Jurnal Teknologi Hasil Peternakan, 6(2): 92-109. September 2025
http://journal.unpad.ac.id/jthp/index
ISSN: 2722-4783
DOI: 10.24198/jthp.v6i2.64650

DAMPAK EKSTRAK DAUN SENGKUBAK DAN LAMA PENYIMPANAN TERHADAP BEBERAPA SIFAT FISIKO-KIMIA DAN MIKROBIOLOGI BAKSO AYAM SIAP SAJI

IMPACT OF SENGKUBAK LEAF EXTRACT AND STORAGE TIME ON SOME PHYSICOCHEMICAL AND MICROBIAL ATTRIBUTES OF READY-TO-EAT BAKSO AYAM (CHICKEN MEATBALLS)

Received : June 25th 2025
Accepted : Aug 22th 2025

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Abstract. *Bakso ayam are chicken meatballs produced using natural additives, and have been found to possess a higher level of safety for regular dietary intake. Sengkubak (Pycnarrhena cauliflora) is an indigenous plant mostly found in West Kalimantan which is commonly used by local communities as a natural food flavoring. In this study, the effect of dry aqueous sengkubak extract concentration (0% as control; 0.25%; 0.5%; and 0.75% of meat weight) and storage time (0 week as control, 4 weeks, and 8 weeks) at ~27°C on some physicochemical and microbial quality of bakso ayam was examined. Other variables examined were the sensory attributes and glutamic acid content. The glutamic acid concentration of sengkubak leaf extract was found to be 5.4 mg/g. A completely randomized design with three replications was performed. Data were analyzed using analysis of variance, and statistical difference were considered significant at P<0.05. The result show that level of sengkubak extract affected most physical qualities parameters, glutamic acid content and sensory attributes, but it did not affect protein content. While, storage time affected water holding capacity (WHC), chemical qualities, total of microbes, but did not affect sensory attributes. The addition of 0.5% sengkubak leaf extract is recommended in this study because it produced the best firmness (7.82 gf), protein content (11.87%), and taste among all treatments. Bakso ayam was safe for consumption until the 8th week of storage at room temperature (±27°C).*

Keywords : *Glutamic acid, Sensory attributes, Natural additives, Microbial quality*

Sitasi :
Amalia, D. N., Kayihura, J. F., Khalisanova, S. Z., Arsita, T. A., Rusman., & Triyannanto, E. (2025). Impact of Sengkubak Leaf Extract and Storage Time on some Physicochemical and Microbial Attributes of Ready-to-Eat Bakso Ayam (Chicken Meatballs). *Jurnal Teknologi Hasil Peternakan*, 6(2): 92-109

INTRODUCTION

Nowadays, the dynamic lifestyles of people have encouraged improvements in the methods of cooking and food processing. One potential impact of a fast-paced and dynamic lifestyle is the reliance on ready-to-eat foods. The reason for the longer processing duration for various food items compared to food products is mostly due to the latter's emphasis on quick and convenient consumption. Meatballs are food items that are typically shaped into spherical or other forms, derived from a combination of meat (with a meat content exceeding 50%), starch, or cereals, and may include food additives such as monosodium glutamate (MSG) and a variety of seasonings (SNI, 2014). They also have the potential to be transformed into ready-to-eat goods due to their nutritional attributes. MSG has been found to have adverse effects on individuals with specific health conditions or diseases (Niaz *et al.,* 2018). Based on study, people with a daily consumption of MSG exceeding 5 g should be considered at risk for metabolic disorder (Insawang *et al.*, 2012). Consequently, scientists are actively seeking plant-derived sources for natural flavorings. Sengkubak has been recognized as a promising plant species in Indonesia, particularly in West Borneo, for several decades.

Sengkubak, a plant indigenous to the forests of West Kalimantan, is commonly employed by local inhabitants as a culinary ingredient. The leaves of this plant are particularly utilized as flavor enhancers. According to Kurihara (2015), the leaves of the Sengkubak plant are known to contain glutamic acid, a compound that has the ability to enhance the flavor profile of food and elicit an umami experience. Thus, it can be used as natural MSG replacers in meat products. According to Ardiningsih (2009), the Sengkubak leaf is also nutritious as it has been shown to have approximately 19.62% protein and 5.8% glutamic acid. The utilization of sengkubak leaf as a food flavour enhancer can, therefore, be achieved through various methods, including the chopped whole leaves, transforming them into a powdered form through drying, or extracting their essence. According to Setyiasi *et al.* (2013), the hedonic sensory score for sengkubak extract in the form of powder was shown to be highest when using concentration level of 0.25%, as opposed to concentration levels of 0.5% and 0.75%. However, there was no studies on the possibility for the development of a nutritious ready-to-eat chicken meatballs which supplemeted with the aqueous sengkubak extract. Therefore, this study was conducted to assess the effect of different concentrations of the dry aqueous extract of sengkubak leaf on some of the important physicochemical and microbial properties of bakso ayam (chicken meatballs) during 8 weeks storage at ±27°C.

MATERIALS AND METHODS

1. Statistical Analysis

The data were analyzed using Analysis of Variance (ANOVA) to determine the effect of treatment on the observed parameters. If the analysis results show significant differences ($P< 0.05$), Duncan's Multiple Range Test (DMRT) is conducted to determine the actual differences between treatment means and to group treatments based on the level of difference.

2. Samples and Data

The sengkubak leaves were collected from a forest located in Putussibau, Kapuas Hulu, Kalimantan Barat. The samples went through a cleaning process and were then subjected to drying under the sun for a duration of 8 hours per day, spanning a period of up to 3 days. The desiccated foliage was weighed packaged, and dispatched on an expedition to Yogyakarta. This study used a completely randomized design with a 4x3 factorial pattern and significance result if $P<0.05$. The treatments used in this study are concentration of the dry aqueous sengkubak leaf extract (0.25%, 0.5%, and 0.75%) and storage periods (0 week as control, 4 weeks, and 8 weeks) of the chicken meatballs.

3. Extraction of Sengkubak Leaf

The extraction of sengkubak leaf was conducted using the infusion method (Setyiasi *et al.*, 2013) with some modifications. The experimental materials consisted of dried sengkubak leaves weighing 900 grams, which were used as the primary substance, and distilled water, which served as the solvent. Before further process, dried sengkubak leaves are crushed with grinding machine being a powder form. The ratio between the dry sengkubak leaves powder and water was 1:4. The extraction process was carried out at a temperature of 90°C for a duration of 15 minutes. The outcome of the extraction process, specifically infusion, is sustained to filtration using filter cloth in order to separate the residual components. The extract is subjected to concentration using a vacuum evaporator operating at a temperature of 40°C in order to decrease the amount of water present. The condensed/concentrated extract underwent a sun-drying process for a duration of 6 hours per day over a period of 3 days in order to obtain the extract in a dry, powdered state. The dry extract was quantified at 31.2 g. By applying the formula (dry extract/raw material of extract) x 100% (Sani *et al.*, 2014), the yield was computed as (31.2 g/900 g) x 100%=3.47%.

4. Application of Sengkubak Leaf Extract to Bakso ayam

Formulation and production process of bakso ayam using the percentage and method described by Ismail *et al.* (2016) with certain modifications. The primary constituent of the bakso ayam are chicken breast, with the proportion of each item expressed as a percentage relative to its weight. Shown below is the table illustrating the formulation of the dough used in the preparation of bakso ayam (Table 1).

The finished dough is shaped into balls, each weighing approximately 15 grams. The meatballs are shaped into balls using a spoon and then placed in warm water at a temperature of around 70°C. The meatballs and water are placed in a labelled retort pouch, which is then packed. Bakso ayam was prepared using sterilization. Each retort-standing pouch had five bakso ayam with a volume of 150 mL broth. Each treatment was replicated three times, resulting in a total sample size of 48 pouches.

The initial stage of the packing process involves the act of sealing. The sealing process is conducted at a temperature of 225°C. Following the sealing process, the subsequent stage involves sterilizing using a retorted machine. The process of sterilization is conducted at a temperature of 121°C and a pressure of 2 atm for a duration of 9 minutes (Triyannanto *et al.*, 2022). The pouches are stored at a constant temperature of around 27°C for a duration of 4 weeks and 8 weeks.

Quality tests are conducted at the end of each storage period. Quality testing was conducted by taking five meatball samples from each treatment, including replicas, under aseptic conditions. Total microbial testing was conducted under aseptic conditions using sterilization treatment, while chemical, physical, glutamic acid content, and sensory testing were conducted by cutting the meatballs according to the proportions required for testing.

5. Physicochemical Qualities Test

The parameters that were examined include pH, glutamic acid content, firmness, and water holding capacity (WHC). The pH is checked using a pH-meter (Mettler Toledo FiveEasy TM F20, Switzerland). The firmness of the samples was evaluated using a texture analyzer, namely Stable Micro System (TA.XT plus C (SMS TA50/650L), UK). The testing of WHC is conducted through the application of pressure, specifically utilizing the Hamm method as described by Hamm (1972). The spectrophotometric ninhydrin method was used to assess the amounts of glutamate amino acids (Apriyantono *et al.,* 1989). A standard calibration curve of glutamic acid (Figure 1) was developed and used to compute the concentration of glutamic acid content in bakso ayam samples.

Table 1. Formulation of bakso ayam with addition of sengkubak leaf extract

Materials	Treatments			
	T_0	T_1	T_2	T_3
Ground chicken meat	750 g	750 g	750 g	750 g
Tapioca flour	75 g	75 g	75 g	75 g
Salt	18.75 g	18.75 g	18.75 g	18.75 g
Sodium tripolyphosphate (STPP)	2.25 g	2.25 g	2.25 g	2.25 g
Pepper powder	3.75 g	3.75 g	3.75 g	3.75 g
Garlic powder	3.75 g	3.75 g	3.75 g	3.75 g
Cold water	150 g	150 g	150 g	150 g
Sengkubak extract	0 g	1.875 g	3.750 g	5.625 g

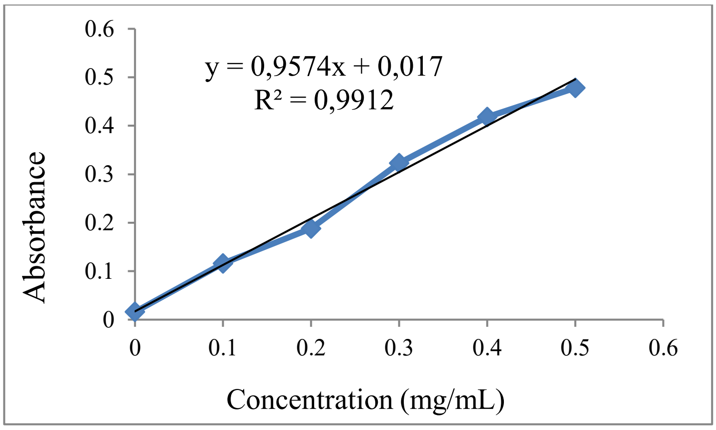

Figure 1. Glutamate standard curve

$$\%\ \text{Glutamic acid content} = \frac{X \times \text{dilution factor x } 100\%}{\text{sample weigh (mg)}}$$

$$X = \frac{y - a}{b}$$

Where:
X = concentration
y = absorbance

6. Total Plate Count

The TPC method depends on the principles outlined in the SNI (2008). The growth of colonies was monitored on each plate and quantified using a colony counter. The number of colonies was determined according to the Standard Plate Count (SPC).

RESULT AND DISCUSSION

1. Physical Qualities of Bakso ayam

1.1 pH Value

The addition of sengkubak extract did not result in a significant change in the pH level of the *bakso ayam*. The addition of sengkubak extract at 0.75% did not result in a statistically significant effect ($p > 0.05$) on the acidity level of bakso ayam. Consequently, there were no meaningful changes in sensory attributes related to acidity, such as sourness, nor in preservative effects that are typically associated with lower pH values. This suggests that at this concentration, sengkubak extract does not influence the acidity-dependent characteristics of bakso ayam, and its functional contributions may be more related to other bioactive components rather than acidification. According to the Indonesian National Standard (SNI, 2014), the pH value of bakso ayam varies in the range of 6 to 7. Additionally, Ismail *et al.* (2016) conducted a study which reported that the pH of bakso ayam varies between 5.5 and 7.2. It is noteworthy that the pH of bakso ayam is mainly influenced by various factors, such as the type of meat utilized as the primary ingredient and the composition of additives and seasonings incurporated into the meatball mixture (Abadi, 2004).

Table 2. Means of physical qualities of bakso ayam during storage

Parameter	Extract (%)	Storage time (weeks) 0	4	8	Mean
pH[ns]	0	6.63±0.02	6.66±0.02	6.62±0.04	6,64±0,03
	0.25	6.62±0.02	6.62±0.01	6.63±0.03	6,62±0,03
	0.5	6.64±0.04	6.64±0.03	6.60±0.05	6,63±0,03
	0.75	6.64±0.01	6.64±0.01	6.63±0.02	6,63±0,03
	Mean	6.63±0.02	6.64±0.02	6.62±0.03	
Firmness (gf)	0	65.26±0.09	80.56±0.12	86.67±0.11	77,49±0,12[k]
	0.25	78.52±0.07	72.39±0.05	84.64±0.22	78,52±0,11[k]
	0.5	72.39±0.07	87.69±0.14	86.67±0.29	82,59±0,18[kl]
	0.75	93.81±0.22	98.91±0.09	96.87±0.06	96,87±0,12[l]
	Mean[ns]	77.49±0.15	84.64±0.13	88.71±0.17	
WHC	0	6,26±0,86[ak]	7,70±1,19[abk]	8,35±1,10[bk]	7.44±1.30[a]
	0.25	7,59±0,63[ak]	6,94±0,49[abk]	8,11±2,10[bk]	7.55±1.23[a]
	0.5	6,97±0,71[ak]	8,46±1,34[abk]	9,56±1,18[bk]	8.33±1.48[ab]
	0.75	8,99±2,18[al]	9,48±0,85[abl]	9,34±0,55[bl]	9.27±1.22[b]
	Mean	7,46±1,50[a]	8,15±1,31[ab]	8,84±1,32[b]	

The storage time did not significantly affect the pH of bakso ayam. Although the effect was statistically non-significant, it should still be discussed, as the variations observed are not attributable to packaging factors but rather reflect the inherent stability of meatball products. Packaging was effectttively restricts the entrance of external oxygen, thereby mitigating the potential deterioration of meatball quality. The primary purpose of packaging is to protect the product from potential contamination while also ensuring the preservation of product quality and safety (Astawan *et al.,* 2015). No significant correlation was observed between the various concentrations of sengkubak extract added and the duration of storage on the pH levels of bakso ayam.

1.2 Firmness

Addition of sengkubak leaf extract led to a significant difference (P<0.05) in the firmness of bakso ayam. As the level of extract is increased, there is a corresponding increase in the firmness value, leading to a greater firmness of the bakso ayam.

The addition of sengkubak leaf extract showed a significant difference (P<0.05) in the tenderness of chicken meatballs. Based on the analysis results, the addition of sengkubak leaf extract at levels of 0% to 0.5% produced the best tenderness values, ranging from 7.44 gf to 7.55 gf. Meatballs with 0.75% sengkubak extract addition resulted in a texture that was too hard. One of the measures or parameters used by consumers to evaluate meatball products is tenderness. Tenderness or firmness can be determined from the breaking force during testing. The lower the breaking force value of the meatball, the softer the meatball; conversely, the higher the breaking force value, the tougher the meatball (Abustam, 2010). Consumers tend to prefer meatballs with a chewy texture and do not particularly like meatballs that are too soft or too hard (Pramuditya and Yuwono, 2014). In addition, the presence of bioactive compounds in sengkubak extract, particularly glutamic acid (Andarwulan *et al.,* 2012) and phenolic constituents (Ozdal *et al.,* 2013), may contribute to the firmness of bakso ayam by enhancing protein–phenolic interactions and improving water-holding capacity during the cooking process

Storage duration had a significant effect (P<0.05) on the firmness of bakso ayam. Meatballs stored for up to 12 weeks exhibited fluctuating firmness, ranging from 7.46 gf to 8.84 gf. An increase in shear force value indicated a decrease in meatball tenderness. Meatballs stored from week 0 to week 8 showed a decrease in tenderness. As we know, bakso ayam are packaged together with broth, so that the meatballs are submerged during storage. Storage by soaking in broth is one of the factors influencing the fluctuation in meatball firmness in this study. Other factors that can influence the firmness value of meatballs include the mixing of meatball dough ingredients and the inconsistency in meatball size during testing. Based on research by Arifiandi and Setiyono (2015), it was found that an increase in storage time can reduce the pH value, water-holding capacity, and tenderness of super free-range chicken meatballs.

1.3 Water Holding Capacity (WHC)

The addition of sengkubak leaf extract at a concentration of 0.75% in *bakso ayam* resulted in a significantly different value (P<0.01) in WHC between the various samples. The addition of Sengkubak leaf extract played an important role in enhancing the WHC of bakso ayam. Scientists have found that Sengkubak contains protease enzymes (Noviyanti *et al.,* 2012), glutamic acid (Kurihara, 2015), and alkaloids with a wide range of biological activities, including properties that kill protozoa, fungi, and bacteria (Sholikhah *et al.,* 2021). Though the most of enzymes were inactive when sterilized, but it might another unknown amino acid constituents existed in sengkubak extract which impacted the WHC. As the level of extract supplied increases, there is a corresponding increase in the presence of constituent elements that serve as supplementary amino acids, which are essential components in the formation of proteins within *bakso ayam*. The amino acids that make up proteins have polar and charged groups that can interact with water molecules through hydrogen bonds and electrostatic interactions. When proteins undergo denaturation and form three-dimensional networks, these groups form a matrix that retains water within it, increasing water-holding capacity and affecting the texture and stability of food products (Zhou and Pang, 2019).

Bakso ayam stored for up to 8 weeks showed a significantly differrence (P<0.05) from the control treatment and 4 weeks of storage. This may result from soaking the bakso ayam in the broth for a protracted period of time in a pouch so that some of the meat protein matrix—such as sarcoplasmic protein or myofibril types—dissolves in water or salt. As we know, sarcoplasmic proteins represent 20 to 40% of the total muscle protein, which has globular form and exhibits solubility in water and diluted salt solutions (Nakagawa *et al.,* 1998). A study proved that the storage of bakso ayam at various temperatures for eight weeks showed a decrease in WHC. Our results are in agreement with those of Song *et al.* (2017) who also stated that frozen storage (-20°C and -40°C) of bakso ayam for 8 weeks resulted in a further reduction in WHC and texture. This reduction in WHC may result in some water exudation contributing to a slight rise in firmness as indicated herein. It was recommended to store the meatballs just for 4 weeks because there was no significant difference compared to no-storage meatballs.

2. Chemical Qualities of Bakso Ayam

2.1 Moisture content.

The level of sengkubak extract addition had a significant effect (P<0.05) on the moisture content of chicken meatballs. The higher the level of sengkubak extract added, the smaller the moisture content of the meatballs. This can be caused by the presence of 5.4 g/100 g dry weight of glutamic acid in the sengkubak extract. Glutamic acid is one of the amino acids that form proteins, while proteins have water-binding properties, so the higher the amount of sengkubak extract added, the higher the proportion of protein, thus suppressing the proportion of other ingredients in the meatballs, including water content. As stated by Meiyani *et al.* (2014) that glutamic acid is one type of amino acid that is bound with other amino acids to form protein structures. The water content in ready-to-eat meatballs tends to have a high value when compared to the water content of meatballs according to SNI (2014), which is a maximum of 70%. This is due to the soaking of meatballs in gravy and the heating process at high temperatures (sterilization) that causes several compounds in the meatballs to hydrolyze, one of which is protein (Meiyani *et al.,* 2014), so that the moisture content of the meatballs is in the range above 70% and even up to 80%.

The storage time of chicken meatballs showed a very significant effect (P<0.05) on the moisture content of chicken meatballs. The moisture content of meatballs tended to increase from week 0 to week 8 and decreased by 0.51% from week 8 to week 12. The significant increase in moisture content at the beginning of production until storage at week 8 can be caused by soaking meatballs in gravy and closed packaging, so that water or gravy can be distributed out and enter freely in meatball products that are submerged without control.

Parameter	Extract (%)	Storage time (weeks) 0	4	8	Mean
Moisture content (%)	0	78,07±0,33^{ak}	79,57±0,06^{bk}	79,60±0,69^{bk}	78,99±0,90^{k}
	0,25	77,98±0,32^{al}	80,27±0,31^{bl}	80,65±0,35^{bl}	79,71±1,36^{l}
	0,5	77,74±0,57^{ak}	79,80±0,44^{bk}	79,97±0,23^{bk}	79,17±1,14^{k}
	0,75	76,48±0,66^{ak}	79,97±0,76^{bk}	79,88±0,23^{bk}	78,77±1,80^{k}
	Mean	77,53±0,76^{a}	79,88±0,50^{b}	80,09±0,63^{b}	
Protein (%)	0	11.73±0,67	12,65±0,08	11,30±0,27	12,28±1,18
	0,25	11,50±0,12	12,26±0,01	11,77±0,64	11,78±0,79
	0,5	11,62±0,24	13,02±0,02	11,71±0,16	12,22±0,83
	0,75	11,79±0,09	12,08±0,05	11,67±0,39	11,83±0,40
	Mean	11,47±0,49^{a}	12,89±0,73^{b}	11,71±0,44^{a}	
Fat (%)	0	2,48±0,07^{cm}	1,70±0,08^{am}	2,03±0,01^{bm}	2,12±0,28^{m}
	0,25	1,54±0,08^{ck}	0,95±0,19^{ak}	1,89±0,10^{bk}	1,40±0,37^{k}
	0,5	2,14±0,07^{cl}	1,31±0,15^{al}	1,72±0,07^{bl}	1,66±0,46^{l}
	0,75	1,59±0,08^{ckl}	1,31±0,06^{akl}	1,88±0,04^{bkl}	1,52±0,33^{kl}
	Mean	1,92±0,49^{c}	1,32±0,36^{a}	1,79±0,19^{b}	

*Values in the same row or column with different superscript letters are significantly different

This can also cause a decrease in the moisture content of meatballs from week 8 to week 12. While the increase in moisture content of meatballs affects the value of water binding capacity. Based on the results of the analysis, the water binding capacity of meatballs experienced a very significant decrease in week 12, as much as 20.03%, so it can be seen that in ready-to-eat meatball products stored in submerged gravy or water conditions there is an increase in the value of water content followed by a decrease in the value of water binding capacity.

2.2 Protein

The level of sengkubak extract addition did not affect the protein content of chicken meatballs. The average protein content of chicken meatballs in the study ranged from 11.29 to 12.50% following the protein content of meatballs according to SNI (2014), which is at least 11%. The main factors that affect the protein content of meatballs are meat and meatball fillers that dominate the total meatball dough, so it is possible that the addition of sengkubak extract does not affect the protein content of meatballs. This study used broiler chicken meat and used tapioca flour fillers in all treatments, so the protein content of meatballs still ranges in a range that is not much different, namely 11.67 to 11.87%. While meatballs with the addition of 0.5% sengkubak leaf extract showed the highest protein content value, namely 11.87%. Harmayani and Susanti (2021) stated that the most protein supplier in meatballs is meat, so the protein content of meatballs is largely determined by the type and amount of meat used as the main raw material. The storage time of chicken meatballs showed a very significant effect (P<0.05) on the protein content of chicken meatballs. The protein content of meatballs increased from week 0 to week 4 then gradually decreased until week 12. The increase in protein content can be caused by an increase in total bacteria, whose cells are composed of protein. In addition, it can also be caused by the dominance of chicken meat at the time of protein testing, or the dough is less well mixed in samples stored for 4 weeks. Meanwhile, a decrease in protein levels can be caused by the dissolution of water-soluble proteins.

2.3 Fat

The level of addition of sengkubak extract has a very significant effect (P<0.05) on the fat content of chicken meatballs. Meatballs have fat content in accordance with SNI (2014), which is a maximum of 10%. The fat content of meatballs with the addition of sengkubak extract tends to be smaller, ranging from 1.44 to 1.61%. This can be caused by the addition of sengkubak extract, which fills the proportion of other compounds in the meatballs, causing the proportion of fat to tend to be smaller when compared to meatballs without the addition of sengkubak extract. Meanwhile, the highest fat content, which is 1.94%, is in meatballs without the addition of sengkubak extract. This can be caused by the absence of other compounds that affect the binding of dough in meatballs and their proportions, so the percentage of fat tends to be higher. The source of fat in meatballs is obtained mostly from the meat used as raw material for meatballs and a small portion from fillers and fatty acids derived from added spices.

The storage time of chicken meatballs showed a very significant effect (P<0.05) on the fat content of chicken meatballs. During storage, there was a significant decrease in fat content until week 4 then an increase again at week 8 and decreased again until storage at week 12. This inconsistent change in fat content can be caused by several factors, namely the submergence of meatballs in the sauce, so that fat substances or compounds easily escape and enter the meatballs. In addition, meatballs soaked in water cause the fat content in them to hydrolyze into fat-derived compounds, as Winarno (2004) states that in the presence of water, fat can be hydrolized into fatty acids and glycerol. Fat content in meatballs is low because it only ranges from 1 to 2% when compared to the fat content of meatballs according to SNI (2014), which is a maximum of 10%. This can be caused by the meat used to make meatballs is broiler chicken breast meat which contains low fat content of around 0.70% (Petracci *et al.,* 2014), while fat from other additives tends to be very small.

Table 4. Means of sensory attributes of *bakso ayam* during storage

Parameter	Extract (%)	Storage time (weeks)			Mean
		0	4	8	
Colour	0	5,80±2,37	6,33±1,95	5,80±1,97	5,98±2,07^{l}
	0,25	6,87±1,77	7,53±1,77	7,40±1,72	7,27±1,74^{m}
	0,5	6,73±1,49	6,33±1,80	6,47±1,77	6,51±1,66^{lm}
	0,75	5,00±2,14	5,00±1,51	4,73±1,49	4,91±1,70^{k}
	Mean	6,10±2,06	6,30±1,94	6,10±1,96	
Taste	0	5,80±2,37	5,40±2,03	5,80±1,47	5,67±1,95^{k}
	0,25	5,40±2,03	6,60±1,72	6,20±1,66	6,07±1,84^{kl}
	0,5	6,60±1,35	6,87±1,77	6,87±1,60	6,78±1,55^{l}
	0,75	6,47±2,33	7,13±1,92	6,87±2,33	6,82±2,17^{l}
	Mean	6,07±2,07	6,50±1,94	6,43±1,81	
Aroma	0	6,73±2,12	6,60±1,88	6,60±2,29	6,64±2,06^{m}
	0,25	5,27±1,67	6,20±1,66	5,53±1,77	5,67±1,71^{l}
	0,5	5,40±2,03	5,53±1,92	5,53±1,92	5,49±1,91^{l}
	0,75	4,33±2,35	4,73±2,12	4,87±2,33	4,64±2,23^{k}
	Mean	5,43±2,18	5,77±1,99	5,63±2,13	

*Values in the same row or column with different superscript letters are significantly different

3. Sensory Qualities of Bakso Ayam

3.1 Colour

The color of processed meat producs plays an important role in consumer acceptance. Based on the analysis, the level of sengkubak extract significantly affected (P<0.05) the hedonic value of meatball color. The addition of sengkubak extract level of 0.25% showed the most favorable value by panelists. This is reinforced by the research of Setyiasi *et al.* (2013) on organoleptic analysis of natural flavoring from sansakng leaves (sengkubak) which showed that the best organoleptic value of sansakng leaf extract powder at a concentration of 0.25%.

3.2 Taste

The The level of sengkubak extract addition had a significant effect (P<0.05) on the presence of savory taste in meatballs. The addition of extract at the level of 0.5% showed the highest favorability value compared to the control. The higher the sengkubak extract added to the meatballs, the higher the hedonic value of the savory taste of the meatballs. This can be caused by the presence of glutamic acid contained in the main ingredients of meatballs, namely chicken meat and spices, and sengkubak extract in the meatball dough. Glutamic acid, inosine monophosphate, and goanosine monophosphate compounds contained in sengkubak leaves provide a savory and delicious taste. These compounds are also an indication of the protein, carbohydrate, and vitamin content in sengkubak leaves (Ardiningsih, 2009). Free glutamate can react with sodium ions (contained in salt) to form gatam MSG (Murdiana, 2012) and glutamic acid is only effecttively applied to meat products, chicken, soups, processed foods from fish and the like (Cahyadi, 2006). Zuhra and Herlina (2012) added that proteins will hydrolyze during the cooking process with high temperatures and then release free glutamate. This glutamate is a key component to obtaining food with umami flavor.

3.3 Aroma

Flavor and aroma of meat or meat products are complex and interrelated sensory properties. Flavor involves smell, taste, texture, and pH. Flavor develops during the cooking process. Leaf aroma is influenced by the addition of sengkubak leaf extract. The results of the hedonic value of meatballs based on respondent assessment showed that the level of sengkubak extract had a very significant effect ($P<0.05$) on the aroma of meatballs. Although aroma and flavor components are volatile during the heating process (Bonnazzi and Dumoulin, 2011), respondent could still smell the leaves in the meatballs. Meatballs with the highest hedonic value on this parameter were meatballs without the addition of sengkubak extract (control), which means that respondent preferred meatballs with a neutral aroma without the aroma of leaves. It is evident from the respondent assessment that the higher the level of extract added, the lower the hedonic value of aroma. Meanwhile, the length of storage did not affect the hedonic value of leaf aroma in meatballs. This could be because the meatballs are submerged in water, so the changes in the aroma of the meatballs in each storage period are not too noticeable.

4. Total Plate Count (TPC)

The results showed that the addition of sengkubak leaf extract does not effects the total number of the total microbial count of the bakso ayam. This could be due to the small percentage of extract added or the disappearance of antimicrobial compounds still present in the added sengkubak extract due to a thermal process during the manufacture of the extract. Meanwhile, the storage time factor had a very significant effect ($P<0.01$) on the microbiological quality of bakso ayam. The total microbial count of chicken meatballs increased throughout the eight-week storage period due to the availability of nutrients and high water content that supportted microbial proliferation. As the applied treatment exhibited no antimicrobial properties, microorganisms were able to metabolize the meat constituents during storage, resulting in alterations of physicochemical and microbiological characteristics (Soeparno, 2015). The increase in the number of microbes was caused by several factors, namely storage time and moisture content. Microbial growth occurs in a short time under conditions of the availability of nutrient content as a source of energy to reproduce (Jay, 2005).

Table 5. Means of Total Microbes (log CFU/g) of *bakso ayam* during storage

Extract (%)	Storage time (weeks)			Mean[ns]
	0	4	8	
0	5.00±0.00	5.66±0.37	6.01±0.49	5.56±0.54
0.25	5.00±0.00	5.19±0.25	5.57±0.05	5.25±0.28
0.5	4.67±0.58	5.07±0.33	5.59±0.70	5.19±0.63
0.75	4.43±0.75	5.11±0.18	5.92±0.47	5.15±0.79
Mean	4.78±0.48[a]	5.31±0.32[b]	5.78±0.46[c]	

*Values in the same row or column with different small or capital superscript letters are significantly different

The results of the total plate count (TPC) in the initial week of storage following sterilization until the eighth week show significant growth. The microbiological values showed a range of 4.78 to 6.19 (log CFU/g). Meatball products with up to eight weeks of storage have shown results that are safe for consumption like Ismail *et al.* (2016) that TPC results at weeks 0–8 are included in the safe, and the total (log CFU/g) of meatballs ranged from 5.30 to 5.86. Based on SNI (2009), food products can be categorized as safe if the total bacterial colonies do not exceed 10^8 CFU/ml. RTE products may still contain microbes even after sterilization because the process does not kill all microbes (especially heat-resistant spores) (Cho *et al.*, 2018), or because of contamination after sterilization due to unhygienic packaging or handling. There was no interaction present between sengkubak leaf extract levels and storage time with total microbial count. However, further research is needed to identify the overall microbiological quality of bakso ayam to ensure their safety.

5. Glutamic Acid Content

The use of Sengkubak leaf extract resulted in a clear significant effect ($P<0.01$) on the glutamic acid content of bakso ayam. Glutamic acid plays a significant role in offering an umami flavor to various food products, and it is found abundantly in plants (Frerot and Chen, 2013). The addition of 0.75% extract to bakso ayam showed efficacy in enhancing the glutamic acid content. The concentration of glutamic acid in bakso ayam ranges between 0.11 g/100 g and 0.24 g/100 g when the quantity of extract addition ranges from 0% to 0.75%. This study demonstrates that the sengkubak extract, while being subjectted to high temperatures of up to 121°C, retains its glutamic acid content and continues to exert an impact on meatball products. The spectrophotometric analysis revealed that the sengkubak leaf extract had a glutamic acid concentration of 5.4 g per 100 g. Furthermore, the glutamic acid content in chicken meat is also influenced by the process of extraction. The glutamic acid contents in several fresh foods exhibited a wide variation,

Table 6. Means of glutamic acid content of bakso ayam during storage

Extract (%)	Storage time (weeks)			Mean
	0	4	8	
0	0.18±0.02[ck]	0.09±0.00[bk]	0.06±0.00[ak]	0.11±0.05[k]
0,25	0.20±0.03[cl]	0.18±0.00[bl]	0.13±0.00[al]	0.17±0.03[l]
0,5	0.27±0.00[cm]	0.21±0.00[bm]	0.14±0.00[am]	0.21±0.06[m]
0,75	0.34±0.02[cn]	0.23±0.00[bn]	0.15±0.00[an]	0.24±0.08[n]
Mean	0.25±0.07[c]	0.18±0.06[b]	0.12±0.04[a]	

*Values in the same row or column with different small or capital superscript letters are significantly different

with zucchini containing 6.7 mg/100 g and walnuts containing 658 mg/100 g. The quantities observed in processed foods; with cottage cheese containing 0.05 mg/100 g and beef stock cubes containing 6.8 g/100 g (Skurray and Pucar, 1988). This finding demonstrates that thermal processing can be a contributing factor to the reduction in glutamic acid content.

The time of storage of bakso ayam showed an extremely significant effect ($P<0.01$) on the concentrations of glutamic acid present in the bakso ayam. The decrease in glutamic acid concentrations occurred in the eighth week of storage. The solubility of glutamic acid in water is quite high. Therefore, when bakso ayam are immersed in broth for a longer period, the glutamic acid content will decrease due to its obvious solubility. According to a study conducted by Yalkowsky *et al.* (2010), it was found that glutamic acid exhibits a solubility of 8570 mg/L at a temperature of 25°C when dissolved in water. The study results show a significant interaction ($P<0.01$) between the addition level of sengkubak extract and the period of storage in relation to the glutamic acid content of bakso ayam.

CONCLUSION

The study indicates that the application of sengkubak leaf extract affects most physical qualities parameters, glutamic acid content and sensory attributes, but it does not affect protein content. While, storage time affects WHC, all chemical qualities, total microbes, but it does not affect sensory attributes. The addition of 0.5% sengkubak leaf extract is recommended in this study because it produces the best firmness (7.82 gf), protein content (11.87%), and taste among all treatments. Meatballs are safe for consumption until the 8th week of storage at room temperature (±27°C).

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