Evaluation of physico-chemical properties and antioxidant activity of bali beef meatballs added Cemba (*Albizia lebbeckoides* [DC.] Benth)

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Abstract. Cemba (*Albizia lebbeckoides* [DC.] Benth) leaf powder contains phytochemical components namely: phenolics, flavonoids, tannins, alkaloids, glycosides, and saponins that potent to be an antioxidant agent. This study investigated the effect of different levels of cemba leaf powder (CLP) on physicochemical and antioxidant activity of Bali beef meatball. Five formulas employed in the study: Control, which without any addition of CLP or BHT; BHT 0.01%; CLP 0.5%; CLP 1%, and CLP 1.5%. All ingredients for each formula were mixed to be the dough and boiled at 80°C for 20 min. The result indicated that the addition of CLP did not change the nutritional properties except for crude fiber. The addition of 1.5% CLP significantly increased the crude fibre. The study also showed that there were no significance different among treatments for cooking loss, WHC, pH value, lightness (L*), and redness (a*) of meatballs. However, the addition 1 and 1.5% CLP increased the greenness (b*) and antioxidant activity of meatballs. In summary, the addition of 1.5% CLP could improve the antioxidant activity and fibre content of the meatballs which is considered as healthy.

1. Introduction

Changes in consumer preferences on ready-to-eat food have intensified the processed meat such as meatball consumption. Indonesian people consider that meatball is the most popular among processed meat products. However, meatballs are perishable during processing due to lipid oxidation. This deterioration can induce indignity of food nutrition and pigments, thereby reducing the grade of the products including color, taste, smell, texture, and even nutritional [1,2].

One of the efforts to retard the oxidative damage is the use of antioxidants that can be sourced from natural or synthetic. The use of synthetic preservatives such as nitrite, acetic acid, phosphate, ascorbic acid, tocopherol, gallic acid, *butylated hydroxyanisole* (BHA), *butylated hydroxytoluene* (BHT), and *tertbutyl hydroquinone* (TBHQ) in the meat industry is one way to inhibit product oxidation [3,4]. However, the use of synthetic agents is not fully accepted by consumers due to potential toxicity and carcinogenic effects [5,6]. It encourages various attempts to develop natural ingredients as a source of antioxidants that are safe and accepted by consumers [7,8].

Natural plant-based ingredients containing secondary metabolites compounds such as diterpenes, polyphenolics, flavonoids, and tannins have prospective for antioxidants and antimicrobials use. Several studies have exhibited the ability of bioactive components of plant extracts in preventing lipid oxidation in processed meat products [5,9,10]. However, the use of the plant extract or powder to meat products...
needs to be assessed on the nutritional content of the product, physical properties, and antioxidant activity.

Cemba is one of the plants that have the potential as a preservative agent, an antimicrobial and antioxidant [9,11,12]. Hajrawati et al. (2019) reported that cemba leaf extract contains bioactive components such as alkaloids, saponins, tannins, phenolics, flavonoids, and triterpenoids [12]. For practical purposes, the use of the plant in powder form is more applicable. However, the capabilities of cemba leave powder need to be evaluated in the use of meat products. This research aimed to assess the nutritional content, physico-chemical properties, and antioxidant activity of beef meatballs added cemba leaf

2. Materials and method

2.1. Materials

The main material used in the study was Cemba (A. lebbeckoides [DC.] Benth) leaves obtained from Enrekang District, South Sulawesi; topside cut of Bali beef obtained from a slaughterhouse in Makassar city, tapioca flour, garlic fresh, white pepper powder, salt, and olive oil. The chemicals were methanol (Merck, Darmstadt, Hesse Germany), butylated hydroxytolune (Himedia, Mumbai, India) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) obtained from Sigma Aldrich, USA.

2.2. Preparation of cemba leaf powder

The cemba leaves were dried at room temperature and then dried at 40°C in an oven for 1 h. The dried leaves were milled using a kitchen blender and sieved through a 35-mesh sieve. Cemba leaf powder (CLP) obtained was stored in a refrigerator (4°C) until used for further study.

2.3. Preparation of meatballs

The manufacturing preparation of meatball referred to Abustam et al. [13] with a slight modification. The Bali beef was minced by using an electrical meat grinder (5 mm plate). Meatball were prepared by making emulsion using a food processor, cooking salt (2%), sodium tripolyphosphate (0.4 %), ice cubes (30%), tapioca flour (20%), fresh garlic (4%), pepper (1%), seasoning (1%) and olive oil as barrier BHT (1%). Treatment consisted of different level cemba leaf powder (control (0%, 0.5%, 1% and 1.5%) and BHT 0.01%). All ingredients were properly mixed in the food processor for sufficient time to make a fine emulsion. The meatball batter was then formed in ball size (13 g) and boiled in water at 80°C for 20 mins. After boiled, all samples were drained and packed in polyethylene pouches before analysis.

2.4. Proximate composition

Moisture, protein, ash, and fat contents CLP and CLP-incorporated Bali beef meatballs was determined using the official standard AOAC’s [14].

2.5. The pH value

The pH of the meatball was measured using “BOECO PT 380” pH meter, which has been calibrated at pH 4 and 7 before measurement. Electrodes were inserted into the inner part of the meatball and pH value was read

2.6. Cooking loss and water holding capacity

The measurement of the cooking loss referred to Das et al. (2012) [15]. Briefly, A-20 g dough in a polyethylene plastic sac was boiled at 80°C for 20 min using a thermostatic water bath. The dough was drained using filter paper and weighed after cooled for 15 min. The cooking loss was calculated as a percentage of the initial weight. The water holding capacity was determined by using a centrifugation Jung and Joo (2013) method [16].
2.7. Color measurement
The color (L*, a*, and b*) was evaluated using a Color meter test (T135) standardized (L=94.76, a= -0.795, dan b=2.200). The measurement was applied on three different spots of meatballs surface immediately after opening packages. The color was determined three times for each sample, and mean values were used.

2.8. Antioxidant activity
The antioxidant activity of meatballs was determined using DPPH method by Hajrawati et al (2019) [9]. A total of 0.2 mL of meatball extract (1 g of meatball in 5 mL of methanol) was put into a test tube then 0.06 mM of DPPH was added and homogenized. The solution was incubated at 37°C for 30 minutes. The determination of absorbance was set at 517 nm using a UV-Vis spectrophotometer. The percentage of DPPH inhibition is calculated as percentage of differences in absorbance between control and sample against control.

2.9. Statistical analysis
The experiments were designed using a completely randomized experimental block design with five treatments (0, BHT, 0.5%, 1%, and 1.5% CLP). The data proximate composition, cooking loss, pH, WHC, color, and antioxidant activities were interpreted by analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS v.9.0. Differences among group means were determined using Tukey differences and were reported as significant at the P<0.05 level.

3. Results and discussion
3.1 Nutritional composition
The nutritional composition of the Bali beef meatballs added BHT and different concentration of CLP are presented in table 1. The nutritional composition of all treatments was not significantly different (P>0.05) except for crude fiber (P<0.05). This result was equal to Najeeb et al (2015), reporting that the moringa leaf powder addition 1% could not significantly affect the moisture, protein, fat, and ash of the restructured chicken product [17]. The moisture content obtained in this analysis was similar to the moisture according to SNI 3818:2014 [18] meatballs which were max 70%. The ash content in the meatballs was 1.96–2.08% and it was lower than the value recommended by SNI (max 3%). The fat content of meatballs was still lower (0.38–1.42%) than the maximum fat (10%) allowed by SNI. The protein content in this experiment was higher (13.99–14.44%) than the standard recommended by SNI (11%).

| Formula       | Moisture (%) | Ash (%) | Protein (%) | Fat (%)  | Crude fiber (%) |
|---------------|--------------|---------|-------------|----------|----------------|
| CLP           | 8.87±0.13    | 8.73±0.14| 8.73±0.13   | 4.52±0.02| 16.11±1.10     |
| CON           | 70.03±0.95   | 2.03±0.42| 14.13±0.42  | 0.37±0.3  | 0.15±0.5       |
| BHT 0.01% (w/w)| 69.99±0.76   | 1.96±0.12| 13.99±0.44  | 1.42±0.06| 0.16±0.05      |
| CLP 0.5% (w/w)| 69.61±0.66   | 2.02±0.10| 14.41±0.41  | 0.38±0.09| 0.18±0.07      |
| CLP 1% (w/w)  | 69.74±0.57   | 2.02±0.13| 14.21±0.29  | 0.41±0.04| 0.24±0.08ab    |
| CLP 1.5% (w/w)| 69.82±0.61   | 2.08±0.05| 14.17±0.36  | 0.42±0.06| 0.38±0.08a     |
| Average       | 69.84±0.68ns | 2.02±0.11ns| 14.18±0.38ns| 0.39±0.07ns| 0.21±0.09     |

ns = non significantly; CON= control ; BHT = butylated hydroxytoluene; CLP= cemba leaf powder; Different superscript in the same column indicates the significantly different (P<0.05).

For crude fiber content of meatball, the addition of BHT 0.01% and CLP 0.5 and 1% exerted the similar content to the control (P>0.05) but the incorporation 1.5% of CLP increased significantly (P<0.05). It indicated that the addition up to 1% resulted in similar characteristics of nutritional composition. The different characteristic was shown at 1.5% addition of CLP. This phenomenon most probably caused by the fact that cemba leaf contains high crude fiber. In this study, CLP was analyzed...
and contained 16.11% (table 1) so that the addition 1.5% affected the crude fiber content of the product. The high content of fiber in food is associated with the health of the food.

3.2 The effect of Cemba leaf powder on pH, cooking loss, and water holding capacity
The effect of cemba leaf powder on pH, cooking loss and water holding capacity of Bali beef meatball is dispensed in table 2. The result of this research showed that there was no effect among treatments on these variables. This occurrence indicated that the incorporation of BHT and CLP did not influence the physico-chemical properties of the meatball. Some researchers also reported that the use of leaf powder in meat product did not affect the pH and cooking loss of the parameters. Biswas et al (2012) and Das et al (2011) observed that pH was not affected by curry leaf powder added in ground poultry and goat meat [19,20]. However, some researchers found that the addition of plant leaf powder exerted a lower pH than that not added. Choi et al (2011) for example, used lotus leaf powder to chicken meat patty and the effect was lowered the pH value of the product [21]. This result confirmed other studies conducted by adding curry lead powder 0.2% in raw ground goat meat patties [23] and drumstick leaf extract 1% cooked ground buffalo meat [20] did not exhibit any significant differences (P<0.05) in the cooking loss compared to the control treatment. The cooking loss is a physical parameter of the meat product. This parameter is affected by some factors and one of them is the capability of the meat to hold the water. The addition of the plant powder usually could boost the capability of the product to hold the water. This result indicated that the WHC was not affected by the CLP. Some researchers have reported that the addition of plants to meat products enhanced the WHC properties of the products. Serdaroglu et al (2018) studied that the beef patties incorporated with dried pumpkin pulp and seed by 5% pumpkin mix resulted in the WHC increased [24]. The different result of the study was most probably caused by the different plant kinds [22] and the number of the plant agent added to the product as conducted by Serdaroglu et al (2018) who added 5% [24].

Table 2. The pH value, cooking loss and water holding capacity Balinese beef meatballs add cemba leaf powder.

| Formula             | pH    | Cooking loss (%) | WHC (%)  |
|---------------------|-------|------------------|----------|
| CON                 | 6.82±0.20 | 3.00±0.37          | 37.26±2.30 |
| BHT 0.01 % (w/w)    | 6.89±0.19 | 3.01±0.59          | 37.14±2.30 |
| CLP 0.5 % (w/w)     | 6.73±0.35 | 2.96±0.52          | 37.33±2.22 |
| CLP 1 % (w/w)       | 6.73±0.25 | 3.00±0.35          | 36.87±1.86 |
| CLP 1.5 % (w/w)     | 6.69±0.16 | 2.90±0.59          | 36.65±2.58 |
| Average             | 6.77±0.19 | 2.97±0.45          | 37.05±2.08 |

ns = non significantly; CON= Control ; BHT = butylated hydroxytoluene; CLP= cemba leaf powder ; Different superscript in the same column indicates the significantly different (P<0.05).

This result confirmed other studies conducted by adding curry lead powder 0.2% in raw ground goat meat patties [23] and drumstick leaf extract 1% cooked ground buffalo meat [20] did not exhibit any significant differences (P<0.05) in the cooking loss compared to the control treatment. The cooking loss is a physical parameter of the meat product. This parameter is affected by some factors and one of them is the capability of the meat to hold the water. The addition of the plant powder usually could boost the capability of the product to hold the water. This result indicated that the WHC was not affected by the CLP. Some researchers have reported that the addition of plants to meat products enhanced the WHC properties of the products. Serdaroglu et al (2018) studied that the beef patties incorporated with dried pumpkin pulp and seed by 5% pumpkin mix resulted in the WHC increased [24]. The different result of the study was most probably caused by the different plant kinds [22] and the number of the plant agent added to the product as conducted by Serdaroglu et al (2018) who added 5% [24].

3.3 Color and antioxidant activity
The meatball's color was expressed in the hunter system including L* (lightness), a* (redness), and b* (yellowness) as shown in Table 3. The degree of lightness and redness of the meatball was not influenced by BHT and CLP (P>0.05). The incorporation of CLP 1 and 1.5% significantly enhanced the yellowness of the meatball (P<0.05), while the addition of CLP below 1%, the color substances was not adequate to influence the yellowness of the meatball. Similarly, Serdaroglu et al (2018) reported that the incorporation of pumpkin into the beef patty did not exert the different lightness of the product color [24]. This study also confirmed a previous study conducted by Selani et al (2015) that found beef burgers added with pineapple, passion fruit, or mango byproducts resulted in no significant differences among the lightness of the product [25].

The excess amount of non-meat ingredients, such as leaf plant powder added to meat products could cause unacceptable color changes, mainly in the red color. The red color is one of the indicators of
acceptability of the meat product influenced by the addition of plants agent. This research indicated that
the addition of CLP did not cause any undesirable red color changes to the meatball. This result
supported Ibrahim et al (2017) that studied the effect of Moringa oleifera leaves powder as an antioxidant
on the quality of meat-rice Kofta during frozen storage. Ibrahim et al (2017) reported that the addition
of Moringa leaf powder could not cause any changes the redness of the product color [26].

The yellowness of the meatball added with CLP was markedly different among treatments (P<0.05). The
addition of CLP 1 and 1.5% of CLP significantly higher than that of addition of CLP 0.5%, BHT,
and control. This result was most probably caused by the existence of color substances of the cemba
leaf. This result confirmed Al-Juhaimi et al (2016) that the increased of yellowness in the moringa seed
flour formulation was probably due to the presence of carotenoid pigments [27]. Al-Juhaimi et al (2018)
also reported that the addition of argel leaf powder on camel patty increased the yellowness than the
control [28]. Similarly, Choe et al (2019) found that the addition of some kinds of leaves in powder
form increased the b* value (yellowness). This study could be that the yellowness increased due to the
color pigment of the leaf [29]. Carotenoids is one of the important pigments that accumulated in the
chloroplast and mostly found in green plants Delgado-Vargas et al (2000) [30], including Cemba (Albizia).
The color substances from the plant could react with the ingredients substances and change
the color of the product.

Table 3. The L*, a* and b* values of Bali beef meatballs added cemba leaf powder.

| Formula     | L*      | a*     | b*     | DPPH (%) |
|-------------|---------|--------|--------|----------|
| CON         | 55.69±3.56 | 8.52±1.65 | 10.33±0.92c | 19.17±1.22d |
| BHT 0.01 %  | 57.52±2.25 | 9.36±1.27 | 10.89±1.13c | 25.66±1.06b |
| CLP 0.5 %   | 54.55±1.14 | 9.50±1.22 | 12.06±0.95bc | 23.18±1.40c |
| CLP 1 %     | 53.11±3.00 | 8.79±1.65 | 13.11±1.74b | 25.66±1.50b |
| CLP 1.5 %   | 53.94±2.54 | 8.42±1.48 | 15.38±0.97a | 30.58±1.70a |
| Average     | 54.96±2.96ns | 8.92±1.40m | 13.37±2.11 | 24.86±4.01 |

ns = non significantly; CON= Control; BHT = butylated hydroxytoluene; CLP= cemba
leaf powder; L* (Ligthness); a* (- = green ; + = redness); b* (- = blue ; + =
yellownes); Different superscript in the same coloumn indicates the significantly
different (P<0.05).

Table 3 ascertains that CLP improved the antioxidant activity of the meatball indicated by the
increase of DPPH inhibition percentage. The increase of the DPPH inhibition of the meatball was along
with the increase of the CLP added and the consequence was retard lipid oxidation of the product as also
reported by Fernandez -Lopez et al (2005) studied the plants extract could inhibit the lipids oxidation of
meatballs during 12 days storage [31]. The inhibition of the oxidation shows that the products have the
capability of antioxidant activity. The incorporation of natural antioxidants into fresh and processed
meat products resulted in preventing off-flavor development, strengthening color steadiness, improving
microbiological quality, and shelf-life, without any decrease in the sensorial and nutritional state [32].
Another study also showed that the use of plant extract in beef patty intensified antioxidant stability of
the product during storage [9].

The higher antioxidant activity of the meatball added with CLP could be caused by the phenolic
compound in the CLP. Hajrawati et al.[12] reported that cemba leaf extract contained a high phenolics
compound. Other researchers also reported that genus Albizia, which is cemba included, rich of phenolic
compound [33,34]. This result indicated that the CLP was rich of bioactive compound that function as
an antioxidant as just like the extract of this leaf. This study was also showed that the addition of CLP
1% was equal to addition of BHT 0.01% on the antioxidant activity of meatball. This result supported
other researchers who found that Albizia leaf powder contained high phenolic compound [35-37].
4. Conclusion
In conclusion, cemba leaf powder is potent to be a mixture substance in the meatball formulation that improve the meatball characteristics. The addition of cemba leaf powder 1–1.5% could replace the use BHT 0.01%, improve the antioxidant activity and increased the fibre content of the meatballs which is considered as healthy food.

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