Jurnal Akademika Kimia, 14(2): 83-89, May 2025
ISSN (online) 2477-5185 | ISSN (print) 2302-6030
http://jurnal.fkip.untad.ac.id/index.php/jak/

Protein Content and Organoleptic Test Central Sulawesi Pumpkin Seed Tempeh (Cucurbita moschata) for Learning Resource

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Received 19 March 2025, Revised 31 March 2025, Accepted 08 April 2025
doi: 10.22487/j24775185.2025.v14.i2.pp83-89

Abstract

The yellow pumpkin (Cucurbita moschata) is a plant that readily adapts to diverse soil conditions; however, the utilization of its seeds remains restricted. Despite its relatively high protein content, which is crucial for growth and energy, it can serve as an alternative raw material for tempeh production. This study aims to generate yellow pumpkin seed tempeh in compliance with SNI 3144:2015, assess organoleptic variations in the protein content of yellow pumpkin seed tempeh from three distinct regions in Central Sulawesi, and produce a Student Worksheet as an alternative resource for chemistry education, validated by expert lecturers. This research employs a quantitative methodology utilizing the Kjeldahl analysis technique to ascertain protein concentrations across three phases: destruction, distillation, and titration. Furthermore, a sensory evaluation was conducted by 20 panelists to analyze the texture, color, flavor, and scent of yellow pumpkin seed tempeh. The resultant tempeh has favorable organoleptic properties, achieving an average preference score of 4. A uniform white coloration, a distinctive tempeh aroma, and a dense texture characterize it. Protein content research revealed that tempeh from Palu City had the highest protein concentration at 24.5%, followed by Palolo District at 23.91% and North Lore District at 20.41%. This suggests the impact of environmental factors on the nutritional composition of the manufactured tempeh. According to SNI 3144:2015, the visual characteristics of yellow pumpkin seed tempeh and its protein content conform to the established quality criteria. The findings of this study were presented as Student worksheets, which achieved a validity rate of 85%, indicating their high suitability as a learning resource. This research enhances the application of yellow pumpkin seeds as a nutritious food source and fosters the creation of engaging and novel educational materials.

Keywords: Kjeldahl, organoleptic, protein, pumpkin seed tempeh, student worksheets

Introduction

Pumpkin is an annual crop that is easy to grow and widely distributed in Indonesia. It has high production in Java, 270.000 tons per year. Sumatra 94.000 tons per year, and Bali 70.000 tons per year (BPS, 2021). Despite its potential, the community's use of it is still limited to fruit due to a lack of knowledge about its nutritional content (Pabidang et al., 2020). Along with the development of various innovations, pumpkin seeds have gained attention as a material with great potential for full utilization. This potential comes from the bioactive compounds' antioxidant properties inside pumpkin seeds. This compound stops the formation of free radicals from peroxide and prevents the oxidation of unsaturated fatty acids in cell membranes. So it is suitable for health, especially in reducing inflammation and protecting the body's cells from serious diseases. These benefits include the presence of amino acids, zinc, magnesium, various essential fatty acids, vitamin C, carotenoids, sterols, and cryptoxanthin (Mumpuni & Khasanah, 2021).

Furthermore, protein can be an alternative energy source when carbohydrates and fats are insufficient for the body's needs. This process involves breaking down proteins into components that can be used to produce energy, thus helping the body function properly in meeting energy deficiencies. Thus, pumpkin seeds, rich in protein, can be an ideal ingredient for making tempeh, which is not only delicious but also has high nutritional value and is part of Indonesian culinary culture (Indonesian National Standard). The protein content of pumpkin seeds makes them a potential alternative material for making tempeh, a typical Indonesian fermented food. Through the fermentation process of soybeans using the Rhizopus oligosporus fungus, tempeh is created, which is nutritious and favored by the wider community. Based on the Indonesian National Standard (2015), some of the criteria for tempeh that need to be considered are having a compact texture, meaning that when cut, the tempeh remains intact and does not fall off easily, the color is evenly white, and it has a distinctive aroma of tempeh

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without the smell of ammonia. Therefore, researchers tried to utilize pumpkin seeds as a substrate in making tempeh.

Previous research has shown that tempeh from pumpkin seeds has a high protein content of 24.66 % and is suitable for use as a learning medium (Hasrina & Nurdin, 2021). Adding pumpkin seed powder has also been shown to affect the chemical properties of tempeh (Mahfud et al., 2021). This research aims to produce pumpkin seed tempeh in accordance with the organoleptic standards of tempeh based on the Indonesian National Standard (2015), this is carried out based on consideration of the appearance of important tempeh and the main thing in public acceptance, especially in ensuring that yellow pumpkin seed tempeh products have good quality and acceptable, especially this study researches tempeh with substrate innovation in the environmental conditions of plant growth that are influenced by nutrients that are absorbed by plants to continue growing, where these nutrients come from the soil related to plant nutrition so it needs to be researched. In addition, the results of this research are expected to be developed into student worksheets as an alternative source for learning chemistry. Student worksheets are a medium that provides learning stimuli to students so that they can think critically and be motivated in learning (Hanik, 2020).

This research is important because the quality of tempeh, both in terms of nutrition and appearance, significantly affects public acceptance. At the same time, the environmental conditions in which plants grow also determine their nutritional content. Based on the description above, this study aims to produce yellow pumpkin seed tempeh in accordance with the Indonesian National Standard (2015), organoleptically test the differences in protein content of yellow pumpkin seed tempeh from three different regions in Central Sulawesi, and create student worksheets as an alternative source for chemistry learning.

Method

Tools and materials

The tools used in this study are divided into tempeh-making tools, including basins, pots, Rinnai RI-201S gas stoves, scales, sieves, spoons, and toothpicks. Tempeh protein testing equipment includes *Sonic Electronic* analytical scales, distillates, *Pyrex* burettes 50 mL, sticks and clamps, 5 mL and 10 mL *Iwaki* measuring cups, 100 mL *Pyrex Iwaki* measuring cups, 50 mL and 100 mL *Pyrex* chemical cups, 5 mL *Pyrex* volume pipettes, 10 mL *Pyrex* glass funnels, 100 mL Kjedhal flasks *Pyrex*, 100 mL *Iwaki pyrex* Erlenmeyer, drip pipettes. Moreover, the ingredients in this study are divided into ingredients for making tempeh, including yellow pumpkin seeds, water, tempeh yeast, and plastic, as well as protein content test materials, including tempeh yellow pumpkin seeds, NaOH 30 %, Kjedhal tablets, concentrated H_2SO_4, HCl 0.1 N, NaOH 0.1 N, methyl red, and aquadest.

Sample preparation

Yellow pumpkin seed samples were taken from three different regions in Central Sulawesi: Palu City (Tinggede Village), Sigi (Bahagia Village), and North Lore (Watutau Village). Hasrina & Nurdin (2021), the sample preparation process began with separating the pumpkin seeds from the fruit, then washing them thoroughly, boil for 10 minutes, drain and separate the yellow pumpkin seeds from the skin, rewash the yellow pumpkin seeds with clean water, and drain again until there is no water, mix 0.1 grams of Raprima yeast of the microbial type *Rhizopus sp.* with 100 grams of yellow pumpkin seeds until evenly distributed, put the mixture of Raprima yeast and yellow pumpkin seeds in plastic, cover the tempeh packaging plastic with fire to cover the plastic, punch a hole in the tempeh packaging with a needle and let it sit for 48 hours.

Analysis of tempeh protein content

Nuryanti et al. (2024) analyzed the protein content in pumpkin seed tempeh samples using a modified Kjeldahl method. The destruction begins by weighing 0.5 grams of the sample, placing it into a 100 mL Kjeldahl flask, and then adding 1 Kjeldahl tablet and 10 mL of concentrated H_2SO_4 solution. The sample is then destroyed until the solution becomes clear. After the solution has cooled, the distillation stage is carried out by diluting the solution resulting from the destruction process with 100 mL of aquades. Next, 5 mL of the sample is taken and put into the distilled tube, then 10 mL of 30 % NaOH solution is added through the wall of the distilled tube. The distillation process is regulated by a condenser and an Erlenmeyer flask containing 10 mL of 0.1 N HCl solution and 3 drops of methyl red indicator. At the titration stage, the distillate solution is titrated with NaOH 0.1 N until the color changes to a clear yellow, indicating the titration endpoint. Make a blank solution using a similar procedure. However, the sample was replaced with aquades.

Organoleptic testing

Organoleptic testing of yellow pumpkin seed tempeh was conducted in three regions in Central Sulawesi to assess differences in product preference based on the panelists' reactions to the samples provided (Purbasari & Putri, 2021). The organoleptic test was carried out by 20 untrained panelists who were students from the Chemistry Education Study Program. Samples of yellow pumpkin seed tempeh were given to the panelists. Each sample was provided raw, and the panelists were asked to fill out a questionnaire with a scale of product preferences on five indicators: color, aroma, texture, and taste, as shown in **Table 1**.

Table 1. Scale and description of organoleptic test assessment

Rating Scale	Description of Assessment			
	Color	Smell	Texture	Taste
1 (very much dislike)	Gray/brownish mycelium	Rotting/stinging/smelling of ammonia	Soft/easily crumbles/not fused	Very acidic/bitter
2 (dislike)	Not uniform /partly yellowish	Not fresh/ sour smell	Less compact/easy to separate	Slightly acidic/less typical tempeh
3 (just likes)	Fairly white/less even	Smell not strong/ slightly bland	A bit compact/a little brittle	A little bland/still acceptable
4 (likes)	Flat white/ no black stains	Typical aroma of tempeh/ no pungent smell	Compact/not easily destroyed	Typical tempeh/not sour/ bitter
5 (very like)	Clean white/ uniform/no stains	Fresh/distinctive/strong/ aroma of tempeh without sour or ammonia smell	Very compact/dense/ stays intact when cut	Very tasty/typical tempeh/savory/not sour/ bitter.

Creation and validation of student worksheets

The initial phase involves creating the Learner Worksheet as a resource for chemistry education. Material and design specialists validate the Student Worksheet to ascertain its suitability as a chemistry learning resource.

The evaluation of the eligibility and validity of the Student Worksheet employs quantitative descriptive calculations utilizing the following method.

$$\text{Percentage} = \frac{\textit{total validation result scores}}{\textit{highest score}} \times 100\% \quad (1)$$

(Sugiyono, 2016)

The eligibility percentage for Student Worksheets is analyzed according to the categories outlined in **Table 2**.

Table 2. Percentage of student worksheet eligibility

Percentage Product Validity (%)	Interpretasi
80 ≥ Score ≤ 100	Very valid
60 ≥ Score ≤ 80	Valid
40 ≥ Score ≤ 60	Quite valid
20 ≥ Score ≤ 40	Invalid
0 ≥ Score ≤ 20	Very invalid

Source: Pattimura et al. (2020)

Analysis of protein content

The assessment of protein levels was conducted by Hasrina & Nurdin (2021), utilizing the subsequent formula.

$$\%N = \frac{Vs - Vb \times N.HCl \times 14 \times P}{mg\ sampel} \times 100\% \quad (2)$$

$$\%\text{Protein} = \%\ N \times 6.25 \quad (3)$$

Information:
Vs = Sample Titration Volume
V_b = Volume of Blank
14 = Atomic Weight of Nitrogen
N.HCl = Normality HCl
6.25 = Conversion Factor
P = Dilution

Analysis of organoleptic test data

The evaluation of organoleptic test results involved determining the feasibility % of yellow pumpkin seed tempeh products across various assessment criteria, including color, aroma, texture, and flavor, utilizing the specified formula.

$$\text{Average} = \frac{\textit{total percentage amount}}{\textit{total panelis}} \quad (4)$$

Results and Discussion

Protein content

The findings of the protein content tests of the yellow pumpkin are shown in **Table 3**.

Table 3. Protein yield data tempeh yellow pumpkin seeds

No.	Sample Tempeh Yellow Pumpkin Seeds	Protein Content (%)			Average (%)
		I	II	III	
1.	Palu City	24.5	26.25	22.75	24.5
2.	Lore Utara	10.5	24.5	26.25	20.41
3.	Palolo	21	24.5	26.25	23.91

The protein content of yellow pumpkin seed tempeh was determined using the Kjeldahl analysis method. The Kjeldahl method is a chemical analysis technique used to ascertain the nitrogen content of a sample. This method relies on converting nitrogen in proteins into ammonia through a destructive process involving sulfuric acid and a catalyst. The resulting ammonia is then quantitatively analyzed using sample titration. The Kjeldahl method was chosen to analyze the protein content in tempeh made from yellow pumpkin seeds (Cucurbita moschata) because it is a precise, standardized, and widely used method in food analysis. Hasrina & Nurdin (2021) showed that the protein content in pumpkin seed tempeh samples was lower than 24.66%. Mahfud et al. (2021) recorded a protein content of 23.07 % in tempeh with the addition of yellow pumpkin seed powder. The ability of this method to measure total nitrogen in fermented foods makes it very reliable, even if the samples are complex or non-homogeneous. In addition, this method can be applied to a small number of samples, making it efficient for laboratory research purposes and the preparation of learning media based on laboratory test results.

The protein content of yellow pumpkin seed tempeh, as indicated in **Table 3**, varies across different regions in Central Sulawesi. The sample from Palu City exhibited the most significant protein level at 24.5 %. Hasrina & Nurdin (2021) indicated that the protein level in the yellow pumpkin seed tempeh sample was inferior to 24.66 %. Lathifah & Hermawati (2019) showed that the protein content of soybean tempeh is 31.85%, which exceeds that of pumpkin seed tempeh. Based on SNI 2015, the three tempeh samples have satisfied the established protein quality standards, which require a minimum of 15 % protein content.

The variation in yield is influenced by other factors, including the volume of titrant required to reach the point of equality, which is indicated by the change in color from purple to yellowish (Yurida et al., 2013). The varied growth location of yellow pumpkin fruits is another factor; Although this plant can thrive in diverse conditions, different environmental conditions in three regions in Central Sulawesi affect soil nutrition, thus affecting the nutritional value of yellow pumpkin fruit. The nutritional composition of yellow squash is influenced by its variety, harvest maturity, and environmental conditions (Tamer et al., 2010).

Optimal environmental conditions promote plant growth, including soil quality, water availability, altitude, and light intensity. Three research locations in the Central Sulawesi region were observed at varying altitudes: Palu City at an altitude of 0 - 700 meters above sea level (BPS, 2025), North Lore Regency at about 1000 meters above sea level (Widyatmoko et al., 2024), and Palolo Regency at an altitude of 500 - 900 meters above sea level (BPS, 2020). This shows the North Lore and Palolo District are highland areas, resulting in lower air temperatures and light intensity. As a lowland, Palu City experiences high air temperatures and light intensity. In Indonesia, temperature variations are determined by altitude. Altitudes below 700 meters above sea level usually experience high temperatures, and altitudes above 700 meters generally experience cooler temperatures. The height of a site above sea level affects the air temperature and light exposure received by plants (Istiawan & Kastono, 2019). Tedianto's (2012) research underlines that environmental conditions affect the nutrient absorption of pumpkin plants, suggesting that an increase in the growth area of yellow pumpkin correlates with a decrease in its biochemical content. Altitude affects temperature, leading to reduced sunlight and increased CO2 concentration, which inhibits photosynthesis and affects the protein composition of plants. Research shows that high soil pH correlates with an increase in the protein content of yellow squash. Nevertheless, increased soil moisture correlates with a reduction in protein content. Hermita et al. (2017) revealed similar findings, indicating that taro leaves at 800 meters above sea level had lower protein levels compared to those at 400 meters above sea level. This is due to the height. The intensity of the received light decreases, and the humidity significantly increases at higher altitudes.

The soil's pH value is a crucial determinant of its appropriateness for plant growth, as it influences nutrient availability. An inadequate soil pH can precipitate numerous issues, including nutrient deficits, diminished microbial activity, reduced yields, and impaired soil health. The soil pH in Palu City ranges from 7.45 to 8.05, indicating that it is neutral to slightly alkaline (Bakri et al., 2016). The pH level of the soil typically varies from 3.5 to 10. Regions with considerable precipitation exhibit a natural soil pH between 5 and 7, whereas arid regions display a pH range of 6.5 to 9 (Taisa et al., 2021).

Organoleptik test

The results of the research obtained regarding the organoleptic testing of yellow pumpkin seed tempeh include the criteria of color, aroma, texture and taste of tempeh for the panelists, the protein content of yellow pumpkin seed tempeh for three regions in Central Sulawesi, including in Palu City, North Lore District, and Palolo District were determined using the Kjedhal method in 0.5 grams of tempeh samples and its use as a source of chemistry learning in the form of Student Worksheets. The following are the results of the organoleptic tempeh submission in **Table 4.**

The findings of organoleptic tests indicate that yellow pumpkin seed tempeh possesses favorable sensory attributes and can potentially be a delectable and high-quality food product. The panelists' evaluation indicates a strong preference for the yellow pumpkin seed tempeh, as its completely white surface signifies the effective growth of the mycelium fungus Rhizopus oligosporus, resulting in high-quality goods.

Wihandini et al. (2012) indicated in their study that the characteristic aroma of tempeh is generated by mold enzymes, which decompose soy protein and fat into a unique scent during fermentation. This aroma received favorable evaluations from panelists, thereby demonstrating the efficacy of the fermentation process.

Table 4. Results of the organoleptic test of yellow pumpkin seed tempeh

Sample	Test criteria	Average panelist rating	Overall average	Rating scale
Palu City	Color	4.2	4.3	Likes
	Smell	4.4		
	Texture	4.3		
	Taste	4.3		
North Lore District	Color	4.5	4.5	Likes
	Smell	4.4		
	Texture	4.5		
	Taste	4.5		
Palolo District	Color	4.35	4.3	Likes
	Smell	4.4		
	Texture	4.3		
	Taste	4.35		

The flat and dense surface of tempeh is caused by molds that produce hyphae and mycelium, thus forming a compact structure (Cempaka et al., 2020). The density of tempeh increases with fermentation time due to loss of water content and tissue formation in soybeans, resulting in favorable evaluations from panelists (Kinanti, 2024). The assessors ultimately appreciated the yellow pumpkin seed tempeh flavor, which resulted from the enzymatic breakdown of the substrate's macromolecules, leading to a unique and palatable taste (Zhao et al., 2018). The outcomes of organoleptic evaluations indicate that yellow pumpkin seed tempeh possesses the potential to be a palatable and high-quality food product, serving as an alternative for individuals seeking healthy and nutritious dietary options. The judges favored yellow pumpkin seed tempeh products in the order of samples from the North Lore District, Palolo District, and Palu City. Nonetheless, the judges' evaluation indicated a preference for yellow pumpkin seed tempeh. The Indonesian National Standard (2015) evaluation for yellow pumpkin seed tempeh indicates that it meets the established organoleptic criteria. The tempeh exhibits a uniform white surface, a characteristic aroma devoid of ammonia, a compact texture that resists disintegration, and the distinctive flavor associated with tempeh.

Student worksheets

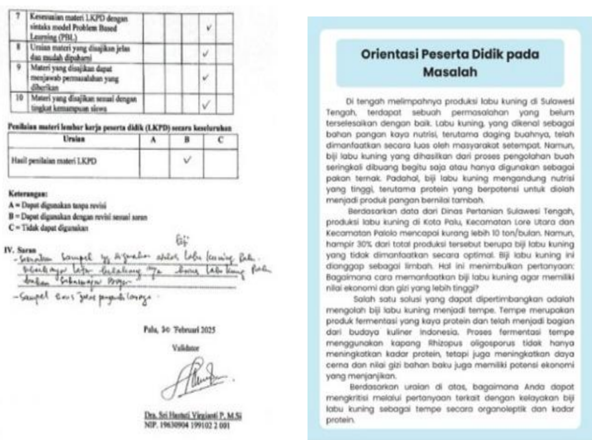
The Student Worksheet is an educational resource that facilitates independent information comprehension and encourages students to actively solve problems. It is anticipated that these worksheets will enhance students' skills. This can be accomplished by supplying high-quality student workbooks. One method is to validate student worksheets (Desmiwati et al., 2017).

The validation of student worksheets assesses their overall practicality (Sari, 2023). Student worksheets are assessed by expert lecturers who serve as validators for both content and design elements. The validation results for the material element indicated that the prepared student worksheets were valid, achieving an average validity score of 80%. In contrast, the design aspect received an average validity score of 90%, categorized as very valid. The validation outcomes by materials and design specialists are displayed in **Table 5.**

Table 5. Validation assessment by experts

Assessment aspects	Rating scale (%)
Material Expert	80
Design Expert	90
Sum	170
Average	85

The validator's evaluation indicates that the student worksheets require improvement based on the recommendations provided. The recommendations and contributions offered serve as a basis for modifying student worksheets. The recommendation for validating the material aspect is to modify the sampling location to align with the background of the student worksheets. Recommendations and enhancements are illustrated in **Figure 1.**



a b

Figure 1. Suggestions and improvements to the validity of material aspects

Description: a). suggestion by material aspect validator; b). improvement of student worksheets

The average evaluation by professionals indicates that these student worksheets are highly suitable as a chemistry learning resource, achieving a rating of 85%. Student worksheets are considered legitimate if the percentage score is greater than or equal to 61 %. The appropriateness of the material in the feasibility component is evaluated based on the alignment between the presented challenge and the learning objectives. The validation results indicate that the arrangement of students in addressing difficulties in worksheets is highly effective. Issues can be resolved by extracting information from many sources and performing analysis. The chemistry study is intrinsically linked to solving chemistry problems using scientific methodology. Consequently, educational tools emphasizing problem-solving are essential for cultivating students' capacity to generate new knowledge and facilitate learning (Wahyuni & Miterianifa, 2019).

Conclusions

This research shows that yellow pumpkin seed tempeh complies with national standards for its appearance in the three regions of Central Sulawesi, with a panelists' preference rating of 4 in the likes category. Protein content samples from Palu City exhibited the highest protein content at 24.5%, followed by Palolo District at 23.91%, and the lowest protein levels were found in North Lore District at 20.41%. The research findings, validated by materials and design specialists, are viable as a chemistry learning resource, achieving an 85 % approval rate. This research shows the potential of yellow pumpkin seeds as a source of protein. It produces valid chemistry learning resources to increase awareness of the importance of using local resources.

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