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Spectroscopic Analysis of Tempeh Protein Content during the Production Process

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Abstrak

Tempeh is a local Indonesian food that is liked by the community, both locally and internationally. The high protein content is the reason for choosing tempeh as a complementary food. In its production, tempeh is made by two important processes, including treatment and fermentation. During this process, the protein can be damaged, resulting in a decrease in the protein content of tempeh. Based on these problems, this study aims to analyze the reduction of tempeh protein content during the production process. The analysis used two spectroscopic methods, including FTIR and UV-Visible. FTIR spectroscopy was used to observe the reduction in total protein content, while UV-Vis spectroscopy was used to observe the reduction in dissolved protein content. The results of the study for FTIR analysis showed that at the pretreatment and fermentation stages, both caused a decrease in the total protein content of tempeh. This was observed with a decrease in the intensity of IR absorption at a wavenumber of 1745 cm^{-1} which is identical to the C=O group and 1543 cm^{-1} which is identical to the N-H group. Based on the results of the UV-Visible study, it shows that the fermentation time affects the amount of dissolved protein. This situation is supported by the increasing pH of tempeh and decreasing water content during fermentation.

1. INTRODUCTION

Spectroscopy is a modern analytical method based on the interaction of matter and energy. There are several types of spectroscopic methods, including nuclear magnetic resonance (NMR) (Capitani et al., 2017), infrared (IR) (McMullin et al., 2015), near infrared (NIR) (Landau et al., 2018), middle infrared (MIR) (Song et al., 2016), Raman, fluorescence (Acković et al., 2018), and UV-Vis spectroscopy (Van de Voort, 1992). In recent years, this method has been widely reported for its use in the analysis of food products, both qualitatively and quantitatively. Several advantages, such as easy sample preparation, fast analysis, sensitivity with high accuracy, are the reasons why this method is widely used (Baeten & Dardenne, 2002).

Among the types of spectroscopic methods above, FTIR and UV-Vis are two spectroscopic methods commonly used in the analysis of a food sample. Generally, FTIR is used in qualitative analysis by studying the chemical properties of the constituents of a sample, such as functional groups (Durazzo et al., 2018; Lucarini et al., 2020). The analysis

was carried out by observing the absorption band at a specific wave number. Where, each sample will produce a different specific absorption band, so on this basis an analysis of the chemical properties of the sample can be carried out. As for UV-Vis, it is used in quantitative analysis. This analysis is intended to determine the level or concentration of a chemical compound (da Silva-Buzanello et al., 2015; Zhang et al., 2015). In the analysis, a chemical compound will produce a different maximum wavelength. This wavelength is then used in determining the level or concentration of a chemical compound. Examples of food analysis of these two methods include carbohydrate analysis of juice samples (Domingues et al., 2014; Leopold et al., 2011), glucose analysis (Yu et al., 2012), lipid analysis of meat products (Candoğan et al., 2021), and others.

The content of chemical compounds in a food product which is also important to analyze is protein. Protein is a group of biochemical compounds that have many roles in the body, for example as a source of energy, providing enzymes and hormones, and supporting the immune system (Wycherley et al., 2012). Based on this, protein becomes one of the important parameters in explaining the quality of a food product.

One food product that contains a lot of protein is tempeh. Tempeh is a processed soybean product that is made through two important stages, including the pretreatment and fermentation processes (Suwanto et al., 2013). Tempeh is not only consumed by local community, but tempeh is widely consumed by the global community. Tempeh has an economical selling price with a distinctive taste, so it is used as a favorite complementary food. In the health sector, tempeh is reported to have many benefits, including as an antioxidant, inhibitor and cholesterol-lowering, preventing the risk of cancer, prostate, and many other benefits (Tahir et al., 2018). In the food industry, tempeh is used as a food additive in the manufacture of fermented soy sauce (Rosmini et al., n.d.).

The minimum protein content in tempeh is 16% (w/w) (Lestari & Mayasari, 2016). This level can be reduced during the production process. As previously explained, the tempeh production process consists of two important stages, namely pretreatment and fermentation. The pretreatment process consists of five stages, including boiling, grinding, washing, soaking, and steaming (Ahnan-Winarno et al., 2021; Damanik et al., 2018; Ferreira et al., 2011). The presence of protein content in this process is strongly influenced by temperature. In general, this process uses very high temperatures, so it will have an impact on damage to the protein structure, and continue to decrease the protein content of tempeh (Schön et al., 2017). Likewise with the fermentation stage. This step is reported to cause the breakdown of complex proteins into simple proteins. The use of time is an important parameter that needs to be considered during this process. The long fermentation time will gradually cause damage to the protein structure (Babu et al., 2009; Chang et al., 2009; Reyes-Moreno et al., 2000). This process will produce protease enzymes that play a role in breaking down the structure of protein complexes into amino acid monomers or simple proteins (Handoyo & Morita, 2006).

Although research on tempeh has been widely reported, the analysis of protein content during the production process has not been reported. So in this work, we specifically report the analysis of tempeh protein content during the production process using two spectroscopic methods, including FTIR and UV-Visible.

2. METHODS

1. Materials

Tempeh as a test sample, soybean as a comparison sample, phosphate buffer pH 8 (Sigma-Aldrich, CAS number : 9011-18-1) as a solvent, biuret reagent (Sigma-Aldrich, CAS number : B3934) as a reagent, aquades as a solvent, and qualitative filter paper (Whatman, Cat No. 1004.110) as a filter.

2. Methods

Tempeh sampling

Tempeh samples were obtained from the Baruga traditional market, Southeast Sulawesi-Indonesia (Fig. 1). Sampling was done randomly, with the selected sample criteria were new tempeh that had not undergone the fermentation process. The tempeh samples obtained were then stored in a sterile room at room temperature. This storage is part of the fermentation process.

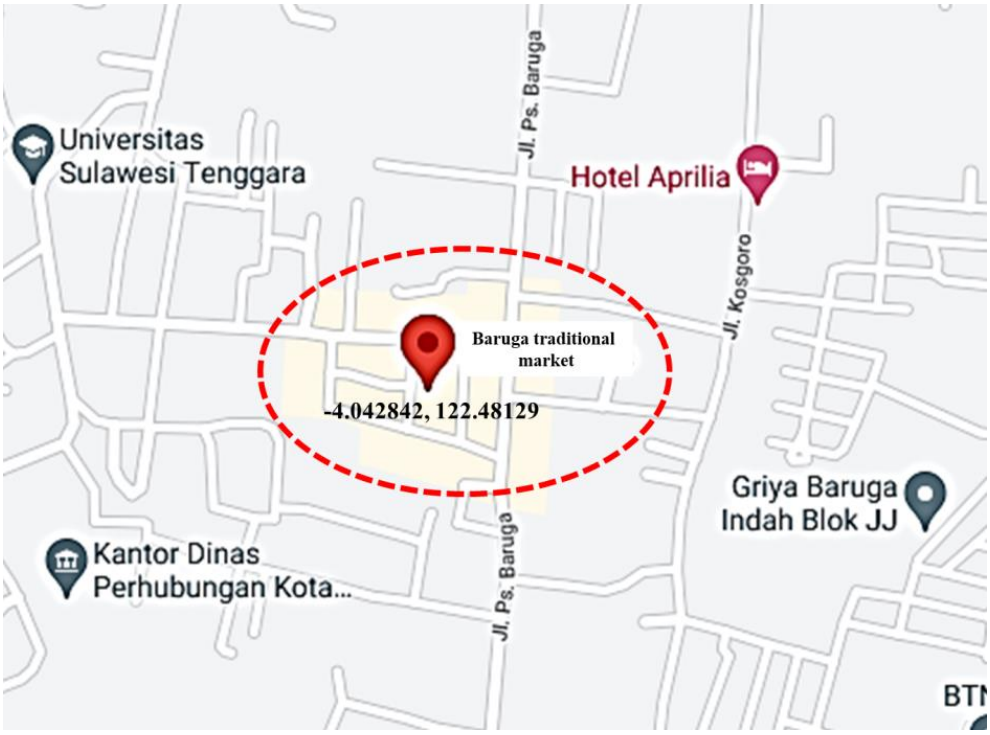

Figure 1. Map of Bagura traditional market.

Analysis of protein content

The protein content of tempeh was analyzed by two spectroscopic methods, including FTIR (IRprestige-21 fourier transform infrared spectrophotometer Shimadzu) and UV-Visible (Agilent 8453). FTIR analysis begins with drying the sample in an oven at 80^{o}C for 24 hours. Tempeh was mashed and filtered using a sieve with a pore size of ± 0.3 mm. Furthermore, it is formed into thin pellets by adding KBr solids and measuring the absorbance of the wave number. The UV-Visible analysis was carried out by weighing 1.0 g of fermented tempeh samples (1 to 5 days), dissolved in 50 mL of phosphate buffer pH 8 and stirred with a magnetic stirrer. Next, the mixture was centrifuged and filtered using filter paper. The filtrate obtained was transferred to a 50 mL volumetric flask, then phosphate buffer pH 8 was added to the mark. 1.0 mL of filtrate was taken, then 3.0 mL of Biuret reagent was added, vortexed and incubated at room temperature for 30 minutes. Then the absorbance was measured at a maximum wavelength (λ_{max}) of 540 nm using a UV-Vis spectrophotometer.

3. RESULT AND DISCUSSION

FTIR analysis of tempeh total protein

The decrease in the amount of total protein, both during the pretreatment and fermentation processes, was observed based on the specific IR absorption of the protein functional groups. Based on their chemical structure, proteins are composed of amino acid monomers linked through amide bonds (Valdar & Thornton, 2001). This bond consists of C=O and N-H groups which will absorb IR at wave numbers 1745 cm^{-1} (specific group for C=O) and 1543 cm^{-1} (specific group for N-H). The structure of the protein amide bond is shown in Figure 2.

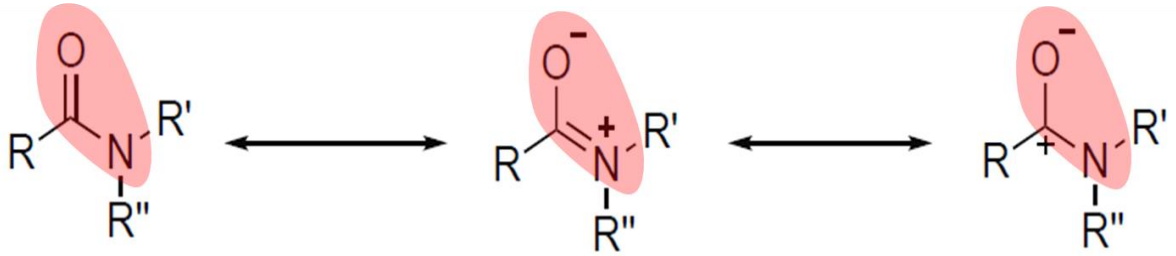

Figure 2. Protein amide bond structure.

The amide bond has a tendency to resonate (see Fig. 2), this situation can describe the stable nature of the peptide bond at high temperature. The resonance process produces a planar structure that prevents the free rotation process around the C=O-N bond. The amide bond will initiate the formation of a more complex protein structure. Through this bond, protein monomers will bind to each other and form a distinctive structure (Fig. 3). Protein monomers are amino acids, which can be grouped into 20 types of amino acids. Everything is used by the body in the metabolic process.

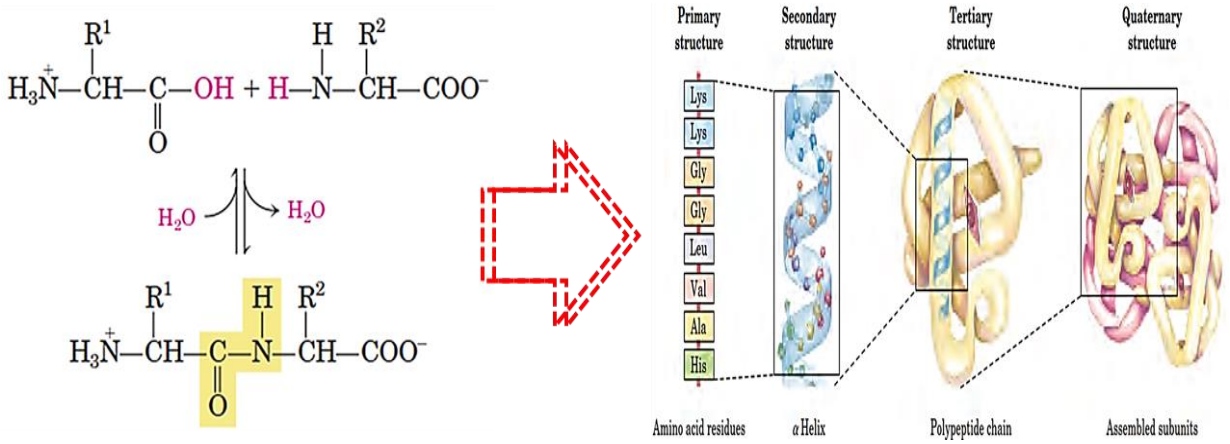

Figure 3. The process of protein formation.

Figure 4 generally shows the IR spectra of samples before tempeh production (Fig. 4a), samples during pretreatment (Fig. 4b) and samples after fermentation (Fig. 4c).

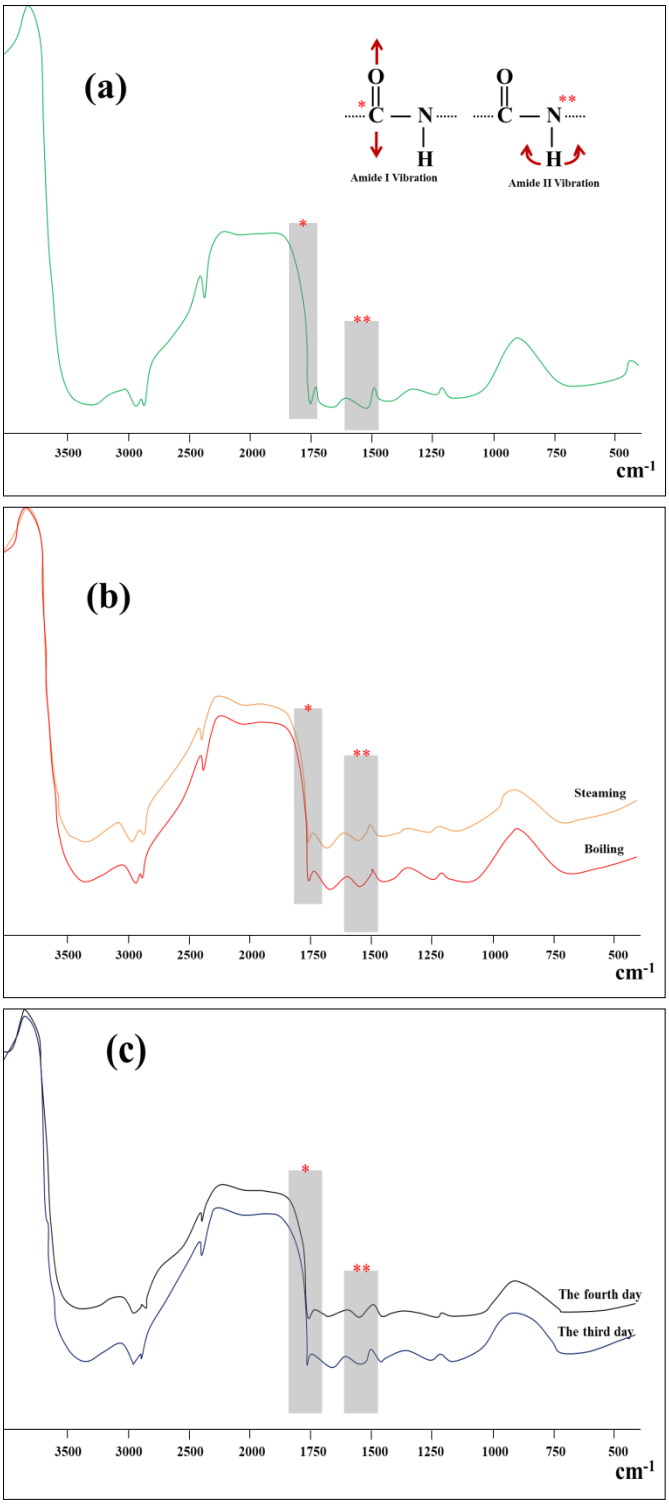

Figure 4. IR spectra of the sample: a) before tempeh production, during pretreatment, and c) after fermentation.

The sample in Figure 4 is observed based on the difference in the intensity of IR absorption at wavenumbers 1745cm^{-1} and 1543 cm^{-1}. The high absorption intensity correlated with the total protein amount contained in each sample. Based on Table 1, it can be seen that there was a decrease in the absorption intensity of the protein during the pretreatment process, both boiling and steaming (IR spectra can be seen in Fig. 4b).

Table 1. Intensity of IR absorption during the tempeh production process.

Wave number (cm-1)	Transmitance (%)				
	Before pretreatment and fermentation	Pretreatment process		Fermentation Process	
		Boiling	Steaming	Day 3	Day 4
1543	26.69 ± 0.1	25.45 ± 0.1	14.80 ± 0.1	13.85 ± 0.1	13.14 ± 0.1
1745	25.51 ± 0.1	22.29 ± 0.1	14.21 ± 0.1	14.11 ± 0.1	13.25 ± 0.1

The same thing was also shown in the samples after fermentation, both on the third and fourth days (IR spectra can be seen in Figure 4c). In the boiling stage there was a decrease in % Transmittance (% T) in the two typical wave numbers of proteins by 4.64 % (for 1543 cm^{-1}) and 12.62 % (for 1745 cm^{-1}). Similar to the boiling stage, in the steaming stage there was also a decrease in % T of 44.54 % (for 1543 cm^{-1}) and 44.29 % (for 1745 cm^{-1}). Meanwhile, during the fermentation process, it was seen that the fermentation time affected the total protein content of tempeh. The decrease in % T in fermentation for 3 days was 48.10 % (for 1543 cm^{-1}) and 44.29 % (for 1745 cm^{-1}), while in fermentation for 4 days it was 50.76 % (for 1543 cm^{-1}) and 48.05 % (for 1745 cm^{-1}). The percentage reduction in % T during the pretreatment and fermentation processes is shown in Figures 5 and 6.

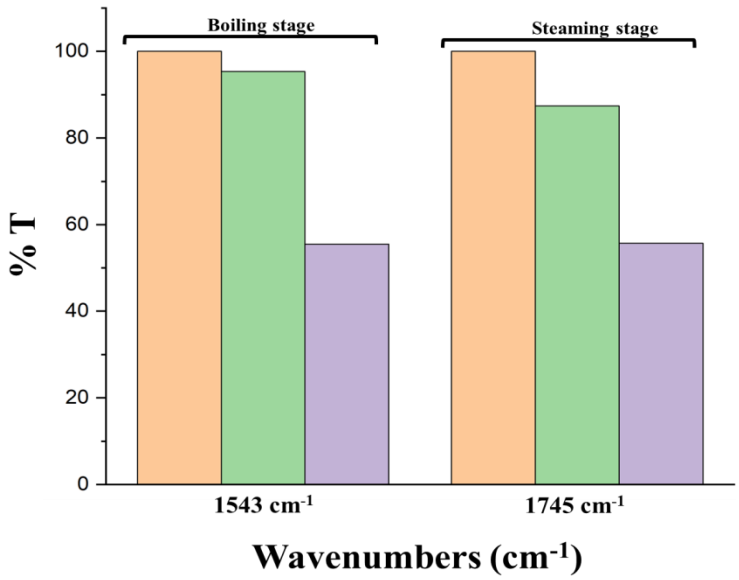

Figure 5. Percentage of % T reduction in the pretreatment process : a) boiling stage, b) steaming stage.

In general, Figure 5 shows that the decrease in % T, both during the boiling and steaming stages, correlates with a reduction in the protein content of tempeh. In addition, based on the

figure, the rate of protein reduction in both pretreatment processes was relatively the same. However, the initial protein reduction occurs more rapidly at the steaming stage.

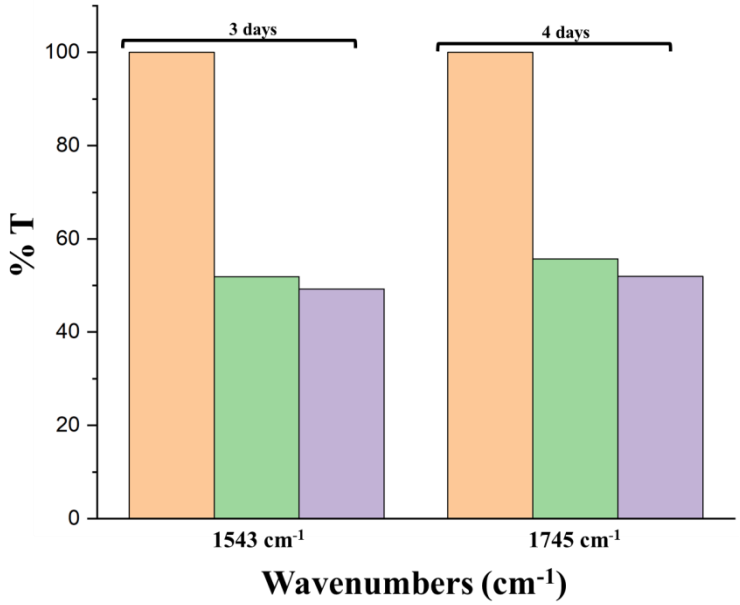

Figure 6. Percentage of % T reduction in the fermentation process.

As in Figure 5, the decrease in % T during the fermentation stage (Fig. 6) correlated with a reduction in the protein content of tempeh. The rate of protein reduction during this process is relatively the same. However, when compared to the pretreatment stage, the rate of protein reduction at this stage is relatively slower and occurs constantly.

UV-Vis analysis on tempeh dissolved protein content

In evaluating the dissolved protein content using UV-Vis spectroscopy, we observed the effect of pretreatment temperature and fermentation time. The use of Benedict's Reagent will cause the formation of a complex between the peptide bond and Cu^{2+} ions, as shown in Figure 7a. The formation of the complex is based on the presence of an electron pair in the peptide bond which is not shared with nitrogen and water oxygen. The formation of this complex produces a purple color with a maximum wavelength of 540 nm (Fig. 7b).

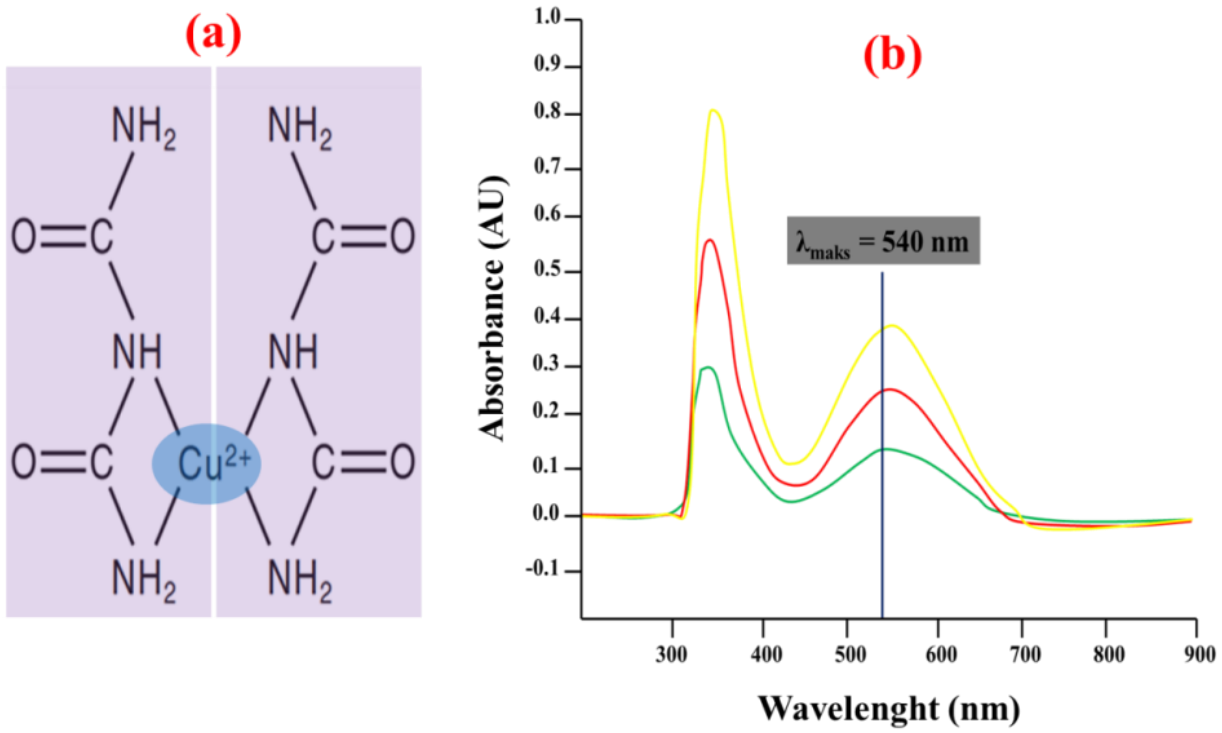

Figure 7. (a) Complex structure of the peptide bond with Cu^{2+}; (b) Maximum wavelength (λ_{max}) of the peptide complex and Benedict's Reagent.

Figure 8 shows the effect of pretreatment temperature and fermentation time on dissolved protein. Based on Figure 8a, it can be seen that the high pretreatment temperature (boiling and steaming) causes the peptide bonds to break, thereby increasing the amount of dissolved protein. These results are correlated with the results of the FTIR analysis as shown in Figure 4b. Based on this data, tempeh pretreatment needs to pay attention to temperature, so as to produce tempeh that meets BSN standards. The results of observations for soluble protein content of tempeh which fermented for 1 to 5 days are shown in Figure 8b. Based on the figure, it can be seen that there is a correlation between fermentation time and the amount of dissolved protein. The amount of dissolved protein will decrease along with the length of fermentation time. This decrease can be attributed to the activity of the protease enzyme produced by *Rhizopus sp*. in breaking the peptide bonds of tempeh protein into amino acids and short chain peptides.

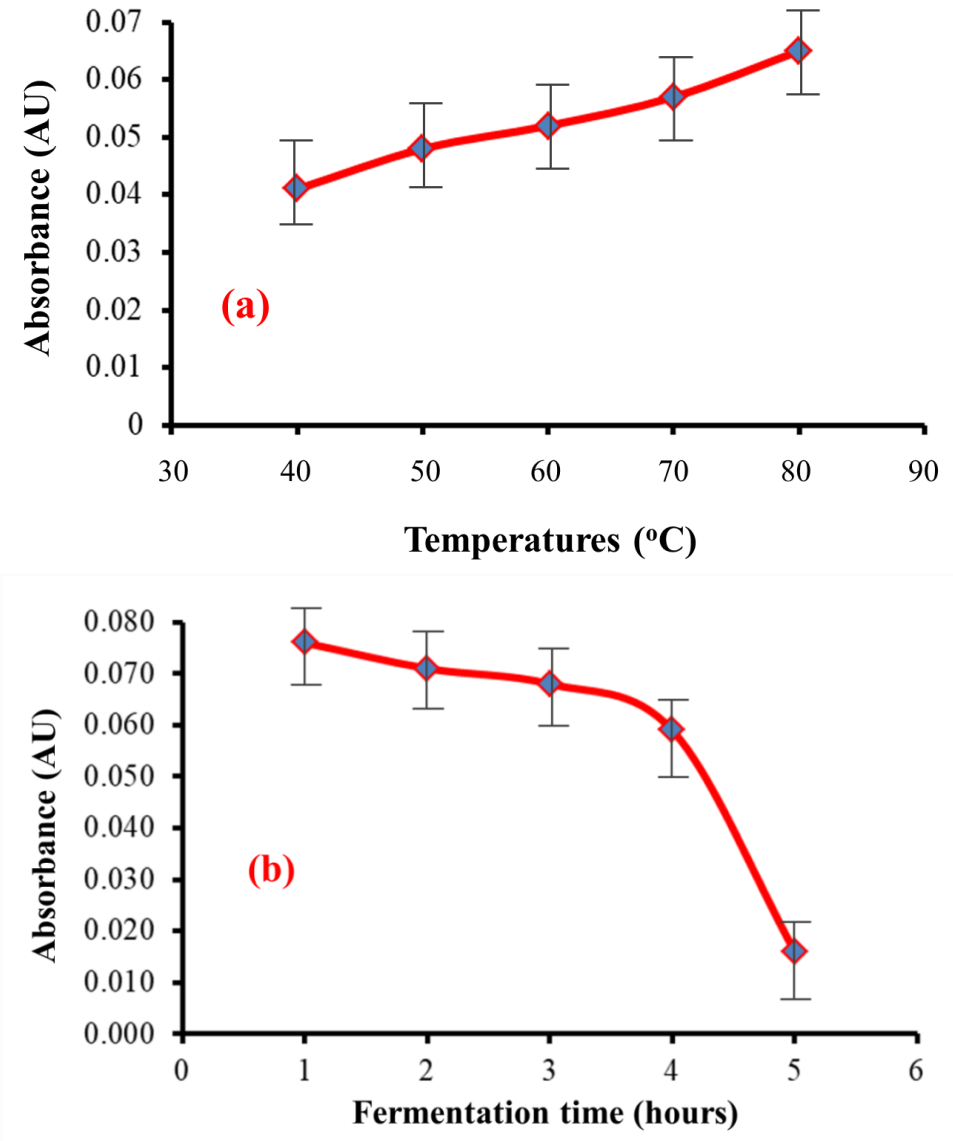

Figure 8. Correlation of dissolved protein to: a) temperature; b) fermentation time.

The formation of short chain amino acids and peptides was indicated by an increase in pH during the fermentation process (Table 2). Gradually, the fermentation time caused the tempeh pH to be higher until it approached neutral pH (pH = 7.0). This property is correlated with the amount of protein that decomposes and the decreased activity of the protease enzyme from *Rhizopus sp*.. The activity of the protease enzyme is strongly influenced by pH, where at an increasingly alkaline pH, the activity of the protease enzyme will decrease. An increase in pH is also associated with the formation of free amino acids (Churchill et al., 2004). Table 2 also shows that another phenomenon regarding the decrease in the amount of dissolved protein can be associated with a decrease in the amount of water content in tempeh. Other enzymes from *Rhizopus sp*. can damage hydrogen bonds, both those formed between the amide group and water molecules or the amide group with other amide groups. Another factor that causes a decrease in water content is the activity of microorganisms that damage the starch component thereby reducing the OH bond in its structure (Kurniadi et al., 2019).

Tabel 2. Correlation of time to pH.

Time (hours)	pH	Water content (%)
1	4.67 (±0.2)	6.40 (±0.1)
2	5.07 (±0.2)	4.17 (±0.1)
3	5.40 (±0.2)	3.09 (±0.1)
4	5.50 (±0.2)	2.74 (±0.1)
5	5.65 (±0.2)	2.04 (±0.1)

4. CONCLUSION

This study succeeded in analyzing the reduction of tempeh protein content during the production process. This analysis is important to do in describing the quality of tempeh. The use of two spectroscopic methods, including FTIR and UV-Vis, was able to effectively analyze tempeh proteins, both total and dissolved proteins. Specific IR absorption of tempeh protein was observed at two wave numbers (1/λ) namely 1543 cm^{-1} and 1745 cm^{-1}. These absorptions are derived from the N-H and C=O groups, respectively, which are connected via amide bonds. Based on the results of the analysis, both FTIR and UV-Vis explained that the tempeh production process was influenced by two conditions, namely pretreatment and fermentation. Both have an effect on the availability of protein, both total protein and soluble protein. The results of this study can then be used as a reference for protein analysis in various other food samples based on FTIR and UV-Vis spectroscopy.

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