The effect of addition of water spinach (Ipomoea aquatica forsk) on physico-chemical characteristics and antioxidant activity of Bali beef meatballs

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Abstract. Nowadays, the use of natural agents for antioxidants and preservatives are increasing for safety and healthy reason. One of the potential natural agents for food application is Water Spinach (Ipomoea aquatica Forsk). The characteristics of physico-chemical and antioxidant activity of the meatball added with water spinach leaf powder (WSLP) were observed in this study. Different four formulas were employed: control, formula without any addition of WSLP; WSLP 0.5%, WSLP 1% and WSLP 1.5% each was added with 0.5, 1 and 1.5% WSLP, respectively. The results of this study indicated that the addition of WSLP lowering the cooking loss and pH value, but increased the WHC of the meatball. The study also showed that WSLP decreased the color parameters (L*, a* and b*), but increased the antioxidant activity. The WSLP did not affect the moisture, ash, protein and fat but significantly increased the crude fiber content. It can be concluded that the addition of 1% of WSLP in the meatball formulation enhanced the physicochemical properties and antioxidant activity of the meatball.

1. Introduction

Meatball has become a special Indonesian food and has been most favorable for Indonesian. Nowadays, the serving of this product varies by frying, grilling, steaming, or boiling with vegetables, noodles, fried tofu in chicken or beef broth. Therefore, the meatball is ethnic food from Indonesia [1]. As just another processed meat product, the meatball is easily spoiled food. On the other hand, recently, consumers have become aware of food products without any addition of synthetic agents for health reasons.

Consequently, the utilization of natural antioxidants and antimicrobials from plants has been developing [2]. Food industry stakeholders also have been interested in substituting synthetic by natural agents, such as from plants [3]. Since meatballs are popular, they are ideal for enhancing their nutritional value and functional qualities such as increasing their antioxidant content to produce healthy food [4]. The employment of plants to processed meat products as a source of antioxidants has been reported by many researchers that it can extend the shelf-life of processed meat products by retarding lipids oxidation [5–8].

One of the prospective plants for use in meatball products is water spinach (Ipomoea aquatica Forsk). This plant is reportedly rich in minerals [9] and contains phenols and flavonoids...
compounds [10]. The addition of water spinach to the meatballs is expected to improve the antioxidant activity and physical characteristics of the meatballs.

2. Materials and methods

2.1. Material
The main materials used in this study were beef meat obtained from Antang slaughterhouse, kale leaf taken from Gowa regency, tapioca flour, ice cubes, garlic, sodium tripolyphosphate (STTP), salt, pepper, olive oil. The chemicals used were methanol (Merck, Darmstadt, Hesse Germany) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) obtained from Sigma Aldrich, USA.

2.2. Leaf powder preparation
The leaves of water spinach were cleaned and then air-dried for several days and oven-dried at 40°C for one hour. The dried leaves were crushed using a blender and sieved using a 35 mesh sieve [11]. The powder obtained was for use.

2.3. Meatball preparation
The manufacture of meatballs with minor modification [12]. The meat was trimmed into smaller size and ground. The ground meat was incorporated with salt, ice cubes, and phosphate and then homogenized using a food processor. Tapioca flour, flavorings, pepper, garlic, olive oil, and kangkung air leaf powder were added according to the treatment (0.5%, 1%, 1.5%; w/w) and then reground. The emulsion was formed into a round and boiled at a temperature of 80°C for 20 minutes.

2.4. Proximate composition
The nutritional composition was determined by using standard proximate analysis (moisture, protein, ash, fat and crude fiber content) on the official standard method [13].

2.5. Cooking loss determination and Water Holding Capacity determination
A 20 g of meatball dough was put into heat-resistant plastic, then boiled using a water bath at a temperature of 80°C for 20 minutes. Cooking loss was determined by measuring the difference in sample weight before and after cooking and calculated [14].

WHC was measured by weighing 10 g of the sample and mixed with 40 ml of distilled water in a tube, then incubated in a water bath at 30°C for 30 minutes. The samples were then centrifuged at 3000 rpm for 30 min. After that, the sample was put back into the water bath for incubation for 10 min at a temperature of 30°C. Furthermore, the filtrate was removed after which the sample was weighted [15].

2.6. Color measurement
The color (L*, a*, and b*) parameters were measured by using an instrument color meter test (T135). The instrument was standardized on a white plate (L*= 94.76, a*=-0.795 and b*= 2.200) before use. Measurements were in three spots of the sample and the average of data were used.

2.7. Antioxidant activity
A 0.2 mL of 20% (w/v) methanolic extract of sample was reacted with 1.8 mL of 0.06 mMol of DPPH radical solution. The solution was homogenized and incubated at room temperature for 30 minutes. The absorbance was measured at a wavelength of 517 nm using a UV-1800 UV-Vis spectrophotometer [11].

2.8. The pH value
The pH value was measured by piercing the meatballs with an electrode from the calibrated pH meter (BOECO PT30).
3. Results and discussion

3.1. Nutritional content of water spinach leaf extract

The nutritional content has an important role and indicates the quality of food. The nutritional composition of this study is depicted in table 1. Only the crude fiber component was affected by the addition of WSLP (P<0.05). The increase of crude fiber content of meatball was along with the addition of WSLP. It was also supported by the fact that WSLP is a source of crude fiber shown by a high content of crude fiber of WSLP (table 1). The composition of water spinach leaves was rich in fiber as much as 17.67% [9]. This study exerted the fiber contained in the WSLP was 9.17%.

| Formula      | Moisture% | Ash%  | Crude Protein% | Crude fat% | Crude Fiber% |
|--------------|-----------|-------|----------------|------------|--------------|
| WSLP         | 12.23     | 9.83  | 33.61          | 5.66       | 8.05         |
| 0%           | 70.50±0.99 | 2.23±0.10 | 13.98±0.44    | 0.58±0.09  | 0.16±0.04    |
| 0.5%         | 69.80±1.06 | 2.16±0.16 | 14.38±0.76    | 0.49±0.05  | 0.18±0.03    |
| 1%           | 70.14±1.33 | 2.09±0.27 | 13.96±0.92    | 0.61±0.20  | 0.21±0.03    |
| 1.5%         | 70.21±0.84 | 2.15±0.24 | 13.80±0.36    | 0.59±0.13  | 0.27±0.06    |

WSLP = Water spinach flour; different superscript in the same column indicate significantly different (P<0.05).

This result also was supported by Indonesia National Standard [16] for moisture content which is allowed max 70% and fat content which is tolerated max 10%, while the protein and ash and protein content were higher than that permitted (ash 3%, protein 11%).

3.2. Cooking loss, pH, water holding capacity

The cooking loss, pH and WHC results are presented in the table 2. This study showed that the addition of WSLP markedly influenced the cooking loss, pH and WHC of meatballs. The cooking loss percentage of the meatball was significantly decreased for WSLP 1% compared to Control (P<0.05). The WSLP 0.5% and 1.5% were neither different from control nor WSLP 1%. The loss of cooking occurs due to the loss of fat and water evaporation. By adding WSLP, probably increases the product’s ability to bind water so that it reduces evaporation and it is reflected in the increased water-binding water of the meatballs added by WSLP. This result is supported by other research using leave powder to processed meat products. The addition of lotus (Nelumbo nucifera) powder to sausage exerted the lower cooking loss and higher emulsion stability of the product [17]. The use of dragon fruit peel powder increased the cooking yield of sausage [18]. One of the factors influencing the WHC is protein, if the protein is denatured, the value of its WHC decreases [19]. The addition of WSLP in this study increased the WHC indicating the possibility of the protein not being denatured, in contrast to the control.

The cooking loss is related to the capability of the meat to hold the water. This study also indicated that the value of WHC was significantly increased (P<0.05) by adding the WSLP. This result supported the reason for improving the cooking yield of the meatball added with the WSLP. The higher WHC of meatball added with WSLP than control was most probably caused by the starch and dietary fiber content in the WSLP which had the emulsifying and gelation properties [20]. Besides, it could be caused by the hardness texture of the product by adding with leave powder [21].

This result also showed that the addition of the WSLP significantly decreased the meatball pH. The lower pH was likely contributed by the leaf powder that is rich in phenolic compounds. The hydroxyl group of phenolic compounds can decline the pH of a substrate [22]. Other research also indicated that the addition of leaf powder to food could decrease the pH value of the product. The addition of lotus leaf decreased the pH of chicken patty [6].
Table 2. The cooking loss, pH value, and water holding capacity meatballs added water spinach flour.

| Formula       | Cooking loss | pH       | WHC       |
|---------------|--------------|----------|-----------|
| Control       | 4.71±0.89\(^a\) | 6.23±0.20\(^a\) | 32.41±3.37\(^b\) |
| WSLP 0.5%     | 4.06±1.43\(^ab\) | 6.10±0.11\(^b\) | 36.51±1.81\(^a\) |
| WSLP 1%       | 2.71±0.53\(^b\) | 6.15±0.11\(^b\) | 35.18±1.99\(^a\) |
| WSLP 1.5%     | 3.41±1.24\(^ab\) | 6.07±0.06\(^b\) | 35.88±2.93\(^a\) |

WSLP= water spinach flour; \(^ab\)different superscript in the same column indicate significantly different (P<0.05).

### 3.3. Color parameters and antioxidant activity

The color parameters of the study are presented in Table 3. The result indicated that the addition of WSLP markedly reduced the lightness and redness (P<0.05) but increased the yellowness of the meatball (P<0.05). The decrease in lightness, redness, and increase in yellowish levels were most likely caused by the green substance of WSLP. As a plant, WSLP contains chlorophyll and it affects the color of meatballs added with WSLP. The results of this study were also in line with Boruzi and Violeta [23] which examined the use of walnut leaf meal (Juglans regia L.) in pork patties. The results showed that the patty had decreased lightness and redness with Juglan leaf powder. A similar result was reported by Choi et al (2011) using lotus leaf powder and Ergezer et al (2018) using plant extracts [5,24]. It can be assumed that the decreased lightness and redness level of processed meat products is due to the presence of chlorophyll.

The increased level of yellowness in meatballs given WSLP was probably due to the role of antioxidant properties in WSLP. The presence of antioxidants can act to block free radicals to preserve pigments and maintain them a yellow color [25].

Table 3. The L*, a* and b* value and antioxidant activity of meatballs added water spinach flour

| Formula       | L*       | a*       | b*       | DPPH     |
|---------------|----------|----------|----------|----------|
| Control       | 54.68±3.24\(^a\) | 13.84±2.46\(^a\) | 13.74±1.29\(^d\) | 23.67±0.58\(^d\) |
| WSLP 0.5%     | 52.11±1.53\(^a\) | 8.06±1.08\(^b\) | 14.43±0.98\(^c\) | 30.27±1.41\(^c\) |
| WSLP 1%       | 48.39±3.85\(^b\) | 4.58±0.26\(^c\) | 15.67±1.14\(^a\) | 33.95±1.21\(^b\) |
| WSLP 1.5%     | 46.49±1.00\(^b\) | 4.33±0.50\(^c\) | 15.03±0.78\(^b\) | 37.08±1.77\(^a\) |

WSLP= water spinach flour; \(^ab\)different superscript in the same column indicate significantly different (P<0.05).

The results of this study showed that the addition of WSLP increased the antioxidant activity significantly (P<0.05) at each level of WSLP addition. This increase was due to the contribution of WSLP's bioactive ingredients. Water spinach has high antioxidant activity and it was a correlation between antioxidant activity and total phenol or flavonoid content [10].

One of the phenolic compounds found in water spinach is carotenoids which can break down DPPH. The scavenging activity of DPPH free radicals is due to their ability to donate hydrogen. The greater the number of hydroxyl groups, the higher the possibility of free radical scavenging ability. Linear correlation between radical scavenging activity and the phenolic compound has been reported in various types of vegetables and fruits [26].

### 4. Conclusion

The addition of WSLP was increased the crude fiber content, improved the physical properties and antioxidant activity, but decreased the color parameters of the Bali beef meatball. The addition of 1% of WSLP exerted the best properties of the meatball characterized by the lowest cooking loss with WHC and antioxidant activity increased markedly from control.
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