

Antioxidant Activity of Indonesian Endogenous Duck Meat Marinated in Ginger (*Zingiber officinale* Roscoe) Extract

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Abstract: This study aimed to determine the effect of marinating in ginger extract on the stability of culled duck meat. The content of fat in culled duck meat is higher than that in younger laying ducks, so it is suspected to be more sensitive to oxidation. Culled ducks are female laying ducks that are not productive anymore (2.5 years old). Rhizome of ginger (*Zingiber officinale* Roscoe) is known to have potency as an antioxidant, antimicrobial and proteolytic compound. Culled ducks from Kedu Region, the magelang local ducks, obtained from duck breeding center in Ambarawa, Central Java, Indonesia. The samples were then marinated in ginger extract with the concentration of 0% (control), 5%, and 10% (w/v) followed by aging for 24, 48, and 72 hours at 10°C. The analyzed variables were fat content, thiobarbituric acid (TBA), antioxidant activity with 2,2 diphenyl-1-picrylhydrazyl (DPPH) analysis, and trimethylamine (TMA). This research indicated that all culled duck meats with marinating treatments were lower in TBA value at the end of aging than that of at control. Marinating treatment with ginger extract was also able to inhibit the increase in TBA value, which have implications for the inhibition of the oxidation process. This inhibition was caused by the effect of polyphenolic compounds (6-gingerol and derivatives) contained in the ginger extract. The ginger extract at higher concentrations able to suppress the formation of TMA. It was suspected that the reduction of TMAO can be suppressed due to the ability of components of ginger extract as antimicroorganism so trimethylamine oxide reduction can be suppressed.

Key words: Duck meat, antioxidant activity, ginger extract

INTRODUCTION

One of the Indonesian commodities that have the potency to contribute the consumption of animal protein is the duck meat. Some of last year's duck meat consumption of Indonesian people have been to increase. Duck meat production in Indonesia since 2009, 2010, 2011, 2012 continued to increase, is 25.782; 25.999; 28.183; and 30.053 tonnes, respectively (Directorate General of Livestock and Animal Veterinary, 2013).

Characteristic of duck meat is interesting to be studied because there is a characteristic combination of red meat and poultry meat. Red meat contains phospholipids as aroma precursors and poultry meat contain a lot of polyunsaturated fatty acids, reaching 60% of total fatty acids. Chemical composition of duck meat varies, influenced by genetic and environmental factors. According to Ali *et al.* (2007) the composition of duck meat are 76.41% moisture, 20.06% protein, 1.84% fat and 0.92% ash. The color of duck meat is darker than chicken because of the higher content of myoglobin. The older ducks contained high myoglobin, so that the color of such meat was darker. Culled ducks are female laying ducks that are not productive anymore (2.5 years old).

Several previous studies have revealed about the properties and changes of duck meat due to some treatment, but it was limited in Peking and Muscovy ducks (Baeza, 2002; Chartrin *et al.*, 2006; Omojola, 2007; Tsai *et al.*, 2011). The aroma of duck meat is relatively stronger than other poultry meats. In a study related to sensory characteristics of duck meat, it found that the flavor of food is positively correlated with lipid content (Chartrin *et al.*, 2006). There are no significant differences in the saturated fatty acid between the breasts and thighs of similar duck species (Aronal, *et al.*, 2012). The high content of saturated fatty acid in Muscovy duck breast meat is similar to the saturated fatty acid content in wild duck. They found that the content of saturated fatty acid in breast meat is 35,1% and higher than that of in wild duck thigh meat (31,1%). Ahn *et al.* (1995) found that the saturated fatty acid content (% total lipid) in broiler chicken breast (28,67%) was slightly higher than that of in the thigh meat (26,38%).

Fatty acid compositional analysis found that oleic acid (C18:1) to be the highest-concentration fatty acid in both of Peking and Muscovy meat. Monounsaturated fatty acid was the highest concentration fatty acid in Muscovy duck, whereas polyunsaturated fatty acid was the highest-concentration fatty acid in Peking duck (Aronal *et al.*, 2012).

Meat of culled duck was more pliant than Peking and Muscovy duck so utilization is also very limited. The content of fat in culled duck meat is higher than that in younger laying duck, so it is suspected to be more sensitive to oxidation.

Rhizome of ginger (*Zingiber officinale* Roscoe) is known to have a potency as an antioxidant, antimicrobial and proteolytic. Components in ginger rhizome can be classified into two, namely volatile components and non volatile components. The majority of the volatile components is ginger essential oil, which is usually give heat and aroma effects of ginger. Non-volatile fraction of volatile components present in the oleoresin. The potential components in ginger as an antioxidant are phenolic compounds, especially 6-gingerol (Jolad *et al.*, 2004). Ressearch on the composition and potential antioxidant on 6 new varieties of ginger has done by Eleazu (2012). Ginger varieties are also found to have strong antioxidant activity, indicated by the activity of scavenging the radical 2,2 diphenyl-1-picrilhydrazyl (DPPH). These study reported that the total phenol contained in ginger was negatively correlated with the total oleoresin. It shows that ginger oleoresin is not derived from phenolic constituents and that oleoresin has only a small contribution to the antioxidant activity of ginger rhizome. In recent years, researchers interested in studying for the ability of a phenolic antioxidant that it can protect the human body from free radicals.

Antioxidant test could be based on the evaluation of lipid peroxidation or on the measurement of free radical scavenging potency. The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers (Roginski and Lissi, 2005). This study aimed to determine the effect of marinating in ginger extract on the stability of culled duck meat.

MATERIALS AND METHODS

Culled ducks (2.5 years old) of magelang local ducks obtained from duck breeding center in Ambarawa, Central Java, Indonesia. The culled ducks are slaughtered and then was taken the meat from the breast and thigh to be marinated.

Preparation of ginger extract: To obtain ginger extract, fresh ginger rhizome (*Zingiber officinale* Roscoe) is washed, peeled and sliced. Furthermore the ginger was blended with cold water $\pm 5^{\circ}\text{C}$ (ginger: distilled water = 2:1 w/w) for 1-2 min, then filtered with a filter cloth. The filtrate obtained was centrifuged for 10 min at 3000 rpm and $\pm 10^{\circ}\text{C}$, then the supernatant was collected and stored as ginger extract. To obtain an ginger extract with a concentration of 5%, 5 g extracted ginger solution was dissolved in 100 ml of distilled water. Similarly, to obtain a concentration of 10%, 10 g dissolved in 100 mL of distilled water.

Preparation of the marinating and aging samples: The way of sample preparation is to immerse the samples of culled ducks meat (approximately 100 g) in 100 mL of ginger extract (5%, 10% and control) for 5 min and aging at 5-10°C for 24, 48 and 72 h. Sample preparation was done in each separate ginger extract concentration.

A randomized factorial design was used in this ressearch. The treatment concentration of ginger extracts were 5, 10% (w/v) and control and aging time (24, 48 and 72 h). Each treatment was replicated 3 times. Variables were fat content, thiobarbituric acid (TBA) (AOAC, 1990), antioxidant activity with 2,2 diphenyl-1-picrilhydrazyl (DPPH) analysis (Thaipong *et al.*, 2006) and trimethylamine (TMA).

Analysis of fat: Measurement of fat content by Soxhlet method (AOAC, 1990). Weighed 2 g of duck meat that has been smoothed. Mixed with sand that had as many as 8 g was burn and put in a tube in a Soxhlet extraction thimble. Extraction tube distillation apparatus mounted on a Soxhlet with petroleum ether solvent enough for 4 h. After stirring the residue in the tube extraction, the extraction was continued again for 2 h with the same solvent. Petroleum ether extract containing fat that has been transferred into a clean bottle and weigh the known weight, then evaporated in a water bath until slightly thick. Continued drying in an oven at 100°C until its weight is constant. Heavy residue in the bottles weigh expressed as weight fat duck meat.

Analysis of TBA value: TBA value analysis procedure as follows (AOAC, 1990). Weighed 3 grams of finely ground meat samples and known water content quantitatively incorporated into the distillation flask was washed with 1000 mL while 48.5 mL of distilled water and added 1.5 ml of 4N HCl. Distillation is done on a hot plate at the highest temperature scale so that as much as 50 mL of distillate obtained during heating 10 min. Distillate obtained was stirred, filtered and transferred as much as 5 to 50 mL erlenmeyer closed then added 5 mL of TBA reagent. TBA reagent was prepared by dissolving 0.02 M thiobarbituric acid in 90% glacial acetic acid. Dissolution accelerated by heating above the *water bath* with stirring. Furthermore, the reference solution was made by the same procedure as above but without the meat samples. After the solution is cooled, the absorbance is read by spektrofotometer at a wavelength of 528 nm and the reference solution as the zero point. Absorbance is used as a comparison scale meat fat oxidation rate.

Analysis of DPPH radical scavenging activity: The DPPH assay was done according to the method of Thaipong (2006). The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1,1 units at 515 nm using the

spectrophotometer. Duck meat sample (0,7 g) were allowed to react with 2850 µL of the DPPH solution for 24 h in the dark. The absorbance was taken at 515 nm.

Analysis of TMA: Trimethylamine in duckmeat was determined by the conway micro diffusion method (AOAC, 1990). Samples that had been cut in a small was weighed as much as 25 g, then added 75 mL of 7.5% TCA solution and mixed for 2 min. The mixture was centrifuged so that the filtrate is clear. As much as 1 mL of solution of boric acid 1% was put in inner chamber of conway cup. With another pipette, 1 mL of the filtrate put into the outer chamber. One milliliter of a solution of potassium carbonate and 0.5 mL of formalin solution was put on the opposite side of the outer chamber. After the cup is closed, the two solutions in the outer chamber were mixed for 1 minute carefully. These procedure is also done at 5% TCA solution as a blanko. All these prepared conway cup were incubated for 2 h at 35°C. After the incubation finished, the boric acid in the inner chamber of blanko cup was titrated with HCL 1/7 N until its color becomes pink. Furthermore, the solution of boric acid in the sample conway cups were titrated until a pink color similar to the color on blank conway cup:

$$\text{Levels of TMA} = (\text{mL sample titration} - \text{mL blank titration}) \times 0.2 \times 100 / 1 \times 100 / 25 \text{ mg N}$$

RESULTS AND DISCUSSION

Fat content: The fat of duck meat was sensitive to oxidation, so duck meat tends rapidly to become rancid. An oxidative reaction in duck meat leads to degrade of fat and protein, resulting in deterioration of flavour, texture and nutritive value and it was considered as one of the major problems in the processing and consumption of duck meat products. The fat of the duck meat in the kind of abdominal fat, is not the marbling fat and it is susceptible for oxidizing. The stability of duck meat against oxidation is different between chicken and turkey. Duck meat have much more unsaturated fatty acids (about 60% of total fatty acids) compared to the chicken meat but the duck meat have fewer antioxidants,

so its fat are more easily oxidized (Russel *et al.*, 2003). Duck meat contains a lot of haeminic pigment (hemoglobin and myoglobin) which is rich in iron (Fe) as the catalyst for the course of the oxidation reaction.

Thiobarbituric acid (TBA): Thiobarbituric acid (TBA) value was used to measure the formation of secondary products of oxidation of fat, such as malonaldehyde. TBA value were often used as one index of the oxidation of fat in marketed meat products. The rancid flavor is early detected in meat products at TBA value between 0.5 and 2.0 mg malonaldehyd/kg (Gray and Pearson, 1987). This ressearch indicated that all marinating treatments produced lower TBA value at the end of aging than its in the control. Generally, TBA value increased during aging time. The phospholipids in muscle membrane provide an ideal substrate for lipid peroxidation. Marinating duck meat with various levels of ginger extracts caused a significant reduction of TBA value compared to the control sample. The control sample had highest TBA value (2, 234 mg malonaldehyde/kg sample) at the end of the aging time. The highest TBA value of the control sample at the end of aging might be due to an interaction between the natural substances (for example, polyunsaturated fatty acids) and catalysts (for example, iron ion) from the duck meat tissue during aging. Duck meat samples marinated with 10% of ginger extract had lowest TBA value (0, 419 mg malonaldehyde/kg) at the end of aging time. TBA values in treatment samples were much lower than the control sample. The inhibitory effect of ginger extract on lipid oxidation might be due to scavenging of free radicals and chelating of transition metals. The results indicated that the high antioxidative effect of polyphenolic compounds in ginger extract was reducing the formation of malonaldehyde during aging (Fig. 1). The components in ginger has potency as an antioxidant that can inhibit the production of TBA as a result of increased fat oxidation reaction. Marinating treatment with ginger extract was also able to inhibit the increase in TBA value, which have implications for the inhibition of the oxidation process. The inhibition caused

Table 1: Fat, thiobarbituric acid (TBA), antioxidant activity and trimethylamine (TMA) value of culled duck meat

Ginger extract (%)	Aging time (h)	Fat (%)	TBA (mg malonaldehyd/kg)	Antioxidant activity (% discoloration DPPH)	TMA (mg N/100 g)
	0	1.598	0.688	9.652	1.200
0	24	1.600	0.518	9.932	3.650
	48	1.904	1.481	9.798	5.600
	72	1.922	2.234	4.578	7.200
	0	1.240	2.162	11.724	1.400
5	24	1.265	1.417	8.814	2.800
	48	1.606	1.451	9.311	5.200
	72	1.596	1.020	11.701	7.080
	0	1.672	1.868	13.796	1.500
10	24	1.935	0.485	8.302	2.280
	48	1.904	0.419	10.227	4.720
	72	1.600	1.329	13.113	6.800

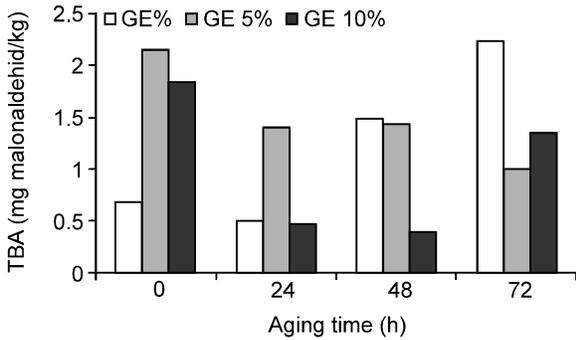


Fig. 1: Graphs of Thiobarbituric acid (TBA) of culled duck meat

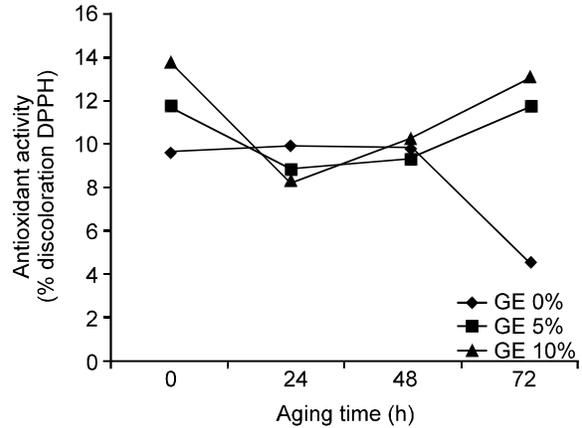


Fig. 2: Graphs of antioxidant activity of culled duck meat

by the effect of polyphenolic compounds (6-gingerol and derivatives) are contained in the extract of ginger.

Antioxidant activity: This high antioxidant capacity may be caused to the high concentration of phenolics in ginger extracts. They can participate in protection against the harmful action of reactive oxygen species, mainly free radical oxygen. These compounds are known also to possess antioxidant activity, due to its ability to reduce free radical stability via electron or hydrogen donating mechanism (Lee *et al.*, 1999; Ruberto and Baratta, 2000). The antioxidant activity of polyphenols is principally based on redox properties of their hydroxyl groups and the structural relationships among different parts of their chemical structure (Rice-Evans *et al.*, 1996). Figure 2 showed the antioxidant activity of culled duck meat during aging. Antioxidant activity in control samples decreased during aging up to 72 h, whereas those of marinated samples with ginger extract 5% and 10% decreased during aging up to 24 h and then increased from 24 h until 72 h. Increased antioxidant activity was influenced by the ability of components in ginger extract as an antioxidant. The results showed that the phenol component in ginger extract was suspected a taking time to be active as an antioxidant.

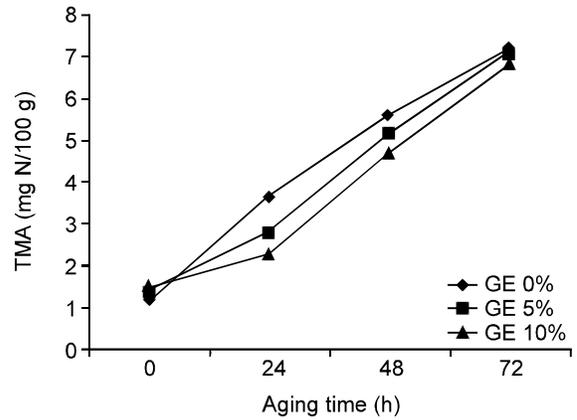


Fig. 3: Graphs of Trimethylamine (TMA) value of culled duck meat

Trimethylamine (TMA): Formation of trimethylamine (TMA) is often associated with spoilage of fish. TMA is the result of the reduction reaction of trimethylamine oxide (TMAO) by microorganisms and enzymes. The reaction depends on the pH of the material. Although TMA measurement is rarely performed in the determination of the freshness of meat, but the results shows that the longer aging time expressed the higher TMA. Figure 3 showed the increasing rate of trimethylamine formation in culled duck meat samples during aging. TMA production continued to increase during the aging. It happened in the control sample (0% GE) and marinated samples with ginger extract 5 and 10% (w/v). There is a similar trend of increasing rates between TMA in the control sample (without marinating)

and treatment samples. It was pointed out that TMA of the duck meat were formed during storage, although not as high as TMA in fish. The TMA line (Fig. 3) of the marinating samples with ginger extract lower than the control sample and marinating samples with ginger extract at a concentration of 10% resulted in a lower rate than the marinating samples with ginger extract concentration of 5% and control. Control samples were aging until 72 h produced the highest TMA, ie 7.20 mg N/100 g, while TMA in marinated samples with GE 5% and 10% were 7.08 mg N/100 g and 6.80 mg N/100 g respectively. It shows that ginger extract at higher concentrations to be able to suppress the formation of TMA. These phenomenon were caused by the components of ginger extract as antimicroorganism, affecting the suppression of trimethylamine oxide reduction. The results showed that the TMA numbers continued to be increased during aging. It has been known that the TMA was the result of the TMAO reduction. It was occurs the release of OH⁻ groups so that the pH was to be increase. This phenomenon was

also occurred in the duck meat, that its pH tends to increase during aging.

Some proteins contain amino acids which are very susceptible to oxidation. These amino acids are cysteine, histidine, methionine, lysine and tryptophan (Xiong *et al.*, 2000). The duck meat contains lysine and tryptophan (Woloszyn *et al.*, 2006), so that the oxidation of the amino acids is also occur in the duck meat. Oxidation reactions involving the side chains of amino acids can lead to the formation of carbonyl groups. The conversion may eventually lead to loss of catalytic activity (enzyme) and increase protein degradation, protein aggregation and loss of solubility. Formation of carbonyls in meat can be caused by several oxidative treatment and has been shown to occur in beef myofibrils during postmortem aging (Rowe *et al.*, 2004).

Conclusion: Culled duck meat has endogenous antioxidants but its could not inhibit lipid oxidation and malonaldehyd formation, although it was kept at low temperature (aging). Marinating with ginger extract could increase the antioxidant activity of culled duck meat. Trimethylamine formation in marinated culled duck meat can be pressed during aging due to antioxidant activity and antimicroorganism in ginger extract.

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