Buletin Peternakan 42 (2): 150-156, May 2018

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Acredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v42i2.30385

The Physicochemical and Microbiological Qualities of Pegagan Duck Meat Preserved with Different Concentrations of Liquid Smoke and Storage Period

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Article history
Submitted: 20 November 2017
Accepted: 19 April 2018

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ABSTRACT

The aim of this research was to investigate the quality of physicochemistry and microbiology of Pegagan duck meat preserved with various concentrations of liquid smoke and storage time. This study used a complete randomized design (CRD) with a 4x2 factorial pattern. The first factor was the concentration of liquid smoke solution consisted of 4 levels, namely 2; 4; 6, and 8% (v/v). The second factor was the storage time which includes 2 levels ie 12 and 24 hours. The replication used was 4 times. The observed variables were physical quality of meat (water holding capacity, cooking loss, and tenderness), chemical quality (moisture content, protein content, and fat content), and microbiological quality (total microbes, pH, acid total, and total phenol). The data were processed by analysis of variance, followed by Duncan's multiple range test (DMRT). The results showed that there was a significant interaction ($P<0.05$) between liquid smoke concentration and storage time to water holding capacity, cooking loss, tenderness, fat content, total phenol, total microbial, total acid, and pH in Pegagan duck meat but did not show a significant interaction with other variables. It could be concluded that the optimum liquid smoke concentration was 4% and the optimal storage time was 12 hours.

Key words: Liquid smoke, Microbiology, Pegagan duck's meat, Physicochemistry, Preservation

Introduction

Duck meat is one of the food that is quite popular by the community and contains a high nutrient. Besides being rich in nutrients, fresh duck meat is also easy to experience quality degradation, especially when stored at room temperature (Oteku *et al.,* 2006). High levels of water in fresh meat can accelerate the development of decomposing microorganisms in meat. On the other hand, meat is also susceptible to damage caused by fat oxidation and protein denaturation due to prolonged storage. The declining nutritional value and the growing number of microbes in meat, ultimately, lead to decrease meat quality (Komariah *et al.,* 2004).

One effective effort that can be done to prevent the degradation of duck meat quality during storage at room temperature is by the process of preservation. This is because preservation can protect meat from damage caused by microbial activity and can extend the storage period. The preservation process can be done by adding preservatives into the meat. One of the potential natural preservatives used is liquid smoke from palm waste. Liquid smoke is a product obtained from high-temperature combustion processes, known as pyrolysis, against wood or materials containing many lignin, hemicellulose, and cellulose compounds and other carbon compounds which are then condensed to form liquids. Liquid smoke from palm wastes contains several chemical compounds such as phenol, carbonyl, and organic acid compounds, such as acetic acid, propanedioic acid and formic acid (Yosi *et al.,* 2016). Phenol compounds known to act as antioxidants that can prevent the oxidation process, while the organic acid compounds act as a coating material that aims to protect the food surface from the activity of microbial decomposition (Abustam and Ali, 2011).

The use of liquid smoke from palm waste has been previously applied to the process of preserving Pegagan duck eggs. The results showed that the use of liquid smoke from palm waste was able to maintain the physical quality of duck eggs, such as egg weight loss, egg white index, haugh unit, albumin viscosity, and egg white (Yosi *et al*., 2016). So far, the liquid smoke used in meat preservation is mostly derived from coconut shells, but from palm waste has not been studied. Associated with the preservation of meat, it was reported that the water content of chicken

meat preserved with liquid smoke with a concentration of 2% was 67.92%, ash content of 2.74%, and total microbial less than 1.0 x 10^1 CFU/g (Werdiningsih *et al.*, 2014). Based on the above, further research is needed on the effect of liquid smoke from palm waste in the process of preserving Pegagan duck meat to the physicochemical and microbiological qualities stored at room temperature.

Materials and Methods

Materials

The ingredients used were duck breast meat (ducks with 2-2.5 months of age obtained from Kota Daro village, liquid smoke from palm waste, aquadest, mixed catalyst ($CuSO_4$: K_2SO_4), NaOH 40%, concentrated H_2SO_4, 0.1 N H_2SO_4, mixed indicator (bromocresol green (BCG) + methyl Red (MR) + 96% alcohol), 0.1 N NaOH, hexane solution, aquadest, phenolphthalein (PP) indicator, 0.1 N NaOH solution, buffer solution 4, media plate count agar (PCA), Nutrient Agar (NA).

The tools used were knives, trays, analytical scales, polyethylene plastic, porcelain cups, oven, desiccators, pincers, soxhlet tools, filter papers, beaker glass, kjedhal distillation, hot plate, destructive gourd, stirrer, micro pipette, erlenmeyer, oven, furnace, Warner Bratzler shear, titration tool, pH meter, aluminum foil, petri dish, test tube, incubator, colony counters, tip pipette, laminar air flow, autoclaf, and cotton.

Methods

Experimental design. This research used a completely randomized design (CRD) with a 4x2 factorial pattern. The first factor was the concentration of liquid smoke solution consisting of 4 levels, which were 2; 4; 6, and 8% (v/v). The second factor was the length of storage with 2 levels, which were 12 and 24 hours. The replication used in these assays were 4 times.

Preparation of liquid smoke solution. Liquid smoke used was first distilled to reduce concentration and eliminate harmful substances such as benzopyrene. Liquid smoke solution was made with concentrations consisting of 4 levels, namely 2, 4, 6, and 8% (v/v), which was dissolved into 1,000 ml of aquadest.The chemical composition of liquid smoke from palm wastes is presented in Table 1.

Preservation of Pegagan duck meat. The preservation process refers to Arizona *et al.* (2011) with modifications. The breast meat of Pegagan duck was cleaned with running water then taken as many as 500 g as the sample for each treatment of liquid smoke concentration, then each meat sample was immersed into a 1,000 ml liquid smoke solution in accordance with the treatment for 30 minutes. The meat was then drained for 1 minute. After drained, the meat was cut into 2 parts as many as 250 g, then packed with polyethylene plastic and each stored for 12 and 24 hours at room temperature. Once stored, the meat was removed from the plastic to be analyzed.

Measured variables. The physical quality of the meat measured includes water holding capacity according to Hamm method (Soeparno, 2005), cooking loss according to Yosi and Sandi (2014), and tenderness according to Farida *et al.* (2006). The chemical qualities of meat, including moisture content, protein content, and fat content, were measured in accordance with AOAC (2005). Microbiological qualities include total microbes according to AOAC (1995), total acids according to Fardiaz (1989), pH according to Yosi and Sandi (2014), and total phenol according to Senter *et al*. (1989).

Statistical analysis

The data of physicochemical and microbiological qualities of meat were processed by analysis of variance, followed by Duncan's multiple range test (DMRT) at 5% probability (Steel and Torrie, 1995). Data analysis was assisted by using SPSS 17 statistical software.

Result and Discussion

The physical qualities of meat

Water holding capacity (WHC). Table 2 showed that there was a significant interaction ($P<0.05$) between the difference of liquid smoke concentration and storage time on water holding capacity (WHC) of duck meat.

The difference of liquid smoke concentration and storage time was also significantly influenced ($P<0.05$) to WHC of Pegagan's duck meat. This suggests that the use of liquid smoke solution up to 8% concentration has been able to influence the water binding capacity of meat. This significant effect is due to the influence of organic acids contained in the liquid smoke. These organic acids can cause a binding strips of myofibril to be looser (Abustam

Table 1. The chemical composition of liquid smoke from palm wastes

Compounds	Level (%)
Acetic acid (CAS) Ethylic acid	27.94
Formic acid (CAS) Bilorin	12.27
Propanedioic acid (CAS) Malonic acid	21.06
2-Propanone, 1-hydroxy- (CAS) Acetol	5.57
(E)-Hex-2-en-4ynal	27.91
Phenol, 2-methoxy- (CAS) Guaiacol	3.91
2-Methoxy-4-methylphenol	0.99
2,5-Dimethoxytoluene	0.35

Source: Yosi *et al*. (2016).

Table 2. The mean of water holding capacity, cooking loss, and tenderness of Pegagan duck meat preserved with various concentrations of liquid smoke during storage of 12 and 24 hours

Variables	Consentration of liquid smoke	Length of storage		Mean
		12 h	24 h	
Water holding capacity (%)*	2%	72.05±2.63	57.82±2.40	64.94±7.96^{c}
	4%	72.13±1.38	56.06±2.84	64.10±8.83^{bc}
	6%	71.05±1.08	51.05±3.20	61.05±10.92^{ab}
	8%	70.75±1.47	47.79±4.95	59.27±12.73^{a}
	Mean	71.50±1.68^{b}	53.18±5.16^{a}	
Cooking loss (%)*	2%	34.29±0.79	44.74±2.11	39.51±5.77
	4%	32.08±2.13	45.99±1.26	39.03±7.61
	6%	32.98±1.59	44.24±1.99	38.61±6.24
	8%	33.88±1.40	42.87±0.95	38.37±4.93
	Mean	33.31±1.64^{a}	44.46±1.87^{b}	
Tenderness (N)*	2%	1.59±0.24	1.69±0.32	1.64±0.27^{a}
	4%	1.46±0.46	3.52±1.25	2.49±1.41^{b}
	6%	1.42±0.10	2.76±1.48	2.09±1.21^{ab}
	8%	1.13±0.22	4.12±0.65	2.63±1.66^{b}
	Mean	1.40±0.31^{a}	3.03±1.32^{b}	

^{ns} non-significant interaction, * significant interaction.
^{a,b} different superscripts at the same column/row indicate significant different (P<0.05).

and Ali, 2011; Yosi *et al*., 2014). As a result, the ability of myofibril to bind the water decreases so that more the WHC decreases. This is in line with the value of WHC obtained, whereby the higher concentration of liquid smoke used in preservation, WHC value of meat tends to decrease. It is also the same as reported by Abustam and Ali (2011) that the more liquid smoke used, the lower WHC of meat. Furthermore, there was a significant decrease in the WHC, almost 25,73%, from the storage period of 12 hours (71.50%) to 24 hours (53.18%). The decrease in WHC is due to the ongoing process of glycolysis during storage. This is as stated by Risnajati (2010) that during the storage process, it will occur anaerobic glycolysis which causes the decreasing ability of the meat in binding water so that more water coming out of meat.

Cooking loss. There was a significant interaction (P<0.05) between the difference of liquid smoke concentration and storage time on cooking loss (CL) of duck meat. Furthermore, the value of cooking loss in flesh is markedly influenced by the length of storage, but not significantly influenced by the concentration of liquid smoke (Table 2). This indicates that the use of liquid smoke solution up to 8% concentration has not been able to influence the CL in duck meat. It is widely known that the CL was used to estimate the amount of fluid in cooked meats. In addition, CL can also be an indicator in determining the quality of meat. Meat with low CL means having good quality because fewer nutrients lost during cooking. This is in line with the statement of Suradi (2006) who reported that meat with a lower CL value is better than that of higher CL. Moreover, there was a significant increase in the CL, which was 33.47%, from the storage time of 12 hours (33.31%) to 24 hours (44.46%). This increase in CL meat is due to an increase in WHC during storage. Suradi (2006) reported that the WHC in meat during storage decreased due to the number of damaged myofibrils protein. As a result, the CL is increasing.

Tenderness. The results showed that the interaction between the concentration of liquid smoke and storage time was significant (P<0.05) in influencing the tenderness (TN) of Pegagan duck meat. Both the difference in the concentration of liquid smoke and storage time have also significant effect (P<0.05) on the TN of duck meat (Table 2). The increase of TN in duck meat is due to the decrease of WHC. The TN of meat has a correlation with WHC (Prayitno *et al.*, 2010; Yosi *et al.*, 2014), whereby the decreasing WHC will increase TN. This is in accordance with the results obtained, whereby the WHC value decreases with the greater the use of liquid smoke concentration. The highest TN value was in treatment of P4 with 8% concentration, that was 2.63 N. Furthermore, TN of meat has increased, which was 1.63 N, from the storage period of 12 hours to 24 hours. The increased TN of meat during storage is due to changes in meat by proteolytic enzymes. It is as stated by Komariah *et al.* (2004) that meat, naturally, could be more tender during storage by the role of proteolytic enzymes contained in meat, especially the enzyme cathepsin through the process of hydrolysis.

The chemical qualities of meat

Moisture content. Table 3 indicated that the interaction between the concentration of liquid smoke and storage period was not significant (P>0.05) in affecting the moisture content (MC) of duck meat.

However, the value of cooking loss in meat was markedly (P<0.05) influenced both by the concentration of liquid smoke and the length of storage. This assumes that the use of liquid smoke solution up to 8% concentration was able to influence the value of MC in duck meat. The difference in water content is due to the activity of organic acids contained in the liquid smoke which

Table 3. The mean of moisture content, protein content, and fat content of Pegagan duck meat preserved with various concentrations of liquid smoke during storage of 12 and 24 hours

Variables	Consentration of liquid smoke	Length of storage		Mean
		12 h	24 h	
Moisture content (%)[ns]	2%	76.71±1.16	73.01±1.21	74.86±2.26[b]
	4%	76.45±0.40	72.12±1.18	74.28±2.45[b]
	6%	77.23±0.42	71.43±1.04	74.33±3.19[b]
	8%	75.21±0.96	71.05±1.43	73.13±2.49[a]
	Mean	76.40±1.05[b]	71.90±1.34[a]	
Protein content (%)[ns]	2%	19.75±1.01	14.45±0.80	17.10±2.96[b]
	4%	19.53±1.00	14.79±0.89	17.16±2.69[b]
	6%	18.74±1.05	13.83±0.46	16.28±273[ab]
	8%	18.18±1.04	13.12±0.45	15.65±2.80[a]
	Mean	19.05±1.12[b]	14.04±0.90[a]	
Fat content (%)*	2%	2.16±0.04	4.02±0.07	3.09±0.10[c]
	4%	1.97±0.09	3.90±0.06	2.93±1.03[b]
	6%	1.80±0.25	3.87±0.11	2.84±1.12[b]
	8%	1.31±0.10	3.79±0.08	2.55±1.33[a]
	Mean	1.81±0.35[a]	3.89±0.12[b]	

[ns] non-significant interaction, * significant interaction
[a,b,c] different superscripts at the same column/row indicate significant different (P<0.05).

diffuses into the flesh during immersion. This caused a binding strips of myofibril to be looser so that much water comes out of the flesh and eventually the water content decreases (Abustam and Ali, 2011; Yosi and Sandi, 2014). It is known that there are several organic acids, including acetic acid (CAS) ethylic acid (13.31%), formic acid (CAS) bilorin (12.27%), and propanedioic acid (CAS) malonic acid (21.06%) (Yosi *et al.*, 2016)**.** This is also in line with the WHC obtained in this study, where the lower the MC, the more the WHC declines. Then, there was a significant decrease in the MC of meat, which was 5.89%, from 76.40% at the storage time of 12 hours to 71.90% at 24 hours. This is because during storage there will be collagen degradation that makes up the cross-link between meat fiber so that it can decrease the ability to hold water (Yosi and Sandi, 2014). Therefore, the longer the meat is stored, the MC will decrease.

Protein content. The results indicated that the interaction between the concentration of liquid smoke and storage time was not significant (P>0.05) in influencing the protein content (PC) of Pegagan duck meat. However, both the difference in the concentration of liquid smoke and storage time have significant effect (P<0.05) on the PC of duck meat (Table 3). It appears that the PC of meat decreased in line with the increasing use of the concentration of liquid smoke in the preserving process of meat. The mean of the lowest PC was in treatment of P4 with 8% concentration, ie 15.65%. This decrease is caused by the activity of some chemical compounds contained in the liquid smoke, especially organic acids and phenols. Organic acids cause changes in the acidity of meat. As a result, this can break the structure of meat proteins. This is in line with Werdiningsih *et al.* (2014) who reported that the organic acids in liquid smoke during the immersion will diffuse into the flesh and cause the protein lysis. In addition, phenol compounds tend to react with the hydrogen sulfur group of proteins that can denature the protein and eventually the PC of the meat decreases. Furthermore, the PC of duck meat also decreased during storage. There was a significant decrease in PC, which was 26.30%, from 19.05% (12 hours) to 14.04% (24 hours). This is because during storage there will be collagen degradation of proteins (Suradi, 2006).

Fat content. Table 3 showed that there was a significant interaction (P<0.05) between the difference of liquid smoke concentration and storage time to fat content (FC) in duck meat. The difference of liquid smoke concentration and storage time was also significantly influenced (P<0.05) to FC of Pegagan's duck meat. Furthermore, FC of meat has increased by 2.08% between the storage period of 12 hours and 24 hours. The high levels of FC are thought to be many primary and secondary oxidative products resulting from the oxidation process of unsaturated fatty acids in meat, such as fat peroxide and malonaldehyde (Valencia *et al*., 2006; Ernawati *et al.,* 2012). The results showed that the higher concentration of liquid smoke can reduce FC of meat. The decrease of FC is due to liquid smoke from palm waste contains phenol compounds that can act as antioxidants to inhibit the oxidation process of fat and prevent the formation of oxidative products. It is known that the phenol contained in liquid smoke from palm waste is about 5% (Yosi *et al*., 2016). The average of the lowest FC lies in the treatment of P4 with a concentration of 8%, namely 2.25%.

The microbiological qualities of meat

Total microbes. The results showed that the interaction between the concentration of liquid smoke and storage time was significant (P<0.05) in affecting the total microbes (TM) of Pegagan duck meat. Both the difference in the concentration of liquid smoke and storage time have also significant effect (P<0.05) on the TM of duck meat (Table 4).

It appears that the TM of meat decreased in line with the increasing use of the concentration of liquid smoke. The lowest TM value was in

Table 4. The mean of total microbes, total acids, pH and total phenol of Pegagan duck meat preserved with various concentrations of liquid smoke during storage of 12 and 24 hours

Variables	Consentration of liquid smoke	Length of storage		Mean
		12 h	24 h	
Total microbes (10^5 CFU/g)*	2%	1.50±0.37	6.16±1.21	3.83±2.50[d]
	4%	1.55±0.21	4.49±1.18	3.02±1.58[c]
	6%	2.10±0.66	3.05±1.04	2.58±0.67[b]
	8%	1.44±0.62	1.95±0.07	1.69±0.49[a]
	Mean	1.65±0.52[b]	3.91±1.63[a]	
Total acids (%)*	2%	0.81±0.08	1.49±0.16	1.15±0.38[a]
	4%	0.99±0.06	1.98±0.09	1.49±0.53[b]
	6%	0.95±0.04	2.62±0.14	1.78±0.90[c]
	8%	1.22±0.13	2.90±0.08	2.06±0.90[d]
	Mean	0.99±0.17[a]	2.25±0.58[b]	
pH*	2%	6.40±0.08	6.08±0.10	6.24±0.19[d]
	4%	6.35±0.06	5.75±0.13	6.05±0.33[c]
	6%	6.25±0.13	5.45±0.12	5.85±0.44[b]
	8%	6.10±0.08	5.08±0.09	5.59±0.54[a]
	Mean	6.28±0.14[b]	5.59±0.40[a]	
Total Phenol (mg TAE/g)*	2%	35.23±0.29	33.02±0.54	34.13±1.25[a]
	4%	38.49±0.81	41.16±0.27	39.82±1.54[b]
	6%	74.53±0.74	71.74±1.09	73.13±1.72[c]
	8%	78.60±0.44	77.33±1.32	77.96±1.14[d]
	Mean	56.71±20.60[b]	55.81±19.69[a]	

[ns]non-significant interaction, * significant interaction.
[a,b,c,d] different superscripts at the same column/row indicate significant different (P<0.05).

treatment of P4 with 8% concentration, that was 1.69 x 10^5 CFU/g. The decrease in TM is due to the phenol contained in liquid smoke has high bacteriostatic and antibacterial properties so that microbes can not grow and multiply (Kristinsson *et al.,* 2007). In line with this, Silva and Junior (2010) reported that phenol compounds have the ability to damage the structure of bacterial cells and inhibit the process of forming cell walls that cause lysis of bacterial cell walls. Then, there was an increase in the TM between the storage time of 12 hours and 24 hours, wich were from 1.65 to 3.91x10^5 CFU/g. This is assumed because the effect of phenol on liquid smoke has decreased at 24 hours of storage time so that it can no longer inhibit microbial growth. In line with this, Prihharsanti (2009) reported that TM will increase even if stored at cold temperatures.

Total acids. There was a significant interaction (P<0.05) between the difference of liquid smoke concentration and storage time to total acids (TA) of duck meat. Furthermore, the value of TA in meat was markedly (P<0.05) influenced both by the concentration of liquid smoke and the length of storage (Table 4). It appears that the meat preserved with higher concentrations of liquid smoke has a higher TA as well. The higher the concentration of liquid smoke, the higher the total acid in the meat. The highest TA value was in treatment of P4 with 8% concentration, that was 2.06%. The increasing value of meat TA is due to the presence of organic acid compounds contained in liquid smoke. It is known that liquid smoke of palm oil waste contains organic acids of about 61.27%, comprising acetic acid, formic acid and propanedioic acid (Yosi *et al.*, 2016). Then, TA of meat has increased by 1.26 % between the storage period of 12 hours and 24 hours. The increase in TA is due to the formation of lactic acid through anaerobic glycogen breakdown process (Suradi, 2006).

Acidity (pH value). Table 4 indicated that there was a significant interaction (P<0.05) between the liquid smoke concentration and storage time to pH in duck meat. The difference of liquid smoke concentration and storage time was also significantly influenced (P<0.05) to pH of duck meat. The pH of meat has the opposite relationship with TA. This is in line with the results obtained, whereby the increasing of TA will decrease the value of meat pH. Furthermore, It seems that the meat preserved with higher concentrations of liquid smoke has a lower pH as well. The lowest pH value was in treatment of P4 with 8% concentration, that was 5.59. In line with this, Rahayu *et al.* (2012) also reported that the pH of the meat will be lower along with the higher concentration of liquid smoke used. This is due to the presence of organic acid compounds that cause the pH to become lower. Moreover, pH of meat has significantly decreased between the storage period of 12 hours (6.28) and 24 hours (5.59). This is because the longer the storage process, the more lactic acid formed from the anaerobic glycogen breakdown process (Suradi, 2006).

Total phenol. The results indicated that the interaction between the concentration of liquid smoke and storage time was significant (P<0.05) in affecting the total phenol (TF) of duck meat. Both the difference in the concentration of liquid smoke and storage time have also significant effect (P<0.05) on the TF of duck meat (Table 4). It appears that the TF of meat decreased in line with the increasing use of the concentration of liquid smoke. This is because the more phenols contained in liquid smoke permeate into the meat

so that the TF in the meat increases. The TF of meat has an opposite relationship with pH (Hardianto and Yunianta, 2015). This is in accordance with the results obtained, whereby the increasing of TF will decrease the pH of meat. The highest TF value was in treatment of P4 with 8% concentration, that was 77.96 mg TAE/g. Moreover, TF of meat has significantly decreased between the storage period of 12 hours (56.71 TAE/g) and 24 hours (55.81 TAE/g). This is due to the increase of evaporated phenol compounds. This is in line with the statement of Komariah *et al.* (2004) that phenol compounds belonging to volatile compounds so that in the longer storage cause the content of phenol in meat to be reduced due to evaporation.

Conclusion

There is a significant interaction between the concentration of liquid smoke from palm waste (2 to 8%) with storage duration (12 and 24 hours) of water holding capacity, tenderness, moisture content, protein content, fat content, total microbial, total acid, pH, and total phenol in Pegagan duck meat. The use of liquid smoke concentration of 4% with 12 hours of storage time provides optimal results in maintaining the physical, chemical and microbiological qualities of Pegagan duck meat.

Acknowledgement

The authors would like to thank to the LPPM of Sriwijaya University who have provided financial support through research of science, technology and art (SATEKS) in 2017 with contract number: 1013 / UN9.3.1 / PP / 2017. Thanks also to Risma Ahya Fitriani, Dwita Fitriyani, Dian Kusuma Putri, and Nyimas Yolanda who participated and assisted in this research.

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