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PERFORMANCE EDIBLE COATING CONTAINING OLEORESIN FROM GINGER EMPRIT (ZINGIBER OFFIVINALE VAR. AMARUM) AND ITS EFFECT ON CONSUMER PREFERENCE PROPERTIES

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ABSTRACT

Ginger oleoresin (GO) as natural compounds becoming widely used to edible film, and coating for extending shelf life product. The objective of this study was to investigate the performance oleoresin and accept of consumer sensory properties from ginger emprit. Preliminary UHPLC-MS assay analysis showed that most components of oleoresin (24.7%) geranial and then gingerdione (10.2%). The methods were active concentration 1%, 1.5% and 2% (wt) GO applied onto edible coating toward beef meatballs. The resulting minimum inhibitory showed GO 1.5% was the optimum concentration against Gram-positive bacteria (S. aureus) 4.3 mm and Gram-negative bacteria (Salmonella) 0.5 mm. During the storage period, minimum meatballs quality was determined based on microbiological (Total Plate Count/TPC) and pH. Meatballs with edible containing GO 1.5% preserve the best quality with resulted in 1.83 log reduction of TPC and average pH value at 5.7. On sensory properties, the attribute was colour, taste, flavour intensity, juiciness, and hardness. Only 2% GO concentration has a negative effect on colour.

1. Introduction

In Indonesia, meatball or known as 'bakso' is popular street food. The consumption of meatball has increased by 18.5% over the last decades due to the relatively convenient purchase and availability (Kurniati et al, 2014; Purnomo & Rahardiyan, 2008). Currently, in Indonesia food adulteration is a major issue in the meatball household-firm and is causing concerns among consumers and local food authority (Rahmania et al, 2015). The presence of prohibited chemical material in food products to gain economic benefits are serious matters in view of consumer health and protection. One of the major authenticity issues of household-firm meatball product using the prohibited material (e.g formalin, borax) for shelf life extension (Ministry of Health, 2017).

Meatball is contained high protein which prompt transforms the meatball to be a susceptible product for the growing of pathogenic and spoilage microorganisms (Kerry et al., 2006). The edible coating is simple method have been developed for shelf life extension to inhibit the growth of undesirable microorganisms and reduce lipid oxidation in the meat-based product. Recently, a variety of

plant resource such as oleoresin for antimicrobial compound applied in edible coatings or films have been developing for application in fresh red meat, fish products and processing food (Horita et al., 2018; Granato, Nunes, & Barba, 2017; Nikmaram et al., 2017; Lorenzo, Batlle, & Gómez, 2014). The oleoresins characteristic is dark brown oil has an unstable mixture of essential oils and resin, non-volatile components that are hydrophobic, and lipophilic in the form of viscous liquids lifting specific scent plant or herbs obtained by extraction (Yasni, 2017).

There are numerous studies on isolation and activities of ginger oleoresins deals with chemistry reactions (Singh et al., 2008; Babu et al.,2018), and anti-bacterial (Auta et al., 2011; Park et al., 2008; Shahidi and Hossain, 2018); however, indigenous ginger from Indonesia emprit (*Zingiber Offivinale var. Amarum*) are not studied so vastly. The purpose of the present study was to investigate ginger emprit oleoresin as alternative antimicrobial agents, against Gram-negative bacteria (Salmonella) and Gram-positive bacteria (S. aureus) and evaluate the effects of using oleoresin when incorporated in beef meatball based coating toward consumer sensory properties. Our goal to find a simple adopting compound and method which can be done by household firms.

2. Materials and methods

2.1. Materials and Media culture

The mature and healthy of ginger emprit (*Zingiber officinale var. Amarum*) were bought from the local market in Malang City, Indonesia. Fresh beef meatball were purchased from household firms.

Glycerol, sulfuric acid (H_2SO_4), Boric acid, NaOH and Petroleum ether used were of analytical grade were purchased from Merck (Darmstadt, Germany). The inhibition zone tests using culture medium of Nutrient agar Merck (Darmstadt, Germany) and resazurin Sigma Aldrich (Missouri, USA) as a metabolic indicator. Clinical isolated pathogenic Salmonella and S.aureus were purchased from Medical Laboratory (University of Muhammadiyah Malang).

2.2. Preparation of oleoresin extraction

The simplicial form ginger was properly weighed to 200 gr put into a beaker glass then added 1L 96% ethanol. The beaker glass perfectly preserved from light and evaporation. The stirring inside the beaker glass was achieved magnetically at 200 rpm for 6 hours. After the extraction, samples were filtrated by a Buchner funnel and Whatman filter paper 42mn and the remaining solvent was removed by rotary evaporation at 50 ºC for 20 min (Fernández-Ronco et al, 2008; Singh et al., 2008). The obtained light yellow colored oil with a pleasant odor was dried over anhydrous sodium sulfate yield was then calculated as the grams and stored in sealed vials at (4 ± 2 ºC) in dark for further use and UPLC-MS analysis.

2.3. Analysis of chemical oleoresins presumptive composition

Ginger oleoresin components identification and presumptive amount present were determined by using UHPLC-MS (Angler Laboratory, Surabaya, Indonesia). Operated in negative ion mode in a capillary temperature of 100°C, gas atomizer with a flow rate of 25 L/hour, the source of voltage +2.9 kV in full scan mode (range 100 −700 m/z) at 30°C temperature, an Acquity UPLC BEH C18 (2.1 mm × 50 mm, 1.7 m; Waters, USA).

Tabel 1. Presumptive identification of the components in ginger oleoresin

t_R (min)	Molecular Formula	Compounds	Fragments (m/z)	Concentration (Peak %)
1.26	$C_6H_{14}NO_5$	Glucosamine	180.0866	0.3
3.51	$C_{10}H_{16}O$	Geranial	224.0716	24.7
4.43	$C_{10}H_{12}O_2$	*Eugenol*	237.1119	36.8
5.1	$C_{17}H_{26}O_4$	6-Gingerol	293.1757	8.9
5.7	$C_{17}H_{24}O_4$	Gingerdione	291.1599	10.2
6.32	$C_{17}H_{24}O_3$	6-Shogaol	275.1651	4.7
6.42	$C_{19}H_{30}O_5$	5-Acetoxy-6-gingerdiol	337.2008	Trace
7.40	$C_{23}H_{34}O_4$	Dehydro-12-gingerdione	373.2375	7.8
9.25	$C_{19}H_{32}ON_5$	2,2-Dimethoxyethyl	368.2431	Trace
Total				93.7%

A ternary gradient elution consisting of 0.1% (v/v) formic acid in water (system A) and 0.1% (v/v) formic acid in acetonitrile (system B), at a flow rate of 0.2mL. min-1 with injection volume of 5 µL. Mass data were processed by the MassLynx V4.1 software.

2.4. Disc diffusion

Both pathogenic strains were cultured in nutrient broth for activation at 37 ºC for 24 h. Afterwards, inoculation into nutrient agar discs which aseptically prepared with the spread plate method. Then, put over sterilized Whatman filter paper no.1 with 5 mm diameter has been dipped into 20 ml of coating solutions containing GO with concentration 1% (1µL/100 µL), 1.5% (1.5 µL/100 µL), and 2% (2 µL/100 µL). The plates were incubated in an upright position at 37 ºC, each plate is examined every next three days (3, 6, 9 and 12). The diameters of inhibition zones (in mm) were measured (Seol et al., 2009; Noori et al. 2018).

2.5. Preparation of coating solutions

Crude ginger weighed 5g dissolved in 100ml distilled water and stirred at a controlled temperature of 80ºC and 1100 rpm for 45 min. 1.2 g (30% wt of crude) glycerol was added as a plasticizer. Then, oleoresin was added with constant stirring to reach a final concentration of oleoresin 1 to 2% wt of crude ginger. After, 10 min stirring the solutions were applied for meatball dipped in a coating solution for 2 minutes and 1h for dried.

2.6. Meatball quality testing

2.6.1. Total Plate Colony (TPC)

Samples 20 g were prepared aseptically and put into sterile plastic 180 ml containing a sterile water mashed up by stomacher® 400C (10^{-1}) for 2 min. 1 mL of solution 10^{-1} dilution was taken by used micropipette and 2 min homogenized in 9 ml sterile water (10^{-2}) using vortex mixer, this step is repeated until reached (10^{-6}) (Wasteson and Hornes, 2009). Serial dilutions 10^{-5} and 10^{-6} were taken 1 mL using the pour plate method on nutrient agar Merck (Darmstadt, Germany). All plates were incubated at 37°C for 48 h, colonies data were transformed into logarithms of the number of colony forming units (Log CFU/g).

2.6.2. pH analysis

Meatballs were measured using digital pH meter (SI analytics LAB 875) instrument after samples (20 g) have been homogenized in distilled water (10 ml).

2.6.3. Sensory evaluation

Semi-trained 20 member panellists (13 females and 7 males) were selected from undergraduate students the department of food science and technology of the University of Muhammadiyah Malang evaluated the total acceptance of samples. Panelists were provided

with an assessment form, plastic bowl, napkin, toothpick, a cup of water, and palate cleansers (plain crackers) to use between samples. All panelists had a background in beef meatball evaluation and were selected based on their sensitivity and limit detection of flavor intensity and taste concentrations of oleoresin. The attributes considered in the sensory evaluation were colour, taste, flavor intensity, juiciness, hardness and overall acceptability using 5 points descriptive scale, where 5 = extremely desirable, 1 = extremely undesirable and score of 3 was taken as the lower limit of acceptability.

2.7. Statistical Analysis

All experiments were performed in twice with analysis of variance (ANOVA) (SPSS Inc, Version 20) performed with a completely randomized design. Duncan's test ($p < 0.05$) was used to detect differences among mean values of meatballs properties in all test intervals.

3.Results and Discussions

3.1. Identification of the components in ginger oleoresin

The Careful identification of GO emprit was carried out HPLC assay result which interpretation contains a specific large number of compounds in Table 1. From Table 1 it is evident that in GO existing four major components of 9 components constituting 93.7% of the total weight. Most of the findings have similarity with other variety of ginger (*var. rubrum and var. officinale*) which has been reported in previous study (Agrawal et al. 2001, Kamaliroosta et al. 2012 and Gurdip Singh et al 2008). The differences only on the amount, Singh et al. (2008) identified Eugenol (49.8) and geranial (25.9%) in our study indicated amount *Eugenol* (36.8%), geranial (24.7%) and glucosamine trace (0.3%) was rarely found in rhizome plants. While specific emperit comparison with Arijanti et al. (2019) identified 6-Gingerol have the main components of GO on Gingerol 1.8%, Shogaol 0.10% and Zingerone 0.86%. There are many factors could be drive differences in the chemical composition of GO such as genetic, production conditions, environmental, weather conditions, distillation conditions and other factors (Rehman et al., 2016; Blair et al., 2001).

3.2. Antimicrobial activity testing

The performance results from antimicrobial activity testing of GO against S. aureus (Gram-positive) and Salmonella (Gram-negative) has different effective concentration is shown in Fig 1. GO concentration starting at 1% (1µL/100µL), 1.5% (1.5 µL/100µL), and 2% (2µL/100µL) was found to be more susceptible to against Salmonella gram-negative.

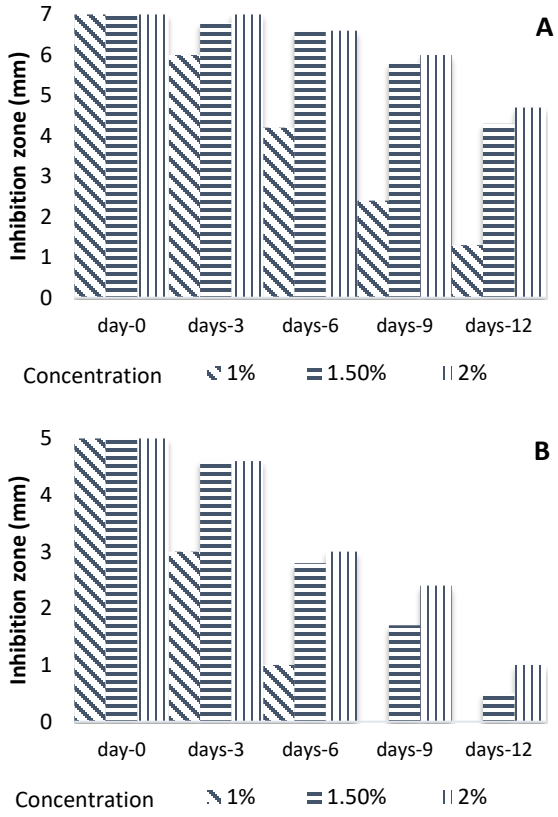

Figure 1. Anti-bacterial activity of meatball edible coatings containing ginger oleoresin against S. aureus (A) and Salmonella (B). Data shown are the means ± standard deviation ($P < 0.05$).

The difference GO was comparatively more effective against S. aureus gram-positive. This discrepancy of the higher resistance of gram-

negative due to the cell wall structural have an outer membrane containing a thin peptidoglycan layer and lipopolysaccharide. It acts permeability barrier lead to reducing bioactive compounds activity by absorption of reactive oxygen species (ROS) thereby affecting the performance of GO (Russell, 2003). The intense inhibiting activity of GO against gram-positive could be due to interactions between principal component properties of phenolic compounds (Eugenol, geranial, gingerdione, 6-Gingerol and 6-shogaol) with the positive charged cell wall (Calo et al., 2015).

In the recently, GO study has been used to inhibit the activity of pathogens which the performance ability dependent on the concentration used to. Our finding, first treatment GO 1% (1µL/100µL) for 72 h storage (3 days) inhibiting activity by more than 90% for S. aureus and 65% for Salmonella. Although the GO (1µL/100µL) be able to inhibit S. aureus for 288 h storage (12 days) but did not with Salmonella which is rotted in early 150 h storage for (6 days 4 hours). In contrast, increasing the GO concentrations to 1.5% (1.5 µL/100µL), and 2% (2µL/100µL) reveal significant performance could inhibit up to 288 h (12 days) these two bacteria with a clear zone for S. aureus (4.3 and 4.7 mm) and Salmonella (0.7 and 1.1 mm).

Interestingly, even though sing et al (2008) study reported that GO is less effective against S. aureus in disc diffusion method. Furthermore, Mesomo et al (2013) result demonstrated that GO has slight inhibition activity against salmonella. However, from our data indicated GO from Emprit variant has high-performance and effect is comparable to chloramphenicol against these bacteria. This suggests that different variant ginger have different strength and characteristics of phenolic compounds which might responsible for inhibition performance.

3.3. Beef meatballs quality testing

3.3.1. Total plate count (TPC)

Total plate count (TPC) indicating the level of microorganism in it during storage study from 0 to 12 days. The control sample has increased significantly ($p<0.05$) 6.12 Log CFU/g at 6 days, because it was completely putrefied was not recorded up to end of a storage.

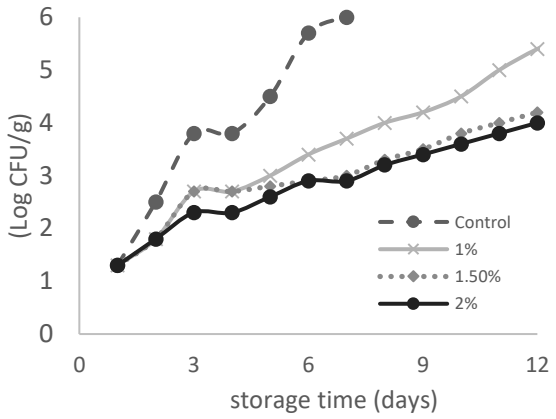

Figure 2. Effect of the edible coatings containing ginger oleoresin on the microbial growth (Log CFU/g) of total plate count (TPC) values in meatballs during storage at $4^0C\pm 1^0C$. Data shown are the means ± standard deviation ($p < 0.05$).

Addition of 1% (1µL/100 µL) coated oleoresin into meatballs significantly decreased value the TPC ±1.2 log CFU/g up to 9 days. Higher addition of oleoresin into meatballs 1.5% (1.5µL/100 µL) to 2% (2µL/100 µL) was significantly control the microbial growth of meatballs under 4.4 log CFU/g at the end of storage time (12 days). Within this result, the meatball can be consumed according Indonesia National Standards which is TPC does not exceed log $1x10^5$ CFU/g (SNI.3818:2014). Similar case on GO Noori et al (2018) result demonstrated the concentration treatment have significant effects inhibit the population of moulds and yeast on chicken breast fillets during storage.

More specific Widayat et al (2017) using liquid smoke containing Eugenol of GO could inhibit inactivates intracellular enzymes for forming process that causes the lysis on the cell wall of microbes.

Table 2. Sensory properties of meatballs edible coating containing ginger oleoresin (*n*=20 panelists)

Storage time (Day)	(%)	Colour	Taste	Flavour Intensity	Juiciness	Hardness	Overall
0	1	6.4±0.04	6.0±0.63	6.4±1.24	6.2±2.07	6.4±0.17	6.0±0.77
	1.5	6.0±0.23	5.9±0.08	6.3±0.32	6.0±1.13	6.1±0.63	6.0±0.33
	2	6.3±0.48	5.9±0.15	6.2±0.67	6.0±0.01	6.0±0.01	6.0±0.61
3	1	5.1±0.44	5.9±0.27	6.0±0.44	6.2±1.20	6.0±0.31	6.0±0.11
	1.5	5.7±0.05	5.5±0.85	6.0±0.25	6.0±0.12	6.1±0.12	6.0±0.22
	2	6.0±0.48	5.7±0.16	6.0±0.05	6.0±0.31	6.0±0.01	6.0±0.90
6	1	4.7±0.57	4.0±0.21	3.7±0.30	6.0±0.53	5.9±0.05	5.8±0.05
	1.5	5.5±0.35	5.5±0.17	5.7±0.05	5.9±0.17	6.1±0.61	6.0±0.12
	2	5.3±0.05	5.1±0.25	4.8±0.23	5.9±0.90	6.0±0.45	6.0±0.91
9	1						
	1.5	4.6±0.23	3.7±0.63	3.9±0.50	5.7±0.54	4.4±0.40	5.2±0.84
	2	4.0±0.12	3.4±0.05	3.5±0.46	5.5±0.12	4.5±0.81	5.1±0.13
12	1						
	1.5	3.4±0.52	3.6±0.22	3.6±0.51	4.9±0.13	3.7±0.82	4.3±0.10
	2	3.0±0.02	2.0±0.47	1.3±0.86	4.8±0.22	3.6±0.21	4.0±0.92
Control		6.6±0.01	6.2±0.20	6.3±0.08	6.2±0.25	6.7±0.25	6.3±0.19

3.3.2. pH Analysis

The control samples had higher pH value ranged from 5.8 to 6.34, while oleoresin treatment samples had the pH value ranged from 5.8 to 6.0 which is differed significantly ($p < .05$). In line with these result, Sallam et al. (2004) study also findings storage time factor had a significant ($p < .05$) influence on increasing of pH values. An increase may be attributed to protein degradation of metabolites by bacterial action in a beef meatball (Karabagias et al. 2011) in this situation spoilage often produce highly malodorous volatile substances (Alfreider et al., 2002; Xiao et al. 2013).

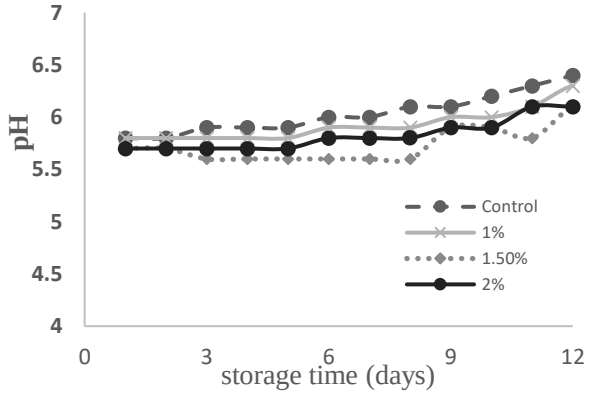

Figure 3. Effect of the edible coating containing ginger oleoresin on pH values in meatballs during storage at $4^0C\pm 1^0C$.

3.3.3. Sensory analysis

Sensory analysis beef meatballs score showed that increased percentage additional oleoresin concentration and refrigerator storage time give effect toward less acceptable in panelist sensory qualities. The sensory preferences on color, taste, flavor intensity juiciness, and hardness levels significantly decreased ($p<0.05$) gradually up to end of the storage (12 days).

Except, oleoresin concentration 1% was not recorded at a day of 9 and 12 because it was completely rotted. In the current study, the colors difference of beef meatballs caused by ginger oleoresin negatively affected the sensory color and flavor intensity evaluation results samples with 2% had higher scores. However, it was evaluated this decrement of preference in color and flavor intensity did not have a negative effect and no significant
differences ($p >.05$) were seen in taste, hardness and overall acceptance of the samples. These

results are in conformity with a study conducted by Turgut et al., (2017) that the addition of pomegranate peel extract in beef meatballs during frozen storage was not a significant difference in terms of taste. Moreover, the taste of the beef with added kaffir lime leaves oleoresin did not induce a significant taste and juiciness of kaffir lime (Utami et al., 2014)

4. Conclusions

These results demonstrated and verified that oleoresins of ginger *emprit* had a potential antibacterial into beef meatball could help to improve shelf life without ignoring consumer preference. The negative impact come from color property which is not affected in taste, flavor intensity and overall acceptance scores. From the inhibit the growth of bacteria oleoresins as natural preservatives have a simple application and low-cost.

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