PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF BUFFALO MEAT PATTY WITH THE ADDITION OF FENUGREEK SEED (Trigonella foenum graecum) DURING STORAGE TIME

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ABSTRACT

Buffalo meat has a good nutritional profile for the development of processed livestock products to meet consumer demand. For some reason, buffalo meat is less desirable to consumers because of its dark colour, coarser fibres, and tougher texture. Innovative product development is necessary as consumer demand tends to shift to ready-to-eat products. Processing buffalo meat into patties is an innovation in product development. Quality products require beneficial ingredients. Fenugreek seeds (Trigonella foenum-graecum) are herbal spices that are beneficial for health, contain protein, fibre, stabilizers, and emulsifiers, and have the potential for product development. The purpose of this study was to determine the effects of anise seed addition on the characteristics of buffalo meat patties. This study used a factorial Complete Randomized Design (4x2) with 4 replications. The factor I was the addition of fenugreek powder, F0 = control or 0%, buffalo meat patty dough + (F1 = 3% fenugreek powder; F2 = 6% fenugreek powder; F3 = 9% fenugreek powder). Factor 2 had a storage time of D1 = 30 days and D2 = 60 days. The results showed that the addition of fenugreek powder had a very significant effect (P<0.01) on cooking shrinkage, protein content, tenderness, and total plate number. The addition of fenugreek powder had a significant effect (P<0.05) on pH, fat content, and moisture content, whereas in the storage period of 30 days (D1) to 60 days (D2), it had no significant effect (P>0.05). The addition of fenugreek powder can reduce pH, cooking weight loss, moisture content, and microbes. The addition of fenugreek powder to buffalo meat patties also increased tenderness, protein content, and fat content. The addition of fenugreek seed powder to buffalo meat patties at 6–9% is recommended because it can improve physicochemical and microbiological quality.

Key words: Patty; fenugreek; physical; microbiological, quality; meat
INTRODUCTION

Buffalo is a ruminant livestock kept by farmers in Indonesia for milk and meat. Buffalo's development around the world is carried out by improving genetic quality, applying technology, and improving husbandry management. Buffaloes have the potential to be developed because buffalo meat is not inferior in quality compared to beef (Borghese et al., 2022). Buffalo meat consumption patterns remain very low because of the habits of people who mostly prefer beef and chicken. The texture and flavour of beef and chicken are considered more palatable than buffalo meat. Buffalo meat is darker in colour and tougher, making it less palatable to consumers.

According to Naveena and Kiran (2014), buffalo meat is not inferior in quality, especially in protein content, which is 20-23% compared to beef with a protein composition of 19-20%. Buffaloes are adaptable to the environment and therefore have the potential to be developed. According to several studies by Mendrova et al. (2016), buffalo meat is rarely consumed because it has a darker colour and sharper aroma. Buffalo meat also has a rough texture, so innovation is needed to increase the added value of the product to increase favorability and marketability. Patty is ground meat that is flattened and moulded into a round shape that is usually used as a filling and served as a dish. Patty is a practical preparation that can be stored frozen.

Quality-processed livestock products require ingredients that have added value and that provide benefits. Fenugreek (Trigonella foenum-graecum) is one of the oldest herbs cultivated in Indonesia and has health benefits. Fenugreek seeds are aromatic, slightly bitter, and sweet and are usually used as spices in Indonesian cuisine. According to Khoshidian et al. (2016), fenugreek seeds contain amino acids, fatty acids, saponins, folic acid, disogenin, gitogenin, neogitogenin, homorientin, saponaretin, neogigogenin and trigogenin. According to Nursetiani and Herdiana (2018), fenugreek exhibits antioxidant, antidiabetic, anti-inflammatory, antibacterial, antifungal, anticancer, and antiatherogenic activities.

Fenugreek seeds contain protein, fibre, stabilizing power, and emulsifiers that can be recommended for development and add value to products (Zaki, 2018). Fenugreek seeds are used as stabilizers, emulsifiers, antioxidants, and anti-fungal agents in food products (Salarbashi et al., 2019). Soaked fenugreek seeds contained (g/100 g) protein 26.45, fat 6.45, crude fibre 6.95, carbohydrate 43.74, ash content 2.92, moisture content 13.96, lysine 5.92 (Hooda Jood, 2002). In a study by Ktari et al., (2017), fenugreek seeds inhibited lipid oxidation and inhibited the oxidative process of fat and myoglobin because of its strong antioxidant content, which can replace vitamin C. Roberts (2011) added that fenugreek seeds contain dietary fibre to emulsify, which has the potential to be widely used in industry because the composition of galactomannan has emulsifying and stabilizing properties.

Fenugreek seeds are an aromatic species that has the potential to be applied to processed livestock products. To date, there has been no research on the use of fenugreek seeds in making buffalo meat patties; therefore, further research is needed to develop quality food product innovations. This study aimed to determine the effect of fenugreek seed addition on the physical and

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How to cite:
microbiological quality of buffalo meat patties during frozen storage.

**MATERIALS AND METHODS**

**Time and Place of Research**

The research was conducted at the Livestock Production Laboratory of the Faculty of Agriculture, University of Sumatra, and the Microbiology Laboratory of MIPA, Universitas Sumatera Utara. The materials used were fenugreek seeds, buffalo meat, wheat flour, table salt, and KCl.

The tools used in this research were a digital pH meter, moisture meter, cooking utensils, scales, and penetrometers. Fenugreek Seeds Powder Manufacturing Procedure Preparation of powder from spices using fenugreek seeds (*Trigonella foenum-graecum*) using the method described by Hegazy (2011), namely fenugreek seeds selected from the traditional market in Medan city with the best quality. The fenugreek seeds were soaked in distilled water at a ratio of 1:5 (weight/volume) for 12 h at room temperature.

Then, Fenugreek seeds were rinsed twice with distilled water, dried at 40°C, or aerated until dry. Fenugreek seeds were ground into a powder using a blender and stored in a glass jar for use.

**Patty making procedure**

Patty making was according to the procedure of Hegazy (2011) modified by the addition of fenugreek according to the treatment. Grind 1000 grams of fresh lean buffalo meat then mix it using 200 grams of wheat flour. Add 15 g of salt and crushed fenugreek seeds or (F) consisting of (F1=3%, F2 =6 %, and F3 =9 %). The patty dough was formed into a round shape with a thickness of approximately 1.5 cm and a diameter of 10 cm. The patty-making formulations are presented in Table 1.

**Table 1. Buffalo meat patty formulation using fenugreek seeds**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>F0 (0%)</th>
<th>F1 (3%)</th>
<th>F2 (6%)</th>
<th>F3 (9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo meat</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Fenugreek powder</td>
<td>-</td>
<td>30</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Salt</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

**pH Measurement Procedure**

Measurement of patty pH using an AMT16M digital pH meter. The pH was measured by inserting a pH meter needle into KCl and then entering Aquadest. Then the tip of the pH meter needle is poked into the patty sample and record the pH value shown on the display screen (Patriani et al., 2021)

**Cooking Weight Loss Measurement Procedure**

Cooking loss was tested using the CSIRO method by weighing a 10-gram sample patty (initial weight) and boiling it at 65 °C for 30 min. The sample was then wiped with tissue and weighed on a digital scale to obtain the final weight of the meat (Prayitno et al., 2020). The cooking loss was calculated using the following formula:

\[
\text{Cooking loss (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\% 
\]

Tenderness Measurement Procedure Patty tenderness was measured using a penetrometer by preparing a piece of patty sample with a size of 1 × 1 × 1 cm3. The pressure was applied to the sample using a penetrometer shear press until the sample broke in units of mm/g/s (Prayitno et al., 2020).

**Patty Chemical Composition Measurement Procedure**

Water content was measured using a DM300R moisture meter (Patriani et al., 2021). The procedure was performed by
turning on the moisture meat meter tool and puncturing the patty sample for 3 min.

After the number on the monitor screen stabilized, the percentage of water content was recorded. Protein levels were determined using the Kjeldahl method (AOAC 2005). Destruction, distillation, and titration constitute the three steps of this procedure. The fat content was measured using the Soxhlet technique (AOAC, 2005).

**Total Plate Count (TPC) Measurement Procedure**

Five (5) grams of patty sample was weighed, 45 ml of Butterfield's phosphate buffer, and then homogenized for 2 min. The homogenate was a 10-1 dilution solution. Then, 1 ml of the homogenate was placed in a bottle containing 9 ml of Butterfield's Phosphate Buffered solution to obtain a sample with a dilution of 10-2. For each dilution, shaking was performed at least 25 times. Do the same for dilutions 10-3, 10-4, 10-5, and so on according to sample conditions (Yunita et al., 2015)

**Research Design**

This study used a randomised complete factorial design (4x2) with 4 replications. The factor I is the addition of fenugreek powder:

- F0 = control or 0%
- F1 = buffalo meat patty dough + 3% *Fungereek* powder
- F2 = buffalo meat patty dough + 6% *Fungereek* powder
- F3 = buffalo meat patty dough + 9% *Fungereek* powder

Factor 2 is the shelf life which consists of:

- D1 = 30 days
- D2 = 60 days

Samples were stored frozen at -18°C. Data were analyzed using analysis of variance (ANOVA) if there was a significant effect, followed by the DMRT test (Steel and Torrie, 1991).

**RESULTS AND DISCUSSION**

**Physical Quality of Patty**

The test results in the study that the physical quality of buffalo meat patties, namely pH, cooking loss, moisture content, and tenderness can be seen in Table 2 below.

**Buffalo Meat Patty pH Value**

The degree of acidity or pH is one of the indicators that determine the physical quality of meat. The highest average pH in the patties without the addition of fenugreek powder (F0) was 7.07 and the lowest average pH value with the addition of 9% *Fungereek* (F3) was 6.28. The results of the analysis of variance showed that the pH value of the patty in the control (F0) was significantly different (P<0.05) with the pH value of the patty added with 3% (F1), 6% (F2), and 9% (F3) fenugreek powder. The pH value of the patties after 30 days of storage (D1) was not significantly different (P>0.05) after 60 days of storage (D2). The pH value can be influenced by the filler or base material used in the patties.

The basic ingredients used, namely, meat and flour, can change the hydrogen balance in the patty and influence the pH value. Mixing the basic ingredients creates the hydrogen balance point [Montolalu et al., 2013]. The DMRT test results showed that there was no interaction between the addition of fenugreek powder and the length of storage on the pH of the buffalo meat patty.

Fenugreek seeds contain high enough protein per 100 g of 23%, which can increase the water binding power because starch can bind water. During cooking, starch molecules bind to proteins through weakened hydrogen bonds, causing water molecules to enter between the proteins and starch molecules. When the patty is cooled,
there is a strengthening of hydrogen bonds between the starch molecules and hydrogen involving water molecules, which increases the pH value. Fenugreek has long been used as a spice to improve food quality [Wani & Kumar, 2018].

Table 2. Physical quality of buffalo meat patty with Funegreek addition during Storage Time

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Addition of Fenugreek powder</th>
<th>Storage Time</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F0: 0%</td>
<td>F1: 3%</td>
<td>F2: 6%</td>
</tr>
<tr>
<td>pH</td>
<td>7.00±0.02a</td>
<td>6.28±0.03b</td>
<td>6.27±0.01b</td>
</tr>
<tr>
<td>Average</td>
<td>7.07±0.07a</td>
<td>6.30±0.04b</td>
<td>6.29±0.04b</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>36.19±0.17a</td>
<td>29.27±0.11b</td>
<td>26.28±0.21c</td>
</tr>
<tr>
<td>Average</td>
<td>37.27±0.23a</td>
<td>29.85±0.21b</td>
<td>26.80±0.28c</td>
</tr>
<tr>
<td>Tenderness (mm/gram/second)</td>
<td>0.0325±0.0018d</td>
<td>0.0456±0.0023e</td>
<td>0.0535±0.0015f</td>
</tr>
<tr>
<td>Average</td>
<td>0.0321±0.0048d</td>
<td>0.0452±0.2400f</td>
<td>0.0537±0.0016e</td>
</tr>
</tbody>
</table>

Note: the mean value with different superscripts shows a very significant difference (P<0.01)

The protein content of fenugreek in 100 g of endosperm is 43.8 and per 100 g of fenugreek protein 25.4 grams (Khorsidian et al., 2016). The fenugreek protein fraction contains lysines and can be compared to soy protein, which affects the dough in addition to increasing the fibre content (Yousef et al., 2009). A pH value lower than the isoelectric point of meat protein has access to positive charges, resulting in the rejection of myofilaments, providing more space for water molecules, and increasing water-holding capacity. During frozen storage, buffalo meat patties supplemented with fenugreek maintained an average pH value. The pH value of the meat can be maintained because the bioactive compounds in fenugreek can inhibit the growth of microbes that hydrolyze meat components into organic acids.

The fenugreek seed extract was acidic, causing the addition of fenugreek powder to the patty dough to be lower than that of the control (F0). The addition of fenugreek powder decreased the pH of buffalo meat patties. According to Kahar et al. (2021), the pH of buffalo meat patties is usually between 6.12 and 7.75. The pH value of the patties in the study conducted by Sembor (2022) ranged from 6.05-6.22. According to the Indonesian National Standard, processed food has a pH value of 6-7, this means that the pH value of buffalo meat patties is within the normal range. The effect of pH can also be attributed to the different types and compositions of ingredients. Physicochemical and structural changes that occur in the protein system during processing affect product quality (Beniwall et al., 2021).

Cooking Weight Loss Buffalo Meat Patty

The results showed that the lowest percentage of cooking weight loss in the patty sample with the addition of 6% fenugreek (F2) was 2.80%, the addition of 9% fenugreek powder (F3) was 26.39%, and the highest percentage of cooking weight loss in the control (F0) was 37.27%. The results of the analysis of variance showed that the percentage of cooking weight loss in the control (F0) was significantly different (P<0.05) from the cooking weight loss of patty samples treated with 3% fenugreek powder (F1). Cooking weight loss in the control (F0) was also significantly different (P<0.01) from that in the patty samples supplemented with 6% (F2) and 9% (F3).
fenugreek powder, while the length of storage was not significantly different. Cooking weight loss in buffalo meat patties is an indicator of the weight of the patty before cooking and the weight of the product after cooking.

Cooking weight loss is related to the release of several nutrients into patties during the cooking process. Cooking weight loss is best if the weight of the patty during cooking has the lowest percentage because the amount of nutrient loss is also low (Patriani et al., 2022; Patriani et al., 2021). The DMRT test results showed that there was no interaction between the addition of fenugreek powder to the patty and storage time on the percentage of cooking weight loss of buffalo meat patties.

The addition of 6% (F2) to 9% (F3) fenugreek flour to patty dough reduced cooking weight loss. This may be because fenugreek flour has gelatinizing properties, which prevent weight loss during cooking. Following research (Gadkari et al. (2019), Zaki (2018), and Roberts (2011) reported that fenugreek is a food-gelling agent. The dietary fibre and gel contained in Funegreek seed flour can maintain the water-holding capacity of the patty and affect the protein content, thereby reducing cooking weight loss. The F1 patty sample (3%) had higher cooking loss than the F2 (6%) and F3 samples (9%) because the composition of fenugreek seeds in the F1 sample (3%) was less than that in F2 (6%) and F3 (9%). This is also related to the protein content in samples F2 (6%) and F3 (9%), which was higher than that in F1 (3%).

The decrease in cooking weight loss is also related to the higher water absorption capacity during cooking because the food fibre and protein content in fenugreek absorb water. According to Yadaf et al. (2014), the decrease in cooking loss is also related to the amount of crude fibre and starch in fenugreek, forming a tight bond with the mixed wheat gluten protein. The addition of fenugreek resulted in lower cooking weight loss because the fibre contained in fenugreek can retain the liquid released during cooking. Fenugreek can improve the retention of meat dough emulsions by binding to the water contained in protein tissues. The gum in fenugreek can bind to the meat particles making the dough patty more stable and can withstand the release of liquid from the meat during the cooking process.

The control sample (F0) had a higher cooking weight loss because there were no emulsifiers or binders that could bind to water. In addition to the gum contained in fenugreek, the decrease in cooking weight loss was also caused by the protein. According to Hellebois et al. (2021), fenugreek contains amylose, amylopectin, and galactomannan. Galactomannan has the potential to be used as an emulsifier or dough stabilizer by controlling the water tension and building viscosity (Llavata et al., 2020). Its emulsification was also superior to that of other galactomannan gums. The gum contained in fenugreek can bind more water so that the dough is more stable and cooking weight loss is lower than that of the control. The best cooking weight loss in this study, which had the lowest percentage, was achieved with the addition of 6% (F2) and 9% (F3) fenugreek flour. Overall, the cooking weight loss percentage was normal.

Buffalo Meat Patty tenderness

Tenderness was tested using a penetrometer based on the amount of pressure and force required per unit area of the processed product (mm/g/s). Buffalo meat patties were first cooked and then tested using a penetrometer. Average tenderness was highest with the addition of 9% fenugreek powder (F3) at 0.0588 mm/g/s and lowest in the control (F0) at 0.0321 mm/g/s. Based on the analysis of variance, the tenderness of the control (F0) was significantly different (P<0.05) from that of the patty sample supplemented with 3% fenugreek powder (F1).

The tenderness value of the control sample (F0) was also significantly different (P<0.01) from that of the samples supplemented with 6% (F2) and 9% (F3)
fenugreek powder, while the length of storage was not significantly different (P>0.05).

This indicates that the tenderness of the patties increased with the addition of 6% (F2) and 9% (F3) fenugreek seed powder. According to Wu et al. (2020), the factors that can increase tenderness are gelatinization and interactions between starch molecules and meat protein myofibrils using the appropriate percentage of ingredients. Fenugreek also contains gum as an emulsifier and stabilizes the emulsion of oil in the water so that the patty dough is better and affects tenderness and texture.

The DMRT test results showed that there was no interaction between the addition of fenugreek powder to the patty and storage time on the tenderness value of buffalo meat patties. Patty products with the addition of fenugreek flour increased tenderness due to the gum content in fenugreek so it can provide higher tenderness than patties without the addition of fenugreek flour. Finesse can also be caused by the effect of flour added during cooking when meat protein shrinks, which is filled with starch molecules, compressing the texture of the product. The addition of hydrocolloids decreases the hardness of patty products (Pemalilleke et al., 2021).

Fenugreek can bind water, which affects the tenderness of protein-based products from processed meat such as patties. Increasing the amount of fenugreek increased the tenderness of the patties. The water content of the dough also affects the tenderness of the patties. The water content of the meat in patty dough causes less water-soluble myofibrillar protein. Myofibrillar protein is an emulsifier that can affect the increase in emulsion compared with other proteins. In processed meat products, myofibrillar proteins exhibit better emulsifying properties when combined with water and oil (Diao et al., 2016). The level of tenderness in this study showed that the greater the addition of fenugreek to the patty, the higher the level of tenderness. This is because the addition of fenugreek up to 9% (F3) did not been able to increase the hardness of the product. The composition of starch in fenugreek that binds to protein during the cooking process is still within normal limits, even though the water content decreases slightly. Firmness can also be affected by the structure of meat myofibrils and the cross-linking of meat fibres. The strength of the meat vein liquid can function as a binding agent for food ingredients during cooking. Based on several approaches, patty tenderness in this study may have been caused by starch in fenugreek and meat protein components. The best treatment to increase the tenderness of buffalo meat patties was the addition of 6% (F2) and 9% (F3) fenugreek seed powder.

**Chemical Composition of Buffalo Meat Patty**

The chemical composition of processed meat products consists of proteins, fats, water, and carbohydrates. The quality of a processed meat product can be assessed based on its food energy and shelf life. Product quality can be assessed based on chemical quality.

Processed meat products contain carbohydrates, fats, proteins, vitamins or minerals. Product quality is good if it is maintained during storage. The chemical quality of processed meat products can be determined by the composition of the ingredients and their changes during the processing process, including damage to certain nutrients caused by the treatment. The results of the buffalo meat patty chemical composition test, namely protein content, fat content and water content can be seen in Table 3 bellow.

**Protein Content**

The highest average protein content in the patty with the addition of 9% fenugreek powder (F3) was 41.02%, and the lowest in the control (F0) was 27.85%. Based on the analysis of variance, the protein content in the control (F0) was significantly different (P<0.05) in the buffalo meat patty sample.
supplemented with 3% fenugreek powder (F1). Protein levels in the control sample (F0) were also significantly different (P<0.01) from those in the samples supplemented with 6% (F2) and 9% (F3) fenugreek powder, while the length of storage from 30 days (D1) to 60 days (D2) showed no significant difference (P>0.05). This indicates that the protein content of the patties increased with the addition of 3% (F1), 6% (F2), and 9% (F3) fenugreek seed powder.

Table 3. Chemical composition of buffalo meat patty with the addition of Funegreek during the Storage Time

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Storage Time</th>
<th>Addition of Fenugreek powder</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F0: 0%</td>
<td>F1: 3%</td>
<td>F2: 6%</td>
</tr>
<tr>
<td>Proteins (%)</td>
<td>D1: 30 days</td>
<td>28.50±0.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30.11±0.25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>D2: 60 days</td>
<td>27.21±0.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31.62±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>27.85±0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30.86±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>D1: 30 days</td>
<td>13.80±0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.41±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>D2: 60 days</td>
<td>13.64±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.12±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>13.72±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.26±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>D1: 30 days</td>
<td>70.10±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.81±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>D2: 60 days</td>
<td>70.62±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.22±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>70.36±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.01±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: the mean value with different superscripts shows a very significant difference (P<0.01)

The DMRT test results showed that there was no interaction between the addition of fenugreek powder to the patty and storage time with respect to buffalo meat patty protein content. This is following research (Hegazy 2011) reported that, in general, patties containing fenugreek emulsion have good nutrient composition even after frozen storage. The higher the amount of fenugreek powder added, the higher the protein composition of the buffalo meat patty.

The increase in protein content in buffalo meat patties may be caused by the high protein content of fenugreek powder compared to the control formulation, which only contains wheat flour. Following the research of Hegazy (2011) and Qureshi et al. (2018), the increase in protein in the patty can occur because fenugreek powder has a high protein content of approximately 32.29%. Meghwal et al., (2012) stated that Fenugreek contains protein and is rich in lysine so it can be compared with soy protein. The protein found in fenugreek also does not affect the cooking process; therefore, the production of functional preparations and therapeutic agents is recommended. Product processing using fenugreek seeds has the potential to increase in vitro starch and protein digestibility (Pandey and Awasthi, 2015). Fenugreek is a legume plant with a high protein content. According to research by Fayzi et al., (2015), the partial replacement of wheat flour with fenugreek flour in the range of 50-200 grams/l showed a significant increase in protein content and the sensory quality of food products and resulted in higher protein digestibility due to lysine content.

Fenugreek has outstanding functional properties, including protein solubility and oil-holding capacity, which can be used for dough formulation and to improve the nutrition, texture, and physical properties of products. Buffalo meat also has a high protein content, which affects the protein composition of the patty. According to the Indonesian National Standard (SNI 8503:2018), the quality requirement for meat patties is at least 13%. Buffalo meat patties with the addition of fenugreek powder during 60 days of frozen storage have a protein content above the SNI 8503 standard:2018, making it suitable for consumption.
**Fat Content**

The highest average fat content in patties treated with 9% fenugreek powder (F3) was 17.00%, and the lowest in the control (F0) was 13.72%. Based on the analysis of variance, the fat content in the control (F0) was not significantly different (P>0.05) from the buffalo meat patty sample supplemented with 3% fenugreek powder (F1). The protein content of buffalo meat patties in the control (F0) was also significantly different (P˂0.05) from the samples supplemented with 6% (F2) and 9% (F3) fenugreek powder, while the fat content during 30 days (D1) and 60 days (D2) of storage was not significantly different (P>0.05). This indicates that the fat content of the patties increased with the addition of 6% (F2) and 9% (F3) fenugreek seed meal.

The DMRT test results showed that there was no interaction between the addition of fenugreek powder to the patty and storage time on the fat content of buffalo meat patties. The fat content of fenugreek powder is the reason for the increase in fat content in buffalo meat patties. According to Ciftci et al., (2011), fenugreek seeds contain oil between 5.8-15.2% which can be utilized in processed products because it can benefit consumers. The fat content in fenugreek can also affect the emulsion stability of patty dough, thus affecting its characteristics. Emulsion stability in patty dough is important because it is related to a decrease in cooking weight loss and an increase in viscosity and texture or tenderness of the resulting patty product.

Higher addition of fenugreek increased the stability of the emulsion in the patties. Surface tension occurs through a protective layer covering more stable globules, and the release of fat from the meat fibre during boiling can be prevented from causing the fat content to remain high. This follows Barbut (2015); Choe and Kim (2019), who found that the fat component is very important in dough emulsion and affects texture, juiciness, wetness, aroma, and cooking weight loss. According to the Indonesian National Standard (SNI 8503: 2018), the maximum fat content in a patty is 20%, and the fat content of the patties in this study is still in a good range. The fat content of buffalo meat patties supplemented with fenugreek is below 20%, which means that the patty product is good for consumption.

**Water Content**

The highest average moisture content in the control (F0) was 70.36% and the lowest in the patty sample with the addition of 9% fenugreek powder (F2) was 61.19%. Based on the analysis of variance, the moisture content in the control (F0) was not significantly different (P˂0.05) from the buffalo meat patty sample supplemented with 3% fenugreek powder (F1). The moisture content of the control sample (F0) was significantly different (P˂0.05) from the samples supplemented with 6% (F2) and 9% (F3) fenugreek powder, while the moisture content of the 30-day (D1) to 60-day (D2) storage periods was not significantly different (P>0.05). This means that the moisture content of the patties decreased with the addition of 6% (F2) and 9% (F3) fenugreek seed flour.

The DMRT test results showed that there was no interaction between the addition of fenugreek powder to the patty and storage time on the water content of the buffalo meat patty. The moisture content in the control (F0) was higher than that in the patties with the addition of 6% (F2) and 9% (F3) fenugreek powder, because the protein and gum content contained in fenugreek can bind water. The high water content in the control sample (F0) and the sample with 3% fenugreek powder (F1) during storage from 30 to 60 days is thought to be due to protein denaturation.

Protein denaturation can occur owing to the presence of bacteria that cause water produced in the metabolic process to add to the moisture content of the patty. The addition of fenugreek causes the water content of the patty to decrease, which can be attributed to the water-retaining properties of fenugreek flour. A decrease in water content can increase the protein...
content. The water content of the patty can also be caused by its constituent components, such as protein and fat. The decrease in water content in the patty can also be caused by the interaction mechanism of fenugreek starch and protein, such that water is not perfectly bound because the hydrogen bonds that bind water are used for the interaction between starch and protein. The decrease in water content in the patty causes the shelf life of the patty to be longer because the higher water content spurs the growth of spoilage microorganisms, which affects the shelf life.

The average moisture content of beef patties is typically between 65%-67% (Mbougueng et al., 2015). Changes in moisture content that occur during storage can be caused by environmental factors such as temperature (Indriyani et al., 2022). Moisture content is related to texture and consumer acceptance (Hafid et al., 2021). According to Noumu et al., (2016), the moisture content of beef patties is 62%-69%. The moisture content of buffalo meat patties treated with fenugreek powder was included in the good category.

### Microbiological Quality of Buffalo Meat Patty

The results of the total plate count test on buffalo meat patty samples show that it can be seen in Table 4 below.

<table>
<thead>
<tr>
<th>Storage Time</th>
<th>Addition of Fenugreek powder</th>
<th>Average</th>
</tr>
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</table>
| D1 (30 days) | F0: 0%                        | 3.60x10^7 cfu/gr
|              | F1: 3%                        | 2.32x10^7 cfu/gr
|              | F2: 6%                        | 2.10x10^7 cfu/gr
|              | F3: 9%                        | 2.12x10^7 cfu/gr
|              | D2 (60 days)                  | 3.92x10^7 cfu/gr
|              |                              | 2.48x10^7 cfu/gr
|              | Average                       | 2.42x10^7 cfu/gr

Note: mean values with different superscripts indicate very significant differences (P<0.01)

The DMRT test results showed that there was no interaction between the addition of fenugreek powder to the patty and storage time on the total plate count of the buffalo meat patty. This means that buffalo meat patty products supplemented with fenugreek can last for 60 days of storage. Following Khorsidian et al., (2016), fenugreek seed flour is an antioxidant and antimicrobial agent in processed beef formulations as a substitute for soy flour that can increase essential amino acids and improve physicochemical quality and microbiological quality during frozen storage. Fenugreek contains saponins and flavonoids. The mechanism of action of

The mean total plate count was highest in the control (F0) at 3.92x10^7 cfu/gr and lowest in the patty sample with the addition of 9% fenugreek (F3) at 1.79x10^4 cfu/gr. Based on the analysis of variance, the total plate count in the control (F0) was significantly different (P<0.05) from that in the buffalo meat patty sample supplemented with 3% fenugreek powder (F1). The total plate count in the control sample (F0) was also significantly different (P<0.01) from that in the samples supplemented with 6% (F2) and 9% (F3) fenugreek powder. The 30-day storage period (D1) was not significantly different (P>0.05) from the 60-day sample (D2). This indicates that the total plate count on the patties decreased with the addition of 3% (F1), 6% (F2), and 9% (F3) fenugreek seed powder.

The total plate count decreased with the addition of fenugreek powder to buffalo meat patties. This can be caused by the antibacterial compounds in fenugreek powder, which can inhibit the development of bacteria during the shelf life of 30–60 days. This is reinforced by research conducted by Norziah et al., (2015), who found that fenugreek has antimicrobial activity, with the highest antioxidant capacity equivalent to vitamin C, as a source of natural bioactive compounds in the food industry to extend the shelf life of food products.
saponins is to reduce the surface tension, causing increased permeability and leakage of bacterial cells.

The mechanism of action of flavonoids as antibacterial agents is to form complex compounds with extracellular and soluble proteins so that they can damage the bacterial cell membrane. Flavonoids can also inhibit the function of the cytoplasmic membrane and energy metabolism in bacteria. Flavonoids function as bactericidal and bacteriostatic agents by damaging the cytoplasmic membrane, inhibiting energy metabolism, and synthesizing nucleic acids (Ahmad et al., 2015). The addition of fenugreek can inhibit bacterial growth because flavonoids can depolarize cell membranes and inhibit DNA, RNA, and protein synthesis. Patty samples with added fenugreek seeds had values below the Indonesian National Standard (SNI 8503:2018), which is a maximum total plate count of 10^6 colonies/g. Patty in the study is still in a good range and suitable for consumption.

**CONCLUSION**

The addition of fenugreek seed powder (Trigonella foenum-graecum) to buffalo meat patties can improve their physicochemical and microbiological properties. The addition of fenugreek seed powder caused a decrease in pH value, cooking weight, moisture content, and microbial count during storage at -18°C for 60 days. The addition of fenugreek powder to buffalo meat also increased tenderness, protein content, and fat content. Fenugreek seed powder is recommended in buffalo meat patties of 6% to 9% because it can improve the physicochemical and microbiological qualities and extend the storage time for 60 days at -18°C. Fenugreek seeds have the potential to increase innovation and add value to high-quality and sustainable food products.

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