Effects of adding andaliman extract to the shelf life of tempeh nugget product

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Abstract. Andaliman is typical spice plant in North Sumatra, has benefits as antimicrobial and antioxidant. Tempeh is a protein source food, has short shelf life, so the tempeh processing technology is needed to create another product with longer shelf life and increase its added value. One of them is by processing tempeh into nugget. The purpose of this study was to determine the shelf life of tempeh nugget added with andaliman fruit extract as natural preservative and to determine which concentration was the most optimal in extending the shelf life of tempeh nugget. This study used completely randomized design with two factors. Factor I was the concentration of andaliman extract (0%, 0.1%, 0.25%, 0.5%) and factor II was the storage time (0 day, 7 days, 14 days, 21 days). The parameters analysed were total microbial, water content, and aroma organoleptic test. The results showed that the addition of 0.5% andaliman extract and 21 days storage time obtained the lowest amount of microbes, the lowest water content was obtained at 0% andaliman extract and 21 days storage time treatment, while the highest aroma organoleptic test was obtained at adding 0.5% andaliman extracts. This is due to the presence of antimicrobial in andaliman.

1. Introduction
Andaliman fruit (Zanthoxylum acanthopodium DC) is one of the typical spice plant from North Sumatra [1]. It has anti-diabetic and antioxidant property, which is often used as a spice in batak cooking, and affect the dishes tend to have a longer shelf life [2]. Tempeh is a source of protein, which is low in calory and cholesterol [3]. The use of tempeh as a food source still has constraints, namely its relatively short shelf life and perishability [4]. Therefore, the alternatives are needed to increase its added value, one of which is to process tempeh into nuggets. Nugget is basically made from animal-based ingredients such as chicken meat [5], but due to the high fat content in chicken, then it can be replaced with a local food source, namely tempeh. Nugget is a processed food with shelf life, so it requires natural preservatives, such as andaliman fruit. Therefore, this study aimed to determine the shelf life of tempeh nugget added with andaliman fruit extracts as natural preservatives with various types of concentrations.

2. Materials and methods

2.1. Materials
Fresh andaliman fruit was obtained from Parbuluan II village, Parbuluan Subdistrict, Dairi Regency. Chemicals and media used were namely NA (Nutrient Agar), MHA (Mueller Hinton Agar), Nutrient
Broth (NB), dimethylsulfoxide (DMSO), physiological NaCl 0.9%, aquades 70%, paper discs (Oxoid), methanol, chloroform, and Plate Count Agar (PCA) media. Equipments used were autoclaves, laminar flow, oven, analytical scale, (Adventurer Ohaus), flow pipettes, oven, visible spectrophotometer, ose needle, spatula, desiccator, Bunsen, vial, analytical scale (Mettler Toledo), incubator, rotary evaporator (Stuart), petri dishes, and colonies counter.

2.2. The making of andaliman fruit extract
Andaliman was washed, cleaned and dried using an oven at 40 °C for 48 hours. The sample was then blended dry until it became powder and ready to be extracted. After that, the simplicia powder was put into 4 pieces of Erlenmeyer and macerated with ethyl acetate solvent. Filtering was conducted at room temperature, avoided to be exposed to direct sunlight and tightly closed with cotton and aluminium foil. After 3 days of operation, the maserat was then filtered. The filtrate obtained was concentrated with a rotary evaporator at 50 °C and evaporated in vacuo, so that the solvent was separated with the thick extract of andaliman [6].

2.3. The making of tempeh nugget
Tempeh was steamed at 65 °C for 30 minutes, then blended. The blended tempeh then mixed with tapioca flour, eggs, onions, garlics, salt, peppers, and nutmeg. The mixture was stirred until it homogeneous, then printed and steamed. Steaming was done for 30 minutes, then followed by the process of drying. The drying process was carried out by dipping the nugget mixture into the egg whites and bread flour.

2.4. Determination of water content (%)
Determination of water content was carried out according to the AOAC method [7]. 5 g of tempeh nuggets were put into an aluminium cup, which had been dried for 1 hour at 105 °C, and their weight were known. The tempeh nuggets were heated to 105 °C for 3 hours. Furthermore, they were cooled in a desiccator and weighed, these processes were repeated several times until a constant weight was obtained. Heating and cooling were repeated until a constant tempe nuggets weight were obtained. The calculation was as follows:

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\text{Moisture content (\%) = } \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100\% \tag{1}
\]

2.5. Determination of total microbes by the total plate count method
Determination of total microbial content was carried out according to the total plate count method [8]. Tofu weighed as much as 5 g was put into an erlenmeyer flask containing 45 mL of sterile physiological salt, diluted decimally from 10¹ to 10³, then each of 1 mL of suspension was put into a sterile petri dish, then 12-15 mL of warmed Plate Count Agar (PCA) solution (40-50 °C) was poured, homogenized. The hardened petri dishes then reversed and incubated at 37 °C for 48 hours. After 48 hours, the number of microbial colonies contained in the cup were counted, with the provision of colony number was between 30-300.

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\text{Total colony = Number of colonies per cup x 1 / FP} \tag{2}
\]

FP = diluent factor

2.6. Organoleptic test of aroma
The aroma organoleptic test was determined using a hedonic value scale and randomly tested by 15 panellists. Testing was done based on sensory hedonic scale.
2.7. Statistical analysis
Data were analysed using SPSS software (22.0). The difference in mean values was tested by analysis of variance, and the level of significance was obtained by the Duncan test. It used significance level of P < 0.05.

3. Results and discussion

3.1. The effect of adding the concentration of Andaliman fruit extracts to the parameter of tempeh nugget
Table 1 shows that the treatment of A₁ (0%) without the addition of andaliman extract in the tempeh nugget obtained the largest number of microbes, amounting to 6.171 (log cfu/mL), while the smallest number of microbes, namely at the A₄ treatment (0.5%). The results of this study prove that the more the amount of andaliman extract added to the tempe nugget, the smaller the amount of microbes remaining, due to essential oil components such as geraniol (14.75%) and geranil acetate (33.44%) found in andaliman, those which can inhibit microbial growth [5]. The highest water content was obtained in the A₄ treatment (0.5%), which was 55.7086%, while the lowest water content was obtained in the A₁ treatment (0%) without the addition of andaliman fruit extracts. This is caused by andaliman fruit having a fairly high water content, which was 14.83% in the form of powder [9]. Organoleptic aroma test revealed that A₄ treatment containing 0.5% andaliman fruit extract was of 3.928 and A₃ treatment containing 0.25% andaliman fruit extract was of 3.606. Therefore, it can be concluded that the panellists liked the aroma of tempeh nugget added with Andaliman fruit extract due to the essential oil content contained in andaliman fruit such as citronellal, limonene, geranil acetate [10].

Table 1. Effect of the additional concentration of andaliman fruit extracts on the parameter of tempeh nugget

| Parameter                  | Treatment |
|----------------------------|-----------|
|                            | A₁ (0%)   | A₂ (0.1%) | A₃ (0.25%) | A₄ (0.5%) |
| Water content (%)          | 55.298±1.779³⁵⁴⁶ | 55.384±1.798³⁸⁹⁰ | 55.379±1.885³⁸⁹⁰ | 55.327±1.806³⁸⁹⁰ |
| Total Plate Count (log cfu/mL) | 95.917±6.511³⁵⁴⁶ | 80.417±8.925³⁸⁹⁰ | 69.500±8.212³⁸⁹⁰ | 54.167±7.183³⁸⁹⁰ |
| Organoleptic test of aroma | 0.922±0.156³⁸⁹⁰ | 2.683±0.038³⁸⁹⁰ | 3.606±0.033³⁸⁹⁰ | 3.928±0.033³⁸⁹⁰ |

³Different letter notations show significantly different effects at 5% (lowercase) level and very significantly different at 1% (uppercase) level according to the DMRT test
⁴Data is the means of three replications

3.2. Effect of storage time on tempeh nugget parameter
Table 2 shows that the B₄ treatment (storage for 21 days) obtained the lowest total microbes, that was 3.463 (log cfu/mL), the longer the storage with the addition of Andaliman in the tempe nugget, the smaller the number of microbes contained in the tempe nugget. This is caused by prolonged freezing temperature results in inactive microbes [8]. At freezing and cold temperatures, there is increase in intracellular solid concentration resulting in physical and chemical change in bacterial cells that cause rot [12]. The longer the storage period, the lower the nugget water content. This is caused by the decrease of water holding capacity, indicated by the increasing amount of free water [11]. Organoleptic test of aroma indicates that the longer the storage, the aroma of tempeh nugget decreases, but when viewed from 0 days to 21 days storage judging by panellists were not significantly different. This is affected
by the concentration of Andaliman fruit extract added only about 0.1-0.5%. This concentration did not significantly cause the distinctive aroma of andaliman and the aroma of essential oil in frozen storage did not evaporate and was stuck in the nugget.

### Table 2. Effect of storage time on tempeh nugget parameters

| Parameter                  | Treatment                |
|----------------------------|--------------------------|
|                            | B₁ (0 day) | B₂ (7 days) | B₃ (14 days) | B₄ (21 days) |
| Water content (%)          | 57.343±0.109ᵃᴬ       | 56.315±0.086ᵇᴮ | 54.405±0.024ᶜᶜ | 53.325±0.072ᵈᴰ |
| Total Plate Count (log Cfu/ml) | 85.500±7.677ᵃᴬ | 75.833±6.770ᵇᴮ | 71.500±6.568ᶜᶜ | 67.167±8.751ᵈᴰ |
| Organoleptic test of aroma | 2.817±1.324ᵃᴬ       | 2.789±1.303ᵃᴬ | 2.778±1.298ᵃᴬ | 2.756±1.472ᵃᴬ |

ᵃDifferent letter notations show significantly different effects at 5% (lowercase) level and very significantly different at 1% (uppercase) level according to the DMRT test

ᵇData is the means of three replications

### 4. Conclusion

The results of the analysis showed that tempeh nugget given 0.5% andaliman extract and stored at freezing temperature (-18°C) produced good quality in accordance with SNI standard for nugget quality until the 21st day of storage time.

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