yanga, Cuitláhuac, Fortín, Omealca, Orizaba, Paso del Macho, Potrero, Tezonapa, and Tierra Blanca.

### Questionnaire Design

The first section of the questionnaire included questions related to demographic information. The second and third sections contained the space for the sample code and the list of attributes, which included: five for the sense of sight, eight for the sense of smell, eight for the sense of taste, and four for the sense of touch. A scale of 1 to 7 was used to collect the intensity values for the perceived attributes.

### Statistical analysis

### Statistical analysis of inoculum collection data.

Two Completely Randomized Designs (CRDs) were performed for: 1) initial medium pH with five levels (4.5, 5.0, 5.5, 6, and 6.5) and 2) temperature with four levels (25, 30, 35, and 40 °C). Each treatment was evaluated in three replicates. The response variables evaluated in both (initial medium pH and temperature) were lactic acid, organic acids, and final medium pH. Data was analyzed using one-way ANOVA. Mean comparisons were performed using Tukey's test to observe significant differences ($p < 0.05$) in lactic acid production, organic acids, and final pH.

### Analysis for RATA test data

Two treatments were evaluated: amaranth-based tempeh and soy-based tempeh. Two approaches were used for data analysis: 1) as binomial data and 2) as rating data. For the first approach, the results were transformed into a binomial scale. All non-zero results were converted to 1. A 0 indicated absence and a 1 indicated presence of the attribute. Subsequently, the results were analyzed using the chi-square ($\chi^2$) test to determine if there was an association between the raw material and the perceived attributes of soy-based tempeh versus amaranth-based tempeh. For the second approach, a t-test was performed to compare the intensity of the attributes between the sensory profiles of amaranth and soy (Meyners *et al*., 2016).

The significance level for the study was $\alpha = 0.05$. The data were processed using R version 4.5.0 ucrt with RStudio version 2025.05.0 Build 496.

## RESULTS AND DISCUSSION

### Respirometry without defined pH and temperature parameters

The $CO_2$ profile recorded during solid-state fermentation reflected the *Rhizopus oligosporus* growth under uncontrolled conditions (Figure 2).

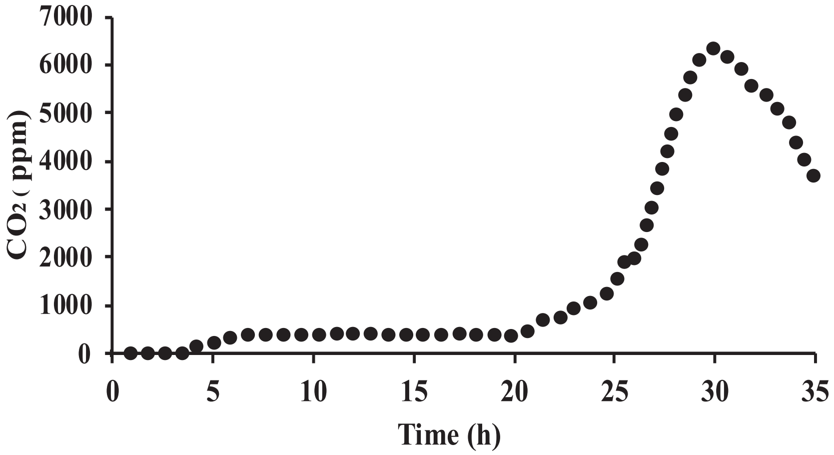

**Figure 2.** $CO_2$ profile without defined pH and initial temperature parameters. The $CO_2$ profile represents the metabolic activity of *Rhizopus oligosporus* over 35 h.

The peak of exponential fungal growth occurred at 30 h, indicating that solid-state fermentation should be stopped. After this time, $CO_2$ release began to decrease, indicating a slowdown in fungal metabolism. The decrease in $CO_2$ may indicate nutrient depletion, where the fungus was limited in resources to continue its growth (Sandoval *et al*., 2024). Therefore, solid-state fermentations to produce inoculum for tempeh preparation were maintained for 30 h. Based on this, some of the physiological products generated during fungal metabolism, such as $CO_2$ and organic acids, were analyzed.

### Effect of adjusting the initial pH of the medium on the production of organic acids

The data obtained (Table 1) indicated that the initial pH of the medium had a significant effect ($p < 0.05$) on organic acids content, and the decrease in pH at the end of solid-state fermentation with rice.

The lowest lactic acid production values at the end of fermentation occurred at pH 5 (1.34 g $L^{-1}$) and pH 6 (1.32 g $L^{-1}$), whereas the highest values were reached at pH 5.5 (1.75 g $L^{-1}$) and pH 6.5 (1.51 g $L^{-1}$). Therefore, adjusting the pH at the start of fermentation increased lactic acid production. Lactic acid content began to increase from pH 4.5 and gradually increased up to pH 5.5.

**Table 1.** Effect of initial pH on organic acid production and final pH of the medium at the end of solid-state fermentation.[†]

| Initial pH | Lactic acid ( g L$^{1}$) | Organic acids (g L$^{-1}$) | Final pH |
|---|---|---|---|
| 4.5 | 1.40 ± 0.145 b | 0.126 ± 0.041 c | 4.53 ± 0.208 ab |
| 5.0 | 1.34 ± 0.076 b | 0.066 ± 0.014 c | 5.16 ± 0.252 a |
| 5.5 | 1.75 ± 0.020 a | 0.675 ± 0.024 a | 3.76 ± 0.551 b |
| 6.0 | 1.32 ± 0.065 b | 0.105 ± 0.027 c | 4.46 ± 0.416 ab |
| 6.5 | 1.51 ± 0.158 ab | 0.375 ± 0.019 b | 4.50 ± 0.265 ab |
| P-value | 0.00366 | <0.0001 | 0.0122 |

[†] Means ± standard deviation with different letters in each column indicate significant differences between treatments (Tukey, $p \leq 0.05$); n= 3.

At the end of fermentation, the highest organic acid content was observed at pH 5.5 (0.675 g L$^{-1}$) and pH 6.0 (0.105 g L$^{-1}$). The pH values 4.5 and 5 remained constant throughout fermentation, whereas pH values 5.5, 6, and 6.5 showed a decrease over the process. This pH reduction suggests higher acidification of the medium associated with increased production of organic acids. These results indicate that *Rhizopus oligosporus* grows adequately using a pH of 5.5, which is ideal for obtaining higher organic acids production.

### Effect of temperature adjustment on the production of lactic acid, organic acids, and final pH of the medium

As expected, the results obtained indicated that temperature had a significant effect ($p < 0.05$) on organic acids content, and the decrease in medium pH during fermentation with *Rhizopus oligosporus* (Table 2).

The highest lactic acid production occurred at 35 and 40 °C, with yields of 1.69 and 1.85 g L$^{-1}$, respectively. A correlation was observed where higher temperatures resulted in

**Table 2.** Effect of temperature on organic acid production and final pH of the medium at the end of solid-state fermentation.[†]

| Temperature (°C) | Lactic Acid (g L$^{-1}$) | Organic acids (g L$^{-1}$) | Final pH |
|---|---|---|---|
| 25 | 1.57 ± 0.036 c | 0.05 ± 0.005 d | 4.5 ± 0.05 a |
| 30 | 1.69 ± 0.031 b | 0.57 ± 0.036 c | 4.2 ± 0.0 b |
| 35 | 1.75 ± 0.050 b | 0.96 ± 0.027 a | 4.2 ± 0.0 b |
| 40 | 1.85 ± 0.021 a | 0.69 ± 0.014 b | 4.6 ± 0.05 a |
| P-value | <0.0001 | <0.0001 | <0.0001 |

[†] Means ± standard deviation with different letters in each column indicate statistical differences between treatments (Tukey, $p \leq 0.05$); n= 3.

higher lactic acid production. The highest organic acid production occurred at 35 °C, with a yield of 0.966 g $L^{-1}$. However, production decreased above 40 °C. Although the initial pH for all four incubation temperatures was 5.5, a lower pH was recorded at 30 °C and 35 °C. These results demonstrate that *Rhizopus oligosporus* is a microorganism tolerant to high temperatures and capable of producing lactic acid at 40 °C.

The production of organic acids is affected by pH and temperature. The results indicated that higher production rates of lactic acid and organic acids are obtained at a pH of 5.5 and a temperature of 40 °C. These conditions are considered suitable for producing the inoculum intended for tempeh preparation. The growth curve of *Rhizopus oligosporus* was also plotted under these conditions.

## Respirometry with selected pH and temperature conditions

The growth curve of *Rhizopus Oligosporus* presents a change over 30 h with an initial pH of 5.5 and a temperature of 40 °C (Figure 3). *Rhizopus oligosporus* behaved differently under adequate pH and temperature conditions, exhibiting higher $CO_2$ rates in a shorter time.

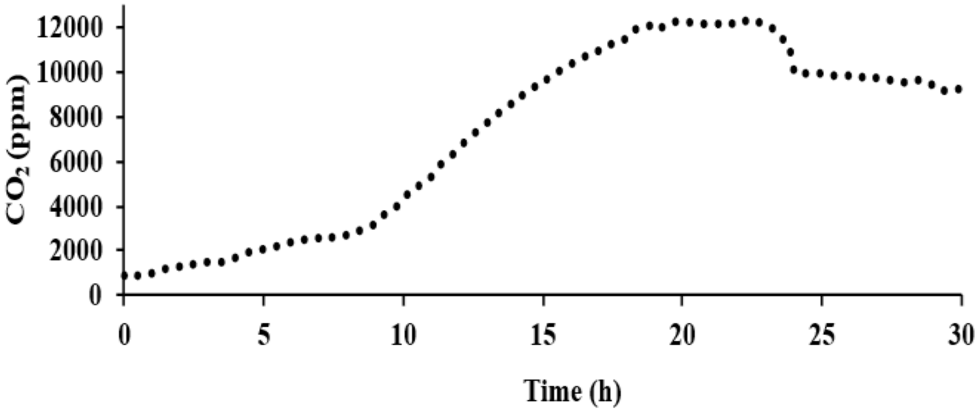

**Figure 3.** $CO_2$ profile under controlled conditions of pH 5.5 and temperature 40 °C. The $CO_2$ profile represents the metabolic activity of over 30 h.

It showed that the exponential phase began at 8 h. The peak of exponential fungal growth, with the highest $CO_2$ release, decreased from 30 to 19 h. After 24 h, fungal growth slowed with low $CO_2$ rates. This may be due to the finite amount of available nutrients in fungi, so after reaching maximum activity, growth continues, but at a slower rate (Sandoval *et al*., 2024). According to the results, using appropriate pH and temperature conditions allows for a reduction in fermentation time. By adjusting the substrate to a pH of 5.5 and using a temperature of 40 °C, the exponential phase of

*Rhizopus oligosporus* occurs at 19 h and a higher production of lactic acid and organic acids is obtained.

### Protein Content

Soybeans and soy-based tempeh had higher protein content than amaranth and amaranth-based tempeh ($p < 0.05$). Soybeans had 31.18 % and soy-based tempeh had 39.53 %. Amaranth grain was 18.93 %, while tempeh had 18.41 %. These protein content results are within the range reported in various studies. Amaranth has been reported to have between 14 % and 19 % protein (Baraniak & Kania-Dobrowolska, 2022), and soy tempeh between 25 % and 45 % (Toor *et al*., 2022).

Amaranth did not show a significant change in protein content after fermentation. However, soy-based tempeh showed an 8.4 % increase compared to its initial protein content. This increase in protein content after fermentation with *Rhizopus oligosporus* has already been described in studies (Ferreira *et al*., 2011; Bavia *et al*., 2012). In our study, this increase in protein is attributed to the dehulling of the soybeans. Dehulling caused a change in the proportions of its components. This change in the proportions of soybean components has already been observed by other authors (Raji *et al*., 2008; Bavia *et al*., 2012). The soybean hull is composed mainly of fiber and constitutes a non-protein fraction. By reducing this fraction, a higher concentration of the remaining components, such as an increase in protein content, is expected (Kohli & Singha, 2024). Soybeans are primarily composed of protein (20-45 %), carbohydrates (36.37-65.71 %), lipids (5-21 %), and fiber (5-9 %) (Raji *et al*., 2008; W; Kohli & Singha, 2024). Despite being an important source of nutrients, soybeans have limited digestibility in their whole form, making it important to remove the hull during processing (Kohli & Singha, 2024).

### Sensory testing

The evaluation of intensity in sensory attributes included students, academics, employees, and merchants primarily from the municipalities of Córdoba (21 %), Cuitláhuac (13 %), Amatlán de los Reyes (12 %), Fortín (10 %), Atoyac (8 %), and Yanga (8 %). Sixty percent were female and 40 % were male. 85 % were between 18 and 24 years old, 7 % between 25 and 34, 5 % between 35 and 44, and 3 % between 45 and 54. 95 % reported never having consumed tempeh, while 3 % reported having consumed it.

### Comparison of Sensory Profiles Using Data Transformed to a Binomial Scale

The results of the Chi-square test revealed significant associations with the type of grain (Table 3).

For amaranth-based tempeh, consumers more frequently reported the presence of the attribute of compactness ($p = 0.049$) perceived through sight and the attribute of roughness ($p < 0.0001$) perceived through touch. For soy tempeh, consumers most frequently indicated the presence of the attribute “fishy smell” ($p < 0.0001$) through the

**Table 3.** Significant attributes that depend on the grain used to prepare tempeh.†

| Attribute | P-value |
|---|---|
| Compact | 0.049 |
| Fishy smell | <0.0001 |
| Fishy flavor | 0.0246 |
| Moist | 0.025 |
| Soft | 0.0044 |
| Rough | <0.0001 |

†$p \leq 0.05$ indicates significant associations between the sensory attribute and the raw material used to prepare tempeh.

sense of smell, the attribute "fishy flavor" ($p = 0.0246$) through the sense of taste, and the attributes "moist" ($p = 0.025$) and "soft" ($p = 0.0044$) through touch. This suggests that the presence of each attribute is related to the type of grain used to prepare the tempeh.

### Comparison of sensory profiles using RATA ratings

The intensities of the attributes perceived through sight, smell, taste, and touch showed significant differences between the two samples (Table 4). The attributes compactness ($P < 0.0001$), fishy odor ($P < 0.0001$), soy odor ($P = 0.0017$), amaranth odor ($P < 0.0001$), baked odor ($P = 0.0039$), fishy flavor ($P = 0.0035$), amaranth flavor ($P < 0.0001$), moistness ($P = 0.0011$), softness ($P < 0.0001$), and roughness ($P < 0.0001$) were perceived at different intensities in the two tempeh samples.

The characteristics of tempeh are usually the result of the enzymatic activity of the fungus, which biotransforms the components of amaranth and soybean grains to ensure its survival during growth (Jeleń *et al*., 2013). The white color and the presence of mycelium are related to the growth of *Rhizopus oligosporus* on the substrate and the network of hyphae that allows it to penetrate, bind, and compact the grains. Hyphal penetration depends on the grain's cell walls, as these act as a physical barrier. However, the fungus secretes enzymes such as cellulase and hemicellulose that degrade the grain's cell wall, binding them together to form a compact cake (Wikandari *et al*., 2021; Tan *et al*., 2024). During fermentation, the raw material interacts with *Rhizopus oligosporus* and produces volatile compounds and amino acids that affect the sensory characteristics of tempeh (Gunawan-Puteri *et al*., 2015). Compounds such as 1-octene-3-ol and 3-octanone impart mushroom-like odors, while 3-(methylthio) propanal contributes to boiled potato-like aromas (Jeleń *et al*., 2013). The amino acids alanine, glycine, serine, and threonine are responsible for the sweet taste (Gunawan-Puteri *et al*., 2015). Another characteristic of various types of tempeh is their meaty and bready flavor. The meaty flavor or notes are associated with umami. This flavor is

**Table 4.** Intensities of attributes perceived through the senses for amaranth-based tempeh and for soy-based tempeh.[†]

| | Attribute | Amaranth | Soy | p-value |
|---|---|---|---|---|
| Sense of sight | Compact | 4.88 | 2.76 | <0.0001 |
| | Spongy | 2.64 | 2.37 | 0.4605 |
| | White | 2.85 | 2.24 | 0.0648 |
| | Granular | 4.61 | 4.24 | 0.3375 |
| | Smell of baking | 3.07 | 3.4 | 0.4332 |
| | Meat | 1.51 | 1.92 | 0.2055 |
| Sense of smell | Fishy smell | 1.21 | 4.03 | <0.0001 |
| | Soy smell | 1.53 | 2.64 | 0.0017 |
| | Cereal smell | 3.35 | 2.52 | 0.0445 |
| | Potato smell | 1.56 | 1.41 | 0.6422 |
| | Amaranth smell | 3.8 | 0.67 | <0.0001 |
| | Smell of baking | 3.31 | 2.2 | 0.0039 |
| Sense of taste | Fungi | 2.21 | 2.89 | 0.108 |
| | Fish | 1.2 | 2.24 | 0.0035 |
| | Fungi | 1.93 | 2.36 | 0.2717 |
| | Meat | 1.19 | 1.51 | 0.3155 |
| | Peanut | 2.12 | 2.87 | 0.0515 |
| | Smoked | 2.57 | 2.23 | 0.369 |
| | Sweet | 1.13 | 0.83 | 0.2339 |
| | Bread | 1.76 | 1.8 | 0.9098 |
| | Amaranth | 4.16 | 0.65 | <0.0001 |
| Sense of touch | Moist | 1.96 | 3.19 | 0.0011 |
| | Soft | 2.2 | 3.87 | <0.0001 |
| | Spongy | 2.49 | 2.93 | 0.2505 |
| | Rough | 4.05 | 2.08 | <0.0001 |

[†] $p \leq 0.05$ indicates significant differences in attributes between treatments. Scores represent the average intensities of attributes perceived in amaranth-based and soy-based tempeh on a scale of 1 to 7, based on 75 consumers.

produced during fermentation with the generation of amino acids grouped as sodium monoglutamate (Gunawan-Puteri *et al*., 2015).

### Sensory Profile of Amaranth and Soy based tempeh

Amaranth-based and soy-based tempeh have different sensory profiles, and these differences are related to the grain used in their preparation (Figure 4).

In amaranth tempeh, the attributes of compactness ($P < 0.0001$), amaranth flavor ($P < 0.0001$), and rough texture ($P < 0.0001$) were perceived more intensely. The higher intensity of the perceived compactness and roughness is related to the size of the grain. Amaranth grains are very small. When the fungal hyphae penetrate and bind

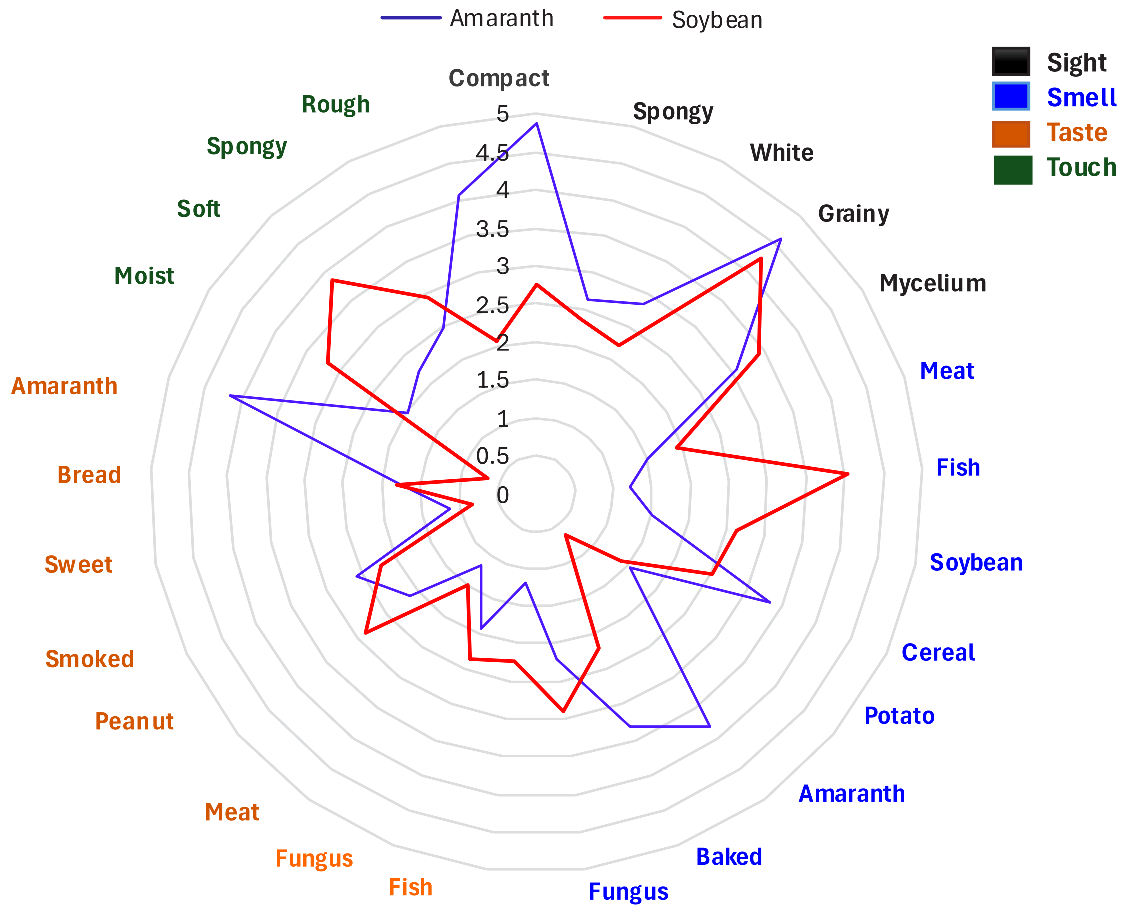

**Figure 4.** Sensory map for amaranth-based tempeh and soy-based tempeh. The sensory map represents the average intensity of the attributes perceived through the senses for both treatments, based on the 75 consumers who participated in the evaluation.

the grains together, a more compact tempeh with a rough surface is produced. Soy-based tempeh had a different sensory profile than amaranth-based tempeh, with the fishy odor ($P < 0.0001$), soy odor ($P = 0.0017$), fishy flavor ($P = 0.0035$), moistness ($P = 0.0011$), and softness ($P < 0.0001$) being perceived more intensely. The perception of soft and moist tempeh is associated to the grain's conditioning process, as the soaking and cooking process softens the grain and causes it to absorb more moisture.

A predominant characteristic perceived in the soy tempeh was its fishy flavor and odor. These sensory characteristics are related to the composition of soybeans.

Soybeans have a significant lipid fraction (Jeleń *et al*., 2013). Isoenzyme lipoxygenase is present in soybean. These enzymes are responsible for catalyzing the oxidation of fatty acids. Soybeans are an important source of fatty acids. They contain between 53 % and 54 % linoleic acid and 8 % $\alpha$-linolenic acid. These polyunsaturated fatty acids are typically unstable and sensitive to oxidation. The interaction of lipoxygenase with fatty acids often results in strong or unpleasant odors and flavors in food (Chedea *et al*., 2013). The degradation of fatty acids through lipoxygenase produces hydroperoxides, these are transformed into volatile compounds such as alcohols (1-octen-3-ol), amines (trimethylamine), sulfur compounds (dimethyl sulfide), aldehydes (heptanal) and ketones (1-octen-3-one) that are responsible for providing the fishy smell and flavor of soy-based tempeh (Chedea *et al*., 2013).

## CONCLUSIONS

The initial pH of the medium and the temperature are factors that affect the content of lactic acid, organic acids, the final pH, and $CO_2$ release. These factors influence the fungus' metabolism, allowing for higher concentrations of organic acids and $CO_2$ under appropriate pH and temperature conditions. The optimal conditions for inoculum production were established at pH 5.5 and a temperature of 40 °C. The peak of the fungus's exponential phase was reduced from 30 to 19 h. Soy tempeh and amaranth tempeh have different sensory profiles according to consumer perception. Amaranth tempeh exhibits characteristics related to the grain, such as the aroma and flavor of amaranth, whereas soy tempeh is predominantly fishy in smell and taste. Using amaranth in tempeh preparation is a viable alternative for diversifying the uses of this grain, as its sensory profile is neutral and it can be combined with various foods.

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