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Soybean Tempeh: Effect of Yeast Concentration and Fermentation Duration on Organoleptic Test as a Learning Resource

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Abstract

Tempeh is a traditional Indonesian fermented food known for its high nutritional value. This study sought to assess the impact of yeast concentration and fermentation length on moisture content, protein content, and organoleptic evaluation of yellow soybean tempeh sourced from Tojo Una-Una, Poso, and Palu. The research included a 3 × 3 factorial design incorporating yeast concentrations of 1, 2, and 3 grams, alongside fermentation durations of 30, 48, and 72 hours. The moisture content was assessed using the gravimetric method, the protein content was determined via the Kjeldahl method, and 20 panelists performed an organoleptic evaluation. The findings indicated that the moisture content of tempeh generally rose with prolonged fermentation time and elevated yeast concentration. The maximum moisture content, 63.22%, was observed in tempeh from Tojo Una-Una, which used 2 grams of yeast and had a fermentation duration of 72 hours. The minimum value, 50.18%, was also from Tojo Una-Una with 1 gram of yeast and 30 hours of fermentation. Protein levels peaked at 48 hours of fermentation with 3 grams of yeast, reaching a maximum of 26.88% in the Palu area. In contrast, the minimum was recorded at 18.55% in tempeh from Tojo Una-Una with 2 grams of yeast and 30 hours of fermentation. The organoleptic test results indicated that panelists favored Poso tempeh in terms of flavor, aroma, and texture. The concentration of yeast and the time of fermentation influence the moisture content, protein content, and organoleptic quality of tempeh. The methodologies and findings of this study serve as a resource for chemistry education in the format of LKPD.

Keywords: Fermentation, organoleptic, yeast, yellow soybean tempeh

Introduction

Soybeans constitute a significant source of nourishment. The nutritional makeup of soybeans changes based on the developed variety and the coloration of the epidermis and cotyledons (Prativi et al., 2023). Yellow soybeans have a substantial nutritional composition of 38% protein, 18% fat, 15% ash, and 29% carbs per 100 grams, along with vitamins and minerals (Afzaal et al., 2022; Moroșan et al., 2023).

Tempeh is a traditional Indonesian dish, originating in Central Java in the 1700s and now recognized globally (Romulo & Surya, 2021). Tempeh is recognized as a fermented product originating from soybean components (Aziez et al., 2022). Tempeh possesses a superior protein content of 18.3% in comparison to other processed soybeans, including tauco at 10.5%, tofu at 7.9%, soy sauce at 5.5%, and soy milk at 2.8% (Subali et al., 2023). Tempeh is a food product derived from the fermentation of soybeans or other legumes utilizing the fungi Rhizopus oligosporus and Rhizopus orizae.

In Indonesia, a wide variety of tempeh, including tempe gembus, tempe mangel kacang, tempe menjes, tempeh benguku, and tempe romtoro. Despite the diversity of tempeh, yellow soybean-based tempeh is more recognized and favored. Yellow soybeans are a superior source of plant-derived protein and are rich in calcium, magnesium, and iron (Jafarzadeh et al., 2024).

One advantage of yellow soybeans is their aesthetic appeal, aligning with customer preferences. The Center for Agricultural Data and Information Systems of the Ministry of Agriculture reported that yellow soybean production in 2015 was 954,997 tons. Consequently, to satisfy the demand, Indonesia imported 2,256,931.68 tons of soybeans. The protein content in yellow soybeans ranged from 31% to 48%. The fat content ranged from 11% to 21%. Soybeans serve as the primary raw materials in the production of tempeh, tofu, and soy sauce due to their high protein content. Yellow soybeans (Glycine max) are typically utilized for

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tempeh production; however, individuals often innovate by substituting soybeans with legumes such as kidney beans (Phaseolus vulgaris) (Safitry et al., 2022).

To date, soybeans have been recognized as a fundamental ingredient for the production of tempeh. The limited storage duration of tempeh fosters innovation in the further processing of yellow soybeans. The procedure for producing tempeh from yellow soybeans and other soybean varieties is identical, encompassing washing, boiling, peeling, soaking, and fermentation. Fermentation is a crucial phase in tempeh production, when yeast is introduced to facilitate the transformation of soybean seeds into tempeh. The fermentation of soybeans for tempeh production necessitates the involvement of Rhizopus sp. mold. The molds commonly utilized for tempeh production are Rhizopus oryzae and Rhizopus oligosporus (Ellent et al., 2022).

Several previous studies have explored the effects of yeast concentration and fermentation time on tempeh quality. For example, Anita (2018) studied black soybean tempeh, while Andika et al. (2023), Hanafi et al. (2023), and Zhao et al. (2024) examined the impacts of fermentation on sensory and chemical properties. However, these studies did not focus on yellow soybean tempeh sourced from diverse regions, nor on its potential as a contextual chemistry learning resource. This study seeks to fill that research gap by analyzing the effects of yeast and fermentation on yellow soybean tempeh from Central Sulawesi (Tojo Una-Una, Poso, Palu) and by developing an LKPD (Student Worksheet) based on the results.

Multiple factors influence the quality of tempeh production, specifically yeast concentration and fermentation duration. Tempeh yeast comprises the fungus Rhizopus sp, commonly referred to as tempeh mushroom. Rhizopus fungus is the predominant species in tempeh yeast, characterized by its white coloration and mycelia that link soybean seeds to tempeh (Teoh et al., 2024). Tempeh production involves two fermentation processes. The primary fermentation process begins when the soybeans are soaked in water. The second fermentation, also known as primary fermentation, occurs when yeast is introduced to soybeans that are prepared for fermentation. The study by Diez-Simon et al. (2020) examines the influence of yeast concentration and fermentation duration on the chemical and sensory characteristics of black soybean tempeh (Glycine soja), sharing similarities in variables and methodologies. The author will highlight the contrasting aspects of the yellow soybean variable utilized as a chemistry learning resource, and will also employ a distinct research location from other studies, specifically three sites in Central Sulawesi.

Methods

Tools and Materials

The tools used are a porcelain cup, a desiccator (*Schott Duran brand*), an analytical balance (*Denver Instrument M-310*), and an oven. For protein content analysis, the following equipment is required: Kjeldahl heater, 100 mL Kjeldahl flask (*Iwaki*), distillation device (*Iwaki*), stopper and clamp (*Pyrex*), Erlenmeyer (*Herma*), 50 mL burette (*Pyrex*), and analytical balance (*Shimadzu AUX220*). The materials required for analyzing the moisture and protein content of yellow soybean tempeh samples include Kjeldahl tablets, concentrated H2SO4, aquades, 30% NaOH, 0.1 N HCl, and methyl red indicators.

Sample preparation

The initial phase involves assembling all necessary tools and ingredients. Begin with 1 kg of yellow soybeans, then sort and wash them. After cleaning, boil the soybeans for 30 minutes to facilitate skin removal. Subsequently, soak the soybeans for 12 hours, then boil them again for 30 minutes. Allow them to cool before adding 1 gram of yeast, along with 2 and 3 grams. Finally, wrap the soybeans in plastic, puncture holes, and ferment for 30, 48, and 72 hours (Anita, 2018).

Tempeh water content analysis

A 10-gram sample of mashed yellow soybean tempeh is placed into a porcelain cup. The mixture is thereafter cooked in the oven at 100°C for approximately three hours. The substance is subsequently cooled with a desiccant for one hour and measured with an analytical balance (Anita, 2018).

Analysis of tempeh protein content

The initial phase is the destruction step, wherein a 1-gram sample of yellow soybean tempeh is placed into a 100 mL Kjeldahl flask, followed by the addition of one Kjeldahl tablet and 10 mL of concentrated H_2SO_4. Subsequently, all the constituents in the pumpkin are denatured (heated) until a transparent solution is achieved. The subsequent phase is distillation, wherein the products of the destruction phase are diluted with 100/10 mL of distilled water and agitated until homogeneous. Subsequently, 10 mL is extracted and transferred into a distillation flask. Introduce 10 mL of 30% NaOH solution into the pumpkin's wall until two distinct layers are established. The flask is subsequently attached to a condenser. Steam from the boiling liquid passes through the condenser. It is collected in an Erlenmeyer flask containing 10 mL of 0.1 N HCl solution, with a red methyl indicator added. The distillate is subsequently titrated with a standardized NaOH solution (0.1 N) using oxalic acid as the titrant. The titration is concluded after the distillate exhibits a pink hue. The latter creates a blank solution, achieved by substituting the sample with distilled water (Nurfadila, 2024).

Data analysis techniques

Determination of moisture content

$$water\ content\ (\%) = \frac{(W_o + W_s)}{W_s} \times 100\% \quad (1)$$

(Badan Standarisasi Nasional, 2009)

Information:

Ws = sample weight before diovened (grams)
Wi = sample weight + cup after ovening (grams)
Wo = weight of empty cups (grams)

Determination of protein levels

$$\% N = \frac{(t_s + t_b) \times HCl \times 14 \times 10}{mg\ sampel} \times 100\% \quad (2)$$

$$\% Protein = \% N \times 5.71$$

(Badan Standarisasi Nasional, 2009)

Keterangan:

V_S = HCl volume of 0.1 N for the titration of the sample, expressed in milliliters (mL);
V_b = HCl volume of 0.1 N for blank titration, expressed in milliliters (mL);
N = normality of HCl solution;
W = sample weight, expressed in milligrams (mg);
14 = atomic weight Nitrogen;
5.71 = protein factor for Soybeans

Organoleptic test

This study engaged 20 untrained panelists from the Chemistry Education Study Program at Tadulako University. Samples of yellow pumpkin seed tempeh were produced in containers designated P1, P2, and P3. Panelists were thereafter provided with samples and questionnaires to provide hedonic evaluations based on aroma, color, taste, texture, and overall product appearance criteria. The panelists' preferences were assessed using a 5-point hedonic scale: 1 (extremely dislike), 2 (dislike), 3 (somewhat like), 4 (like), and 5 (very like). Subsequently, the panelists were requested to complete a questionnaire and engage in a discussion regarding the provided sample (Tanone & Prasetya, 2019).

Creation and validation of student worksheets (LKPD)

The initial Student Worksheet (LKPD) was developed to encompass work practices and research findings shown in the accompanying draft, intended as a resource for chemistry education. The LKPD's validity was evaluated by two specialists: a content expert and a design expert. The validity criteria are deemed practical if they receive a minimum score of 2.75.

$$Persentase = \frac{sum\ of\ validation\ result\ scores}{highest\ score} \times 100\%$$

The assessment results will be evaluated using a Likert scale with the following classifications: 5 - very good; 4 - good; 3 - moderate; 2 - adequate; and 1 - poor. LKPD is deemed viable if the percentage score of 61% of the Student Worksheet eligibility percentage is classified within the eligibility category outlined in **Table 1**.

Table 1. Percentage of student worksheet eligibility

Skor percentage (%)	Category
81– 100	Highly Valid
61– 80	Valid
41– 60	Quite Valid
21 – 40	Less Valid
20	Very Invalid

Source: (Riduwan, 2012).

Results and Discussion

The results of the moisture content analysis in soybean samples from Tojo Una-Una, Poso, and Palu City are presented in **Table 2** after being analyzed and calculated.

Table 2. Average moisture content

Region	Moisture content
Tojo una-una	56.58
Poso	56.29
Palu	58.08

The determination of moisture content in this study uses the gravimetric method, which involves drying the sample at 105°C until it reaches a constant weight. Moisture content is calculated from the difference in weight before and after drying and expressed in percent (Zambrano et al., 2019).

In this study, tempeh moisture content was influenced by soybean origin, yeast concentration, and fermentation duration. Longer fermentation generally increased moisture content, especially in soybeans from Tojo Una-Una and Palu, due to the enzymatic activity of Rhizopus sp., which softened the substrate. In contrast, soybeans from Poso showed relatively stable moisture content, presumably due to a denser seed structure. Increasing yeast concentration also tended to increase moisture content due to the higher intensity of mold metabolism.

In theory, prolonged fermentation can lead to enzymatic activity from Rhizopus sp. molds, which hydrolyze soybean components like proteins into smaller compounds. This process can draw water into the tempeh tissue, leading to an increase in moisture content (Hanafi et al., 2023). However, several technical and biological factors can cause the moisture content to decrease as fermentation time increases. This was observed in the results, where an increase in mold respiration activity led to denser mold growth with longer fermentation. The molds perform respiration that produces heat, which can cause greater water evaporation. This causes the water content of tempeh to decrease.

Sulistiyawati & Lusiani (2022) explained that the increased fermentation temperature due to mold metabolism can accelerate water evaporation. Besides the temperature and air circulation during fermentation, whether carried out in the open or with good air circulation, the water released during metabolism will evaporate, especially from the second day onward. The longer the fermentation lasts under these conditions, the more water is lost, and as the texture of tempeh becomes denser, the yeast concentration increases. The fermentation time is extended. The faster the mold forms thick mycelium, the denser the tempeh becomes, which binds water more strongly or even causes it to lose water as the texture hardens and dries.

Thus, in general theory, the water content increases with fermentation time. However, the results of this study show that the process does not always occur linearly and can be influenced by environmental conditions and local raw materials. The microbial metabolic process produces water as a by-product of aerobic fermentation (Mori et al., 2023).

The results of the moisture content analysis in soybean samples from Tojo Una-Una, Poso, and Palu City are presented in **Table 3** after being analyzed and calculated.

Table 3. Average protein content

Region	Protein content
Tojo una-una	21.75
Poso	24.34
Palu	22.18

The results showed that the protein content in yellow soybean tempeh increased with both the yeast concentration and the length of fermentation time. Tempeh fermented for 48 hours with a yeast concentration of 3 grams had the highest protein content compared to other treatments. This aligns with research by Widodo et al. (2021), which states that the increase in protein levels in tempeh results from the activity of protease enzymes produced by Rhizopus sp. These enzymes can hydrolyze complex proteins into simpler, easily measurable forms. The increase in protein levels can also be caused by the growth of mold biomass during fermentation. Rhizopus sp. grows and develops on the surface of soybean seeds. This mold mycelium itself contains protein, so it contributes to the total protein content of tempeh. During fermentation, the process of breaking down complex compounds degrades antinutritional compounds, such as trypsin inhibitors, which inhibit protein digestion. This increases the availability of protein that can be measured chemically. Besides that, it can be seen from the Optimal Fermentation conditions that during the 48-hour fermentation with 3 grams of yeast, temperature and humidity tend to approach optimal conditions for mold growth, allowing the fermentation process to occur more completely.

The protein content of tempeh varied among soybean origins (Palu, Poso, and Tojo Una-Una) due to differences in varieties, fermentation environmental conditions, and physical characteristics of the seeds. This finding aligns with Andika et al. (2023), who stated that high yeast concentration and optimal fermentation can increase protein content. The best treatment was obtained at 3 grams of yeast and 48 hours of fermentation, while Tojo Una-Una soybean with 2 grams of yeast also showed optimal protein levels. In theory, fermentation by Rhizopus sp. increases protein levels through the synthesis of mold biomass, the breakdown of complex proteins, and the reduction of antinutritional compounds.

The high protein yield in tempeh from Tojo Una-Una supports the theory of Widodo et al. (2021) that moderate yeast concentration, when combined with good soybean quality, can optimize enzymatic activity. Tojo Una-Una soybeans are thought to have high initial protein, ideal seed size, and stable moisture, resulting in efficient fermentation even with only 2 grams of yeast. This finding demonstrates the efficient use of yeast without decreasing yield, relevant for an economical biochemical learning example.

In contrast, tempeh from Palu and Poso showed lower protein levels despite using more yeast. This indicates that increasing yeast and fermentation duration does not always increase protein content, as the initial soybean quality and fermentation conditions are also influential. Soybeans from Palu and Poso had lower initial protein levels, with Poso experiencing excess moisture that inhibited mold growth. In addition, the use of excessive yeast in some treatments actually decreased fermentation efficiency.

According to Hanafi et al. (2023), too high yeast concentration can trigger microorganism competition and disrupt fermentation stability, thereby reducing tempeh quality. Inappropriate fermentation time, especially if it is too long, can also prevent significant increases in protein levels due to protein degradation or microbial contamination.

This study shows that the protein content of tempeh meets the quality standards, with the best results obtained at a yeast concentration of 3 grams and optimal fermentation conditions. The less yeast and the faster the fermentation, the higher the protein content tends to be. However, adding yeast to the optimum point also increases the activity of the proteolytic enzyme Rhizopus oligosporus, which breaks down complex proteins into simple compounds (Perdani & Utama, 2020; Zhao et al., 2024).

The increase in protein is also supported by an increase in soluble nitrogen and free amino acids. However, excess yeast can increase the fermentation temperature and accelerate protein degradation, thus reducing protein levels (Surbakti et al., 2022). Therefore, the balance between yeast concentration and fermentation time is essential to produce tempeh with the best chemical and sensory quality (Lusiana et al., 2023).

Organoleptic test

The organoleptic test used in this study is the Hedonic Scale Scoring method, as one of the consumer acceptance tests. This test used 20 untrained panelists. Organoleptic analysis was carried out by preparing test samples and questionnaires. For the hedonic test, the test parameters contained smell, color, taste, and texture of the product. The results are shown in **Figure 1**.

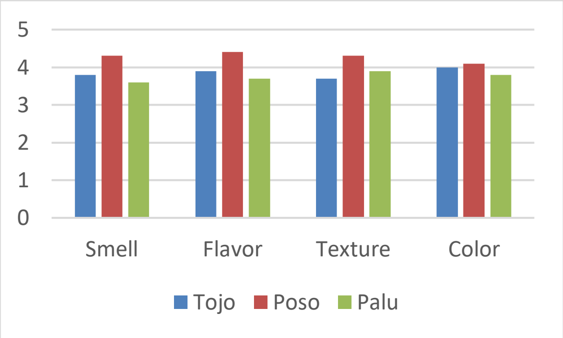

Figure 1. Organoleptic test

The results show that tempeh from Tojo Una-Una received the highest scores in texture (3.95) and taste (3.73). The superior texture indicates a well-developed mycelium network of Rhizopus sp., resulting in a firm but tender tempeh structure. This aligns with the findings of Nirmalasari et al. (2024), who stated that optimal fermentation yields a compact, fully bound structure with white, dense mycelium covering the surface.

The high taste score also reflects the balance of moisture and protein levels, leading to favorable fermentation by-products such as free amino acids and volatile compounds that enhance the flavor profile. These sensory characteristics suggest that fermentation in Tojo Una-Una proceeded under stable and favorable conditions.

Poso-produced tempeh achieved the highest aroma score (3.91), slightly surpassing Tojo Una-Una (3.90). This suggests that the fermentation process in Poso produces a more appealing tempeh aroma, potentially due to environmental factors or higher-quality soybean substrates that promote optimal microbial metabolism. According to Sulistiyawati & Lusiani (2022), aroma formation during fermentation is highly influenced by temperature, humidity, yeast concentration, and the generation of volatile compounds such as esters, alcohols, and aldehydes. Poso also showed consistent scores in taste and texture (both 3.70), indicating overall uniform sensory quality. This implies that soybeans from Poso are highly responsive to fermentation, resulting in well-accepted sensory characteristics.

In contrast, tempeh from Palu consistently received the lowest scores across all parameters, particularly in aroma (3.30) and color (3.02). The lower color rating may be due to uneven fungal growth, which results in patches, discoloration, or black spots on the tempeh surface. The relatively low aroma score could result from rapid or uncontrolled fermentation due to higher ambient temperatures, which lead to the production of sharp or undesirable odors.

Although the texture of Palu tempeh (3.45) is still acceptable, it lags behind the other regions. This may be related to fluctuating humidity or suboptimal environmental conditions that disrupt consistent mold growth during fermentation.

Implications for chemistry learning beyond sensory analysis, these findings provide meaningful insights for contextualized chemistry education. The differences in organoleptic scores across regions can be used to design student worksheets (LKPD) that incorporate real data, encouraging students to explore the connection between fermentation, biomolecules, enzyme activity, and sensory perception.

By analyzing data from local food products, students can relate chemistry concepts to everyday experiences. This not only improves scientific understanding but also fosters appreciation for local wisdom and traditional food biotechnology. The integration of tempeh into chemistry learning enhances engagement and relevance in the classroom. According to **Table 4** of the results, it was determined that learning resources in the form of LKPD are very suitable for use as a learning medium. The results achieved were 80% for material experts and 90% for design experts. Consequently, the LKPD (Student Worksheet) is deemed legitimate as a learning resource, since it satisfies the percentage criteria, achieving a range of 80% to 61% (Riduwan, 2012).

Table 4. Average validation assessment of the LKPD Aspect Assessment Rating Scale

Aspect Assessment	Rating Scale (%)
Material Expert	80
Design Expert	90
Total	170
Average	85

Source: Data from Material Expert and Design Expert Validation Results

The LKPD developed contains research data, including water content, protein content, and organoleptic scores. Students are directed to analyze the data and relate it to chemical concepts such as biomolecules, enzymes, and changes in compounds during fermentation. By using tempeh as a learning medium, students can develop critical thinking skills and understand the application of chemistry in everyday life.

Conclusions

The study indicates that the optimal yeast concentration for producing yellow soybean tempeh meets desirable chemical parameters (moisture and protein content) and offers superior organoleptic quality. This concentration produces elevated protein levels and suitable moisture content, and is organoleptically favored. The optimal fermentation duration for producing yellow soybean tempeh with satisfactory chemical and organoleptic quality is 48 to 72 hours, contingent upon the specific qualities of the soybeans from each location. Fermentation for 48 hours yields ideal protein levels, whereas 72-hour fermentation markedly elevates moisture content and alters the texture of tempeh. The validation test results of the LKPD (Student Worksheet) indicate its appropriateness as a learning resource.

Conflict of Interest

The authors declare that there are no conflicts of interest associated with the publication of this article. Each author contributed equally and without pressure from other parties.

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