Chitosan-garlic essential oil incorporation on beef meatball edible coatings as antioxidant-based functional food

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Abstract. In addition to providing protection, edible coatings can also improve the functional properties of the coated food through the incorporation of bioactive compounds, such as antioxidants. The study aimed to investigate the potential of chitosan and/or garlic essential oil (EO) incorporation into the edible coatings of beef meatballs in enhancing the antioxidant properties. The potency of chitosan, garlic EO and its combination in increasing the antioxidant properties of meatballs were examined by comparing them toward meatballs without bioactive compounds incorporation (control) for 18 days of storage at 4±1°C. This study used a completely randomized design and the data were analyzed using an analysis of variance at p>0.05. Results showed that incorporation of chitosan and garlic EO into edible coatings increasing both either RSA or RP of coated meatballs. RSA of meatballs increased 3.7 times (chitosan), 4.6 times (garlic EO) and 7 times (chitosan-garlic EO), while the RP increased 2.6 times (chitosan), 2.9 times (garlic) and 3.0 times (chitosan-garlic EO). During storage, all of the meatballs tested showed a reduction in both RSA and RP. Incorporation of chitosan-garlic EO into the edible coating produces a synergistic effect in RSA but it showed antagonist effect in RP.

1. Introduction

Beef meatballs are one of the popular and favorite beef products in all classes of Indonesian society especially the youngsters. Dewi and Mahmudiono [1] reported that beef meatballs consumption of Indonesian people reaches 1-2 times a week on average. Akcan et al. [2] proved that beef meatballs coated with edible coatings prepared using whey protein isolate incorporated with Laurus nobilis and/or Salvia officinalis extracts were increases the antioxidant activity of beef meatballs during frozen storage.

Active packaging (AP) is a packaging which was incorporated with bioactive compounds so that in addition to imparting product protection its also improves the product functional values [3]. One of the bioactive compounds that can be incorporated into AP is antioxidants [4]. Antioxidants are compounds which are capable to prevent oxidative stress through their abilities to neutralize reactive oxygen species (ROS), therefore provide various health benefits such as anti-diabetic, anti-cancer, and anti-atherosclerotic activities [5,6]. Antioxidants define as “any substance that delays, prevents, or removes oxidative damage to a target molecule”[7].

Chitosan and essential oil of garlic are natural ingredients which have been proven to exhibit antioxidant capabilities [8,9,10,11,12,13]. The combination of two or more antioxidant may show...
synergistic, antagonistic or additive effects [14]. Incorporation of essential oil (EO) in chitosan edible coatings increased the antioxidant capacity, such as EO of Zataria multiflora Boiss [15], EO of Thymus [16], EO of cinnamon [17], and EO of caraway [18].

Previous researchers have proven that chitosan and/or EO added to meat products inhibits the oxidative deterioration [19,20,21,22,23]. The addition of antioxidant directly into food products decreases its antioxidant capacity due to interactions with the food matrix. Incorporation of antioxidants into edible coatings is one simple strategy to minimize the decrease in antioxidant effectiveness. Ponce et al. [24] reported that the direct addition of chitosan did not show antioxidant effects but in the form of chitosan edible coatings showed inhibition of polyphenol oxidase and peroxidase enzymes.

Kanatt et al. [25] showed that application of chitosan coatings on chicken meatballs capable to inhibit the lipid oxidation and microbial growth. Yuan et al. [26] reported that chitosan coatings have the potential to extend the shelf life of meat and fish products. Several studies have examined the incorporation of EO in edible coatings to inhibit oxidation and microbial growth in meat and meat products, such as Ginger EO [27], Ziziphora clinopodioides EO [28], Origanum virens EO [23], Ziziphora persica EO [22], and Satureja thymbra EO [29]. To the best of our knowledge, study on the incorporation of chitosan and/or garlic EO in meatballs edible coatings to improve the antioxidants properties have not been reported.

This study examined the potential of chitosan and/or garlic EO incorporated in beef meatballs edible coatings to improve the antioxidant properties as an alternative of antioxidant-based functional food development. The potential of the coated beef meatballs as functional food was determined by measuring its antioxidant activity for 18 days of storage at a temperature of 4 ± 1ºC. The potential of chitosan and garlic EO as antioxidant ingredients were evaluated by measuring the radical scavenging activity and reducing power.

2. Methods

2.1 Beef Meatballs Preparation

Beef meatballs were prepared refers to Komariah and Hendrawati [30]. The silverside beef minced using a meat chopper (Philips HR2939) with the addition of ice water (20%). Salt (3%) was added to pulverized meat, followed by addition of tapioca flour (20%), it was then mixed until homogenous. The resulting dough was molded into balls (15g of each ball). The raw meatballs was boiled until cooked (for about 30 minutes) and then drained.

2.2 Preparation of Edible Coatings

Edible coatings prepared by the method previously described by Amalia and Putri [31] with slight modifications. Briefly, dispersion of cornstarch in water at a concentration of 5% was added with 1% (v/v) glycerol and then heated and stirred using hotplate magnetic stirrer (Heidolph MR 3001 R) for 30 minutes at 75ºC and 50 rpm. This edible coatings solution is further cooled until the temperature reaches 37ºC. In this study, edible coatings were prepared using 4 formulas, namely control (without any addition), chitosan addition, garlic EO addition, and the addition of chitosan-garlic EO mixture. Chitosan was purchased from CV. Bio Chitosan Indonesia (Jakarta, Indonesia), whereas garlic EO was obtained from CV. Happy Garden (Jakarta, Indonesia). Chitosan solution of 1% (in 1% acetic acid solution) was added as much as 1% (v/v) after glycerol added (before heating treatment). Incorporation of garlic EO of 0.5% (v/v) was carried out after the temperature of the edible coating solution reached 37ºC.

2.3 Application of Edible Coatings on Beef Meatballs

The method used for edible coating applications on beef meatballs as previously described [32]. Meatballs are dipped into edible coatings solutions for 60 seconds, lifted and air dried. The coated beef meatballs then stored at a controlled temperature of 4±1ºC. Antioxidant activity evaluation
(radical scavenging activity and reducing power) of beef meatballs were conducted at 0, 5, 10, 14 and 18 days of storage.

2.4 Radical Scavenging Activity (RSA) Analysis
RSA of beef meatballs was determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) Antioxidant assay [33]. The RSA was expressed as Gallic acid equivalent activity (GAEA) (mg GA/kg sample).

2.5 Reducing Power (RP) Analysis
RP test was conducted using the FRAP (ferric-reducing antioxidant power) method [34]. The RP of beef meatballs was expressed as Ascorbic acid equivalent activity (AAEA) (mg AA/kg sample).

2.6 Statistical Analysis
Data were presented as mean and standard deviations. The data of radical scavenging activity and the reducing power were analyzed using the program of IBM SPSS Statistics 22 (SPSS Inc., Chicago, USA) by analysis of variance (ANOVA). Independent sample t-test was used to evaluate the significant differences (p<0.05) on the potency of chitosan and garlic EO as antioxidant agent. Duncan’s multiple range test (DMRT) was used to evaluate the significant differences (p<0.05) on the antioxidant activity among samples (chitosan, garlic EO, chitosan-garlic EO, and control).

3. Results and Discussions

3.1 The potential of chitosan and garlic EO as antioxidant agent
Antioxidant potential of chitosan and garlic EO which were used in this study was evaluated by measuring its radical scavenging activity (primary antioxidant) and reducing power (secondary antioxidant), the results were presented in Table 1. Primary antioxidant retards oxidation by radical scavenging, whereas secondary antioxidant inhibits oxidation by a mechanism that does not involve direct scavenging of free radicals[35].

Table 1. Radical scavenging activity (Gallic acid equivalent activity) and reducing power (Ascorbic acid equivalent activity) of chitosan and garlic essential oil.

| Bioactive compounds | RSA (mg GA/kg) | RP (mg AA/kg) |
|---------------------|----------------|---------------|
| Chitosan            | 2867.00±5.19a  | 51452.00±51.50a |
| Garlic EO           | 3820.16±5.19b  | 56477.00±51.50b |

Different letter within a similar column means significant differences (p<0.05)

Garlic EO showed higher antioxidant activity rather than chitosan, in term of both radical scavenging activity (RSA) and reducing power (RP) (Table 1). Previous studies reported similar results. The RSA of garlic EO and chitosan at a concentration of 1 mg/ml were 64.1% and 38.03 % respectively [10, 36]. Lawrence dan Lawrence [10] dan Wu et al. [37] indicated that garlic EO exhibited higher reducing capacity rather than chitosan. The reducing power of garlic EO at a concentration of 2.5 ppm equal to that of 500 ppm chitosan.
3.2 Antioxidant activity of coated meatballs with various edible coatings formulas during storage of 4±1°C.

3.2.1 Radical scavenging activity (RSA)

Table 2. Radical scavenging activity (Gallic acid equivalent activity) of coated beef meatballs with various edible coatings formulas during storage at 4±1°C.

| Edible Coatings Formulas            | RSA of coated meatballs during storage (mg GA/kg) |
|-------------------------------------|--------------------------------------------------|
|                                     | 0 day 5 days 10 days 14 days 18 days             |
| Control                             | 103±0.01a 9±0.02a 9±0.02a 0±0.00a 0±0.00a       |
| Chitosan                            | 370±0.01b 136±0.01b 127±0.01b 73±0.01a 70±0.01a |
| Garlic essential oil                | 463±0.01c 202±0.01c 193±0.01bc 181±0.01bc 175±0.01ac |
| Chitosan-garlic essential oil       | 700±0.01d 670±0.01d 667±0.02b 586±0.01b 586±0.01b |

Different superscript in the same rows and different subscript in the same columns mean significant differences (p< 0.05)

Data presented in Table 2 indicated that incorporation of chitosan and/or garlic EO into beef meatballs edible coatings significantly improve RSA of the coated meatballs reach to 3.7 times (chitosan), 4.6 times (garlic EO) and 7 times (chitosan-garlic EO) respectively. Beef meatballs coated with either chitosan or garlic EO edible coatings have significantly higher RSA than that of control in all storage periods. Both chitosan and garlic EO proved to be good radical scavenging agents (Table 1), thus its incorporation into edible coatings improved RSA of the coated meatballs. During storage, chitosan edible coatings produce coated meatballs with a lower RSA rather than the garlic EO ones. This result was consistent with the RSA data in Table 1 which shows that garlic EO exhibits significantly higher RSA than chitosan. Beef meatballs coated using chitosan-garlic EO edible coatings provide the highest RSA during storage periods. The sum of the RSA value of chitosan edible coatings treatment and garlic EO edible coatings treatment was lower than the RSA value of chitosan-garlic EO edible coatings treatment, indicated that there was a synergistic interaction between chitosan and garlic EO on RSA.

Synergistic interaction of chitosan and garlic EO on RSA related to the different radical scavenging mechanisms in both bioactive compounds so that there is no competition in scavenging free radicals. DPPH classified as a method utilizing both Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) mechanisms [38]. The radical scavenging capacity of chitosan via the electrons donor, whereas garlic EO through the protons (hydrogen) donor. The antioxidant activity of chitosan as a free radical scavenger generally due to the presence of nitrogen groups in C-2 which can eliminate various types of free radicals [39]. Pasanphan and Chirachanchai [40] stated that the mechanism of chitosan in scavenging free radicals is related to the electrons donor ability. The hydrogen donor ability of chitosan is relatively low due to strong hydrogen bonds both intermolecular and intramolecular. Diallyl disulfide was reported as one of the major compounds of garlic EO [12,41]. The level of this organosulfur compound in commercial garlic EO reached 25.3% [42] dan 25.9% [43]. Colin-Gonzales et al. [44] stated that antioxidant capacity of organosulfur compounds related to the thiol groups which can easily donate its proton to an electrophilic species, thereby neutralizing them. Radical scavenging mechanism of garlic organosulfur compound involves H-atom transfer from the methylene of the allyl group on the divalent sulfur to the radicals [45].

Table 2 showed a decreasing in radical scavenging activity along with increasing storage time. This is related to the stability of the chitosan and garlic EO during storage. Dhawade and Jagtap [46] reported increasing of chitosan molecular weight during storage due to the oligomers changes. Chien et al. [47] stated that high molecular weight chitosan (HMWC) showed a lower DPPH radical scavenging activity than low molecular weight chitosan (LMWC). The decrease in RSA of coated meatballs with incorporation of garlic EO is related to evaporation of the EO volatile compounds during storage [48]. Politeo et al. [49] stated that the decrease in volatile compounds of essential oil resulted in a decrease in
its radical scavenging capacity. Kim and Kobayasi [50] explained that the concentration of diallyl disulfide decreases during storage. Diallyl disulfide is one of the major compounds of organosulfur antioxidant in garlic EO [51].

3.2.2 Reducing Power

Table 3. Reducing power (Ascorbic acid equivalent activity, AAEA) of coated beef meatballs with various edible coatings formulas during storage at 4±1°C.

| Edible Coating     | 0 day          | 5 days         | 10 days        | 14 days        | 18 days        |
|--------------------|----------------|----------------|----------------|----------------|----------------|
| Control            | 3701.72±10.30<sub>A</sub> | 3695.77±10.30<sub>A</sub> | 3677.93±10.30<sub>A</sub> | 3671.98±10.30<sub>A</sub> | 3671.98±10.30<sub>A</sub> |
| Chitosan           | 9759.70±51.50<sub>B</sub> | 9551.60±68.13<sub>B</sub> | 9521.60±77.25<sub>B</sub> | 9343.50±77.25<sub>B</sub> | 9343.50±77.25<sub>B</sub> |
| Garlic EO          | 10651.00±25.75<sub>C</sub> | 10071.00±25.75<sub>C</sub> | 10057.00±44.60<sub>C</sub> | 9566.50±44.6<sub>C</sub> | 9536.7±68.13<sub>C</sub> |
| Chitosan-garlic EO | 11172.00±0.00<sub>D</sub> | 11082.00±89.20<sub>D</sub> | 10993.00±44.60<sub>D</sub> | 10413.00±44.04<sub>D</sub> | 10354.00±92.84<sub>D</sub> |

Different superscript in the same rows and different subscript in the same columns mean significant differences (p<0.05)

Table 3 indicated that the control sample exhibits reducing power. According to Udenigwe and Aluko [52], amino acids with sulfur groups (methionine and cysteine) have the higher reducing capacity than other amino acids. Beef has methionine and cysteine contents of around 1.99% and 1.21% respectively [53]. Meatball samples with the incorporation of chitosan or garlic EO showed higher AAEA than the control sample. The AAEA of beef meatballs coated with chitosan, garlic EO, and the chitosan-garlic EO edible coatings were 2.6 times, 2.9 times and 3.0 times higher than that of the control ones. This is related to the reducing power of chitosan and garlic EO (Table 1). The reducing power of chitosan due to its amine group (-NH₂) [54]. EO showed reducing capability [10] related to the organosulfur compounds. Meatballs coated with chitosan edible coatings exhibited a lower reducing power than that of garlic EO edible coatings ones. This result was in line with the reducing power data of garlic EO and chitosan presented in Table 1.

The value of RP in meatballs with chitosan-garlic EO treatment showed an antagonistic effect, indicated by its lower RP value compared to the sum of RP values of the individual treatments (chitosan treatment and garlic EO treatment). Antagonistic effects that occur may be attributed to the competition between the two compounds to reduce Fe<sup>3+</sup> ion or the limited of potassium ferricyanide available as reduced substance in the reaction system.

Similar to the RSA, the RP also decreased with increasing storage time. As explained in the RSA section, this result also related to an increase in chitosan molecular weight as well as reduced of garlic EO volatile compounds during storage. The RP of LMWC was higher than that of HMWC [55,37]. The decrease in the concentration of essential oil volatile components also resulted in a decrease of reducing power [49].

4. Conclusions

At all storage periods, meatballs coated with edible coatings incorporated with chitosan, garlic EO, and chitosan-garlic-EO showed significantly higher antioxidant capacity than control (without any incorporation). Incorporation of chitosan-garlic EO garlic into edible coatings provides the highest antioxidant activity of coated meatballs. The antioxidant activity of beef meatballs coated with chitosan-EO garlic edible coatings showed a synergistic effect as a primary antioxidant (radical scavenging activity) and antagonistic effect as a secondary antioxidant (reducing power). It can be concluded that the incorporation of chitosan-garlic EO into beef meatballs edible coatings provide an alternative antioxidant-based functional food development, especially as the radical scavenger.
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