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Optimation of Red Mould Rice Addition as Curing Material on Fatty Acid Profiles, Cholesterol and Thiobarbituric Acid (TBA) of Spen Hen Meat Sausages

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ABSTRACT

Background: Sausage is one of the diversified products of the unproductive layer's meat utiliation. Addition of red mould rice as curing material is expected to be able to influence the fatty acid profiles, cholesterol, thiobarbituric acid of culled laying hens sausage. This study aims to analyse the saturated fatty acid profile, the unsaturated fatty acid profile and cholesterol content of the laying hens meat sausage with an addition red mould rice as curing material.

Methods: The saturated and unsaturated fatty acid data were descriptively analysed and tabulated. The cholesterol and TBA data of the sausage were analysed using ANOVA and continued with the Tukey test. The experiment used a complete randomized design with 4 treatments (R1 = 0%, R2 = 0.5 %, R3 = 1%, R4 = 1.5%) and 4 replications.

Result: The results showed that the highest saturated fatty acid value was methyl palmitate 28.42% at the level of 1.5% while the smallest saturated fatty acid value <0.1% was Methyl hexanoate, methyl docosanoate and methyl tricosanoate at the level of 0% - 1.5%. While the highest unsaturated fatty acid value was methyl linolelaidate 36.05% at the level of 1.5% and the smallest unsaturated fatty acid value, 0.1% was myristoleic acid methyl ester, methyl cis-11,14-eicosadienoate and methyl cis-11,14,17-eicosatrienoate at the level of 1.5%. Addition of 1% red mould rice is considered to be optimal to increase the nutrition quality and the oxidative stability of the sausage. This finding supports the use of red mould rice as an effective natural preservative for healthy processed meat products.

Key words: Cholesterol, Red mould rice, Saturated fatty acids, Sausage, Unsaturated fatty acids.

INTRODUCTION

Layers are one of the poultry most commonly reared to give eggs and potential to yield meat after the productive period is over (Fenita *et al.*, 2009). According to Chueachuaychoo *et al.* (2011) and Okarini *et al.* (2019), the unproductive layer also contains has 35.6% saturated fatty acids (SFA), 44.57% monounsaturated fatty acids (MUFA) and 19.79% polyunsaturated fatty acids (PUFA).

Efforts to improve the quality of meat from rejected laying hens can be done through food diversification into processed chicken sausage products. Sausage is a processed meat product that is popular among the public. It is generally made from minced meat, flour and other additives, then inserted into a casing (Herlina *et al.*, 2015; Ashari *et al.*, 2023). The unproductive layer's meat is one of the raw materials used to make sausage. It has harder texture and higher fat content than the broiler chicken (Purnamasari *et al.*, 2012). However, the problems in making sausages from rejected laying hen meat is the high content of saturated fatty acids and cholesterol. High levels of saturated fatty acids and cholesterol can increase the risk of cardiovascular disease. Therefore, efforts need to be made to reduce the content of saturated fatty acids and cholesterol in the meat sausages of cull laying hens.

In general, fatty acids can be divided into saturated acids and unsaturated acids. The former seems to increase cholesterol in the blood, whereas the later can give positive effect on heart health (Sartika, 2008). In food industry context, unproductive layer's meat sausage becomes an interesting alternative due to utilizing a neglected protein source.

Red mould rice, a fermented rice product of fungus *Monascus purpureus*, is very beneficial for health and and provides high nutritional value. The secondary metabolites contained in this red mould rice are sterols (b-sitosterol, campesterol, stigmasterol and sapogenin), isoflavone, glycocide isoflavone, zinc, selenium and monounsaturated

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fatty acid (Ma *et al.*, 2000). It is also a natural substance usable to improve the fatty acid profile, reduce the cholesterol content in the processed meat product and as a natural dye (Ma *et al.*, 2000; Zhang *et al.*, 2016; Mhalaskar *et al.*, 2017).

The unproductive layer's meat becomes an interesting alternative due to taking benefit of unused protein source (Palandeng *et al.*, 2016). Nevertheless, some people believe that consuming meat can increase the cardiovascular disease risk. This belief is related with the fact that sam the saturated fatty acid in the meat can increase low-density lipoprotein (LDL) level (Hoenselaar, 2012).

Previous research has been conducted by Rumondor *et al.* (2016), that the addition of red mould rice affects the physical, chemical and organoleptic properties of discarded duck meat sausages at a level of 1%. Then Rumondor *et al.* (2024) examined the effect of the physicochemical and antioxidant properties of discarded dry chicken meat sausages added with red mould rice as a curing agent, which also had a significant effect up to a level of 2%.

This study is aimed to optimize the red mould rice concentration as curing material in chicken meat sausage processing. This finding is expected to be able to increase the quality of the chicken sausage as good nutritive and secure food material, containing lower cholesterol, lower TBA and balanced fatty acid profiles.

MATERIALS AND METHODS

The study was carried out in the laboratory of livestock product technology, faculty of animal husbandry, sam ratulangi university, Manado, using the unproductive layers. The examination on the saturated and unsaturated fatty acids and cholesterol content was done in the Laboratory of Integrated Research and Testing of Gadjah Mada University, Yogyakarta. The saturated and unsaturated fatty acid data were descriptively analysed and tabulated. The cholesterol and TBA data of the sausage were analysed using ANOVA. The experiment used a completely randomized design with 4 treatments (R1 = 0%, R2 = 0.5%, R3 = 1%, R4 = 1.5%) and 4 replications, thus obtaining 16 treatment combinations. Because there was a significantly different effect, the analysis was continued with the Honestly Significant Difference test at the 5% level (Steel and Torrie, 2001).

The raw material for sausage processing was the 20-24 months old-unproductive layer obtained from a chicken saler in Kleak, Manado. Red mould rice was obtained from the supermarket. Chicken meat is cleaned and separated from bones and skin and cut into 2 × 2 cm and prepared into 4 treatments. The meat was then cured by adding 1,5% salt, 1.5% sugar, 0%. 0.5%, 1% and 1.5% red mould rice of the meat weight. It was administered in powder form and kept at ± 5° C for 24 h. The meat was then ground and added with filler and binding materials as follows: 1.5% garlic, 0.5% pepper, 0.75% ginger, 0.5% nutmeg powder, 10% oil, 5.7% tapioca flour, 3.5% skimmed milk, 17% ice cube and 0.3% STPP (Sodium TriPoly Phosphate*).* The dough was ground and put into a 10 cm long and 1.65 cm diameter tube, then steamed at 85°C for 30 min. After cooled, the sausage was wrapped in a standing pouch seal-typed plastic and analysed (Fig 1).

Analysis procedure

Saturated and unsaturated fatty acid measurements

The unsaturated fatty acids were measured using gas chromatography (GC) (Jacoeb *et al.,* 2020). The measurements

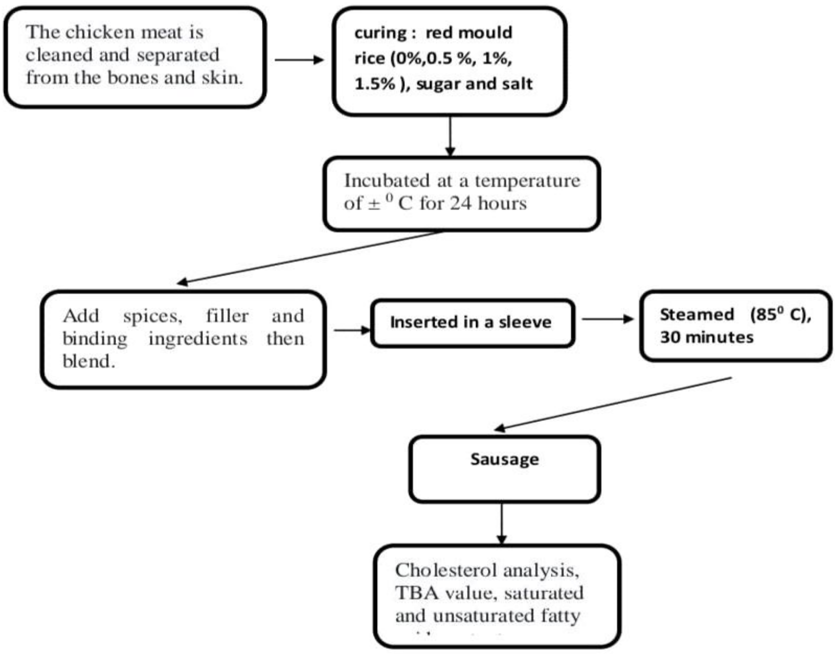

Fig 1: Making sausage.

of saturated and unsaturated fatty acids were conducted through several phases: In hydrolysis phase, as much as 5 g sample was put into big flask, added with 10 ml of strong HCl, heated at 80°C until boiling for 3 h. It was then cooled, extracted with 25 ml of diethyl ether and petroleum ether at 1:1 ratio, vortexed and left until it settled. The upper layer in oil form was collected and evaporated in the waterbath using N_2.

Methylation

As much as 0.5 mL of oil was inserted into a small flask, added with 1.5 mL of methanolic sodium, covered, heated at 60°C for 5-10 min., shaken, cooled and added with 2 mL of Boron trifluoride metanoic. It was then heated at 60°C for 5-10 min. while stirred and cooled, then extrscted with 1 mL of heptane and 1 mL of saturated NaCl. The upper layer was collected and inserted into a GC vial, then injected as much as 1 µL of sample into GC.

Cholesterol content

Cholesterol content was measured by homogenizing the sample, prepared as much as ±5 g and added with 10 mL of 0.25N KOH. The sample was then heated in a waterbath at 80°C for 3 h while stirred once a while and cooled to warm. As much as 20 mL of ethanol was added, extracted in 10 mL of mixed diethyl ether and petroleum benzene (1:1) solution and deposited overnight. The upper layer was collected and thickened in a waterbath at 40°C. The oil product was then dissolved in 10 mL of toluent and vortexed to homogenous. If the solution is turbid, it was centrifuged. One mL of sample was then taken and inserted into a GC vial. Afterwards, the standard solution of cholesterol was made and inserted 1 µL of sample solution and standard solution into the GC vial.

$$\text{Cholestrol content (mg kg}^{-1}\text{)} = \frac{\text{GC readingt}}{\text{Sample weight}} \times \text{end volume}$$

TBA (Thiobarbituric acid) measurements

TBA value of the chicken sausage was measured following modified method of Salih *et al.* (1987) and Kusrahayu *et al.* (2009). As much as 5.0 g of sample was mixed with 15.0 mL of 20 g mL^{-1} trichloroacetic acid dan 20 g/mL Butylated hydroxytoluene, homogenized at 10,000 rpm for 3 min. In an ice bath, deproteinated for 2 h and centrifuged at 2,000 × g for 10 min. at 4°C. The supernatant was filtered through a multiple slow paper filter and diluted with bidistilled water to 50 mL. As much as 5.0 mL filtrate aliquot was mixed with 5.0 mL of 0.02 mol L^{-1} 2-thiobarbituric acid solution, left to react at 90°C for 30 min., cooled to room temperature and the absorbance was read on a UV-Vis spectrofotometer at 532 nm wavelength. The TBA value is expressed in mg malonaldehyde per kg of sample, The TBA content was calculated as:

$$\text{Thiobarturic Acid} = \frac{3 \times \text{ absorbance} \times 7.8}{\text{Sample weight (g)}}$$

RESULTS AND DISCUSSION

Saturated fatty acid profile

The present study found that addition of red mould rice could reduce the saturated fatty acid of the chicken sausage. The higher the red mould rice is added, the lower the saturated fatty acid detected (Table 1).

The highest saturated fatty acid was recorded in methyl palmitate ($C_{17}H_{34}O_2$), whereas the lowest (<0.1) was found in methyl hexanoate ($C_7H_{14}O_2$), Methyl undecanoate ($C_{12}H_{24}O_2$), Methyl tridecanoate ($C_{14}H_{28}O_2$), Methyl docosanoate ($C_{23}H_{46}O_2$) and Methyl tricosanoate ($C_{24}H_{48}O_2$) in all red mould rice concentrations. Methyl palmitate is an esther methyl of palmitic acid belonging to saturated fatty acid. Palmitic acid is a saturated fatty acid with 16 carbon

Table 1: Saturated fatty acids composition of unproductive layer's meat sausage added with red mould rice.

Saturated fatty acids	Red mould rice (%)			
	0	0.5	1	1.5
Methyl butirate ($C_5H_{10}O_2$)	0.42	0.39	0.35	0.32
Methyl hexanoate ($C_7H_{14}O_2$)	<0.1	<0.1	<0.1	<0.1
Methyl octanoate ($C_9H_{18}O_2$)	<0.1	0.15	0.13	0.10
Methyl decanoate ($C_{11}H_{22}O_2$)	0.17	0.13	0.13	0.12
Methyl undecanoate [$CH_3(CH_2)_9CO_2CH_3$]	<0.1	<0.1	<0.1	<0.1
Methyl laurate ($C_{13}H_{26}O_2$)	0.45	0.43	0.41	0.39
Methyl tridecanoate ($C_{14}H_{28}O_2$)	<0.1	<0.1	<0.1	<0.1
Methyl myristate ($C_{15}H_{30}O_2$)	3.34	3.26	3.23	3.19
Methyl pentadecanoate ($C_{16}H_{32}O_2$)	0.12	0.11	0.11	0.09
Methyl palmitate ($C_{17}H_{34}O_2$)	29.06	28.92	28.64	28.42
Methyl heptadecanoate ($C_{18}H_{36}O_2$)	0.13	0,.13	0.14	0.12
Methyl stearate ($C_{19}H_{38}O_2$)	0.19	0.16	0.19	0.15
Methyl arachidate [$CH_3(CH_2)_{18}COOCH_3$]	14.30	14.27	14.24	14.22
Methyl heneicosanoate [$CH_3(CH_2)_{19}COOCH_3$]	0.18	0.17	0.14	0.13
Methyl docosanoate ($C_{23}H_{46}O_2$)	<0.1	<0.1	<0.1	<0.1
Methyl tricosanoate ($C_{24}H_{48}O_2$)	<0.1	<0.1	<0.1	<0.1

atoms found in animals or plants (Moghadasian and Shahidi, 2017). Okarini *et al.* (2019) found that the unproductive layers contain 26.5% palmitic acids, whereas the present study found a higher range (Table 1). The change in methyl palmitate could result from the addition of red mould rice indicating lower content with the red mould rice concentration. The addition of red mould rice containing active compounds, such as monacholine K, can influence the chemical structure and feature of methyl palmitate. As the red mould rice concentration increases, methyl palmitate content declines. It could result from the interaction between the active compound in the red mould rice and the methyl palmitate structure. The addition of red mould rice can trigger the reaction to change the esther form or increase the breakdown of fatty acid eventually reducing methyl palmitate concentration. The higher the red mould rice concentration is added the lower the concentration of methyl palmitate is. It indicates that red mould rice can function as a converting agent affecting the stability and the presence of the fatty acid. According to Fassah *et al.* (2012), the addition of natural antioxidant compounds can reduce total SFA (Saturated Fatty Acids) and increase PUFA (Polyunsaturated Fatty Acids) content of the meat and increase the stability of meat oxidation, then increase the meat quality. Hoenselaar (2012) stated that the saturated fatty acids in the meat can increase cholesterol content of low-density lipoprotein (LDL) that can cause cardiovascular disease risk. Furthermore, Reddy *et al.* (2022), stated that the PUFA influences in the level of nutrient metabolization in the body and as the proportion increases a smaller deposition of lipids.

Unsaturated fatty acid profiles

The present study found that the addition of red mould rice increased the unsaturated fatty acid of the chicken sausage. The higher the red mould rice addition, the higher saturated fatty acid is identified (Table 2). The highest unsaturated fatty acid was recorded in methyl linolelaidate ($C_{19}H_{34}O_2$), 35.48 - 36.05%, whereas the lowest (<0.1) was found in myristoleic acid methyl esther ($C_{15}H_{28}O_2$), methyl cis-11eicosenoate ($C_{21}H_{40}O_2$), methyl cis-11,14-eicosadienoate ($C_{21}H_{38}0_0$) and methyl cis-11,14,17-eicosatrienoate ($C_{21}H_{36}O_2$) at all red mould rice concentrations. The addition of red mould rice in unproductive layers sausage processing can increase the unsaturated fatty acid content. It could result that red mould rice contains bioactive component, such as monacholine, influencing metabolism and fatty acid synthesis (Lee and Pan, 2011). Methyl linolelaidate is an esther of linoleic acid, a polyunsaturated fatty acid (PUFA). This compound has a molecular formula of $C_{19}H_{34}O_2$ and is known to be useful for health. Methyl linolelaidate is obtained from the oil added in the sausage processing. It functions to reduce the cholesterol LDL level and increase HDL level. It is also beneficial to reduce the cardiovascular risks (Khandelwal *et al.*, 2013; Abeywardena *et al.*, 2001). According to Vangaveti *et al.* (2016), polyunsaturated fatty acid (PUFA) increases in the cell with oxidative stress and yields various bioactive compounds, in which linoleic acid is a very important component.

Cholesterol

The cholesterol analysis in the chicken sausage added with different concentrations of red mould rice gave a

Table 2: Unsaturated fatty acids composition of unproductive layer's meat sausage added with red mould rice.

Unsaturated fatty acids	Red mould rice (%)			
	0	0.5	1	1.5
Myristoleic acid methyl ester	<0.1	<0.1	<0.1	<0.1
Methyl cis-10-pentadecenoate	1.35	1.27	1.27	1.30
Methyl palmitoleate	0.82	0.92	0.96	0.99
Methyl cis-10-heptadecenoate	0.31	0.32	0.34	0.37
Methyl trans-9-elaidate	6.12	6.15	6.18	6.25
Methyl cis-9-oleate	0.25	0.27	0.22	0.23
Methyl linolelaidate	35.48	35.52	36.01	36.05
Methyl linoleate	0.14	0.14	0.14	0.14
gamma-Linolenic acid methyl esther	0.27	0.27	0.27	0.27
Methyl cis-11-eicosenoate	<0.1	<0.1	<0.1	<0.1
Methyl linolenate	-	0.35	0.37	0.37
Methyl cis-11,14-eicosadienoate	-	<0.1	<0.1	<0.1
Methyl cis-8,11,14-eicosatrienoate	-	0.2	0.2	0.2
Methyl cis-11,14,17-eicosatrienoate	-	<0.1	<0.1	<0.1
Methyl erucate	-	0.13	0.13	0.13
Methyl cis-5,8,11,14- eicosatetraenoate	-	3.68	3.75	3.78
Methyl cis-13,16-docosadienoate	-	0.54	0.57	0.57
Methyl cis-5,8,11,14,17- eicosapentaenoate	-	0.51	0.53	0.53
Methyl nervonate	-	0.41	0.46	0.47
Methyl cis-4,7,10,13,16,19- docosahexaenoate	-	0.29	0.32	0.37

significant effect. The highest cholesterol content of the chicken sausage was recorded in the sausage production without red mould rice addition, 188.41 mg kg^{-1}, whereas the addition of red mould rice in the present study effectively diminished the cholesterol level (Table 3). The cholesterol reduction mechanism is related with the interaction between the active compound contained in the red mould rice, such as monacholin K and lipid of the meat. Monacholin K is known as an HMG-CoA reductase inhibitor functioning in cholesterol synthesis (Patakova, 2013). The inhibition of the enzyme makes the red mould rice be able to reduce the cholesterol production in the body. It is in agreement with Nguyen *et al.* (2020) that red rice fermended by mould *Monascus purpureus* producing monacholin K is used as statin to inhibit cholesterol biosynthesis. According to Venero *et al.* (2010), red reduce total cholesterol mould rice can, LDL and triglycerides level and can increase HDL level in the blood. Moreover, Verhoeven *et al.* (2013) found that red mould rice can reduce the cholesterol level as much as 22%.

Thiobarbituric acid (TBA)

TBA is a compound used to determine the rancidity of fat and oil (Laila *et al.*, 2020). In relation with sausage, TBA measurement is crucial to evaluate the meat quality and the additives used. High TBA indicates lipid oxidation that can affect food taste, aroma and safety. The unproductive layers-based sausage has certain characteristics which can affect the oxidative stability. The layers tend to contain different fat levels that can influence the oxidation level (Lichovnikova *et al.*, 2009).

The effect of red mould rice on thiobarbituric acid (TBA) content of the chicken sausage can be seen in Table 4. TBA is a parameter used to measure the fat oxidation level, particularly malonaldehyde, formed as a result of lipid destruction in meat-based food. The higher the TBA level, the higher the oxidation occurs, which impacts the sensory quality (Rumondor *et al.*, 2016). According to Purba *et al.* (2022), the increase in the TBA value of sausages is caused by damage to fat which causes a rancid odor and taste due to oxidation reactions which can take place by auto-oxidation and the formation of compounds resulting from the breakdown of hydroperoxides. Furthermore, Rumondor *et al.* (2024), stated that meat sausages from rejected laying hens are susceptible to rancidity so they need to add antioxidant compounds. Low fat content can affect the TBA value of sausages (Chowdhury *et al.*, 2015).

The present study indicates that red mould rice, which is rich in monascorubrin and monascorubramin, has an antioxidant feature that can suppress lipid oxidation in meat product, including sausage. It contributes to TBA reduction by inhibiting the fat peroxide compound formation which eventually maintains the fat stability and quality of the sausage (Swastike *et al.*, 2021). This finding is supported by Rumondor *et al.* (2019) that red mould rice can reduce fat content in certain concentration due to lovastine compound contained in the red mould rice functioning as HMG-CoA reductase (cholesterol biosynthesis-promoting enzyme) inhibitor. Red mould rice also significantly influences the fat oxidative stability in processed meat products (Rumondor *et al.*, 2016). It is in agreement with Bastari *et al.* (2023) that the use natural antioxidant, such as plant extract or red mould rice pigment, is effective in maintaining the sensoric quality and could extend the durability of the meat product. Therefore, the addition of red mould rice into the chicken sausage could significantly reduce the TBA value, so that the product could be more durable to the oxidative destruction and have good quality during the storage.

Table 3: Mean cholesterol content of the chicken sausage added with red mould rice.

Red mould rice (%)	Cholesterol (mg/kg)
0	$188.41^{a}\pm0.3145$
0.5	$69.24^{b}\pm0.3145$
1	$65.57^{c}\pm0.3145$
1.5	$63.36^{d}\pm0.3145$

Note: Values are presented as mean±SD, n = 5. Values with different superscript within the same row are significantly different (<0.05).

Table 4: Mean TBA content of the chicken sausage.

Red mould rice (%)	TBAI ()
0	0.64 ± 0.02^{a}
0.5	0.45 ± 0.01^{b}
1	0.41 ± 0.01^{b}
1.5	0.39 ± 0.02^{b}

Note: Values with different superscript within the same row are significantly different (<0.05).

CONCLUSION

The addition of red mould rice as curing material in the unproductive layer's meat sausage could optimize the fatty acid profiles, reduce the cholesterol level and inhibit fat oxidation. Addition of 1% red mould rice is considered to be optimal to increase the nutrition quality and the oxidative stability of the sausage. This finding supports the use of red mould rice as an effective natural preservative for healthy processed meat products.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the committee of experimental animal care and handling techniques were approved by the university of animal care committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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