The Effect of Antioxidant Activity of Liquid Smoke in Feed Supplement Block on Meat Functional of Muscle *Longissimus dorsi*

E. Abustam[1*], M.I. Said[1] and M Yusuf[1]

[1]Departement of Animal Production Faculty of Animal Science Hasanuddin University, Makassar.

Corresponding E-mail: effendiabu@hotmail.com

Abstract

This study aims to look at the role of liquid smoke as an antioxidant added in feed supplement block and administered to cattle for 45 days on the functional properties of meat. The level of liquid smoke in the feed and the time of maturation in Muscle *Longissimus dorsi* after slaughtering cattle were the two treatment factors observed for the functional properties of meat. The study used a complete randomized design in which factor 1 was a 10% smoke level in the feed (0, 1, 2%) and factor 2 was maturation time (0, 2, 4, 6, 8 days). The parameters observed were water holding capacity (WHC), raw meat shear force (RMSF), fat oxidation rate (thiobarbituric acid reactive substance) and antioxidant activity (DPPH). The results showed that liquid smoke levels lowered the WHC, RMSF more or less the same, increased fat oxidation rate, and antioxidant activity more or less the same. While maturation tends to increase WHC, increase RMSF, fat oxidation rate, and antioxidant activity. It can be concluded that liquid smoke as an antioxidant in the diet of block supplements can maintain the functional properties of Muscle *Longissimus dorsi* of Bali cattle during maturation.

1. Introduction

The functional properties of meat decreased postharvest, especially water holding capacity and shear force value of meat. One of the causes of the decline in the functional properties of meat is stress due to the uncomfortable treatment of livestock before slaughter resulting in oxidation of the functional properties. Oxidation in the functional properties of meat can occur in fats and proteins; fat oxidation results in increased rancidity, while oxidation of proteins results in decreased functional properties of meat [1].

Efforts to provide concentrate feed during cattle fattening before slaughter and post-slaughter handling are expected to reduce or inhibit stress due to transport and uncomfortable treatment of livestock. Cattle that experience stress during transport and do not get adequate rest before slaughter resulted in decreased meat quality marked by dark red meat, firm and dry texture [2]. WHC decline and tenderness of the meat and increased cooking loss is an implication of livestock who experience stress before slaughter [2,3].

Giving antioxidants to the feed or the meat and its processed products is an attempt made to reduce or inhibit oxidation in the functional properties of meat. The researchers provided antioxidants to the meat to inhibit the oxidation in which free radicals would be bound by antioxidants so that the functional properties of the muscles persisted during postharvest handling. One such antioxidant is liquid smoke from pyrolysis at 400^0C, producing 4.13% phenol, 11.3% carbonyl and 10.2% acid [4].

The phenol compound is one of the chemical content of liquid smoke that acts as an antioxidant and as a preservative.

Some researchers use liquid smoke as an antioxidant in meat and its processed products; [5] suggest that liquid smoke as an antioxidant can increase WHC and decrease cooking loss in meatballs products. Similarly, [6] reported that the functional properties of Bali beef increase with the administration of liquid smoke in *Longissimus dorsi* muscle during maturation.

The use of liquid smoke in mice was conducted by [7] in which in 14 days of observation, the body weight of mice increased at all levels of liquid smoke exposures. Meanwhile, the liquid smoke as an antioxidant in catfish was studied by [8]. [9] concluded that the protein of tilapia (*Oreochromis niloticus*) increased to 79% with the use of liquid smoke concentration of 5% as a preservative.

Another way of giving liquid smoke as an antioxidant is to add it to the block supplement feed. Previous studies have shown that the higher concentrations of liquid smoke in feed supplement block decreased shear force value to 3.23 kg /cm^2 at a concentration of 20%. Similarly, WHC increases with increasing maturation time [10]

This study aims to improve or maintain the functional properties of post-aging flesh through the provision of liquid smoke as an antioxidant into the block supplement feed; UCSMB.

2. Materials and Methods

This study utilized the post-rigor *Longissimus dorsi* (LD) muscle, from 9 males of 2-year-old cattle fed with Urea Coconut Smoke Multi-nutrient Blocks (UCSMB) and elephant grass (*Pennisetum purpureum)* as a source of roughages for 45 days. *Longissimus dorsi* muscle-derived from Bali cattle raised in Maiwa Breeding Center Hasanuddin University, 198 km north of Makassar capital of South Sulawesi. Muscle extraction and retrieval were performed at the slaughterhouse Tamangapa Makassar. The liquid smoke concentration of 10% added to the UCSMB feed at 1 to 2% (w/w) functioned as an antioxidant to preserve meat, especially during the maturation time. *Longissimus dorsi* muscles (LD) were dissected from all of the nine male cattle after the slaughtering and aged for eight days with two days interval the measurement.

Table 1. Composition of feed materials in UCSMB[1)]

Feed materials	Composition (g/kg) at Levels of liquid smoke concentration of 10%		
	0%	1%	2%
1. Coconut water	30	29	28
2. Urea	5	5	5
3. Rice Bran	30	30	5
4. Cornmeal	10	10	10
5. Copra meal	10	10	10
6. Cement	10	10	10
7. Cow Mineral	2	2	2
8. Table salt	3	3	3
9. Liquid smoke	0	1	2

[1)] Abustam *et al*. (2015b)

Urea, coconut water, liquid smoke multi-nutrient block (UCSMB) is a modification of UMMB (urea-molasses block multi-nutrient) where molasses replaced with coconut water, and liquid smoke was added as an antioxidant [11]. There were three kinds of UCSMB used with liquid smoke in concentration 10% treatment adapted to the levels of 0% (control), 1%, and two % in the formulation (w/w). The composition of the feed material in UCSMB shows in Table 1.

This research used an entirely randomized design of factorial pattern of 3 x 5. The first factor was the levels of liquid smoke in the feed block (0, 1%, and 2%) and the second factor was the aging time (0, 2, 4, 6, 8 days) at a temperature of 2 - 5^0C replicated for three times. Thus, the variables measured included raw meat shear force value (RMSF), water holding capacity (WHC), fat oxidation by the thiobarbituric acid reactive substance (TBARS) and antioxidant activity (DPPH). Every animal received feeds in the form of 500 g of UCSMB, and 20 kg of *Pennisetum purpureum* per day for 45 days.

The shear force measured by using CD Shear Force, in which the meat samples in cylinder form with 1 cm length and a diameter of 0.5 inches placed in the hole on CD shear force. Thus, the samples have torn by using CD shear force knife with 1 mm thickness. The bigger the weight to tear the meat samples, the harder the meat tenderness. SF value was in kg/cm^2 [2].

Measurements on WHC of raw meat was carried out by using Filter-Papers Press Method as proposed by Hamm 1986 [2]. TBARS test was performed to measure the level of rancidity caused by fat oxidation during maturation [12].

Measurement of antioxidant activity by methods DPPH (1, 1-diphenyl-2-picrylhydrazyl) [13] was slightly modified. An aliquot of 3.9 mL of 0.1 mM DPPH radical in methanol was mixed with 0.1 mL of methanol extract samples. The mixture of DPPH reagent was shaken vigorously and allowed to stand at ambient temperature in the dark for three h. Measurement of the absorbance of the sample used with a UV-VIS spectrophotometer (Shimadzu brand) at 515 nm.

Processing of the data by utilizing analysis of variance (ANOVA) and testing between average uses LSD based on [14] with SPSS (SPSS 16.0, SPSS Ltd., West Street Woking, Surrey, UK).

3. Results and discussion

Variance analysis showed that liquid smoke level did not significantly affect RMSF even though there was a tendency of RMSF to increase with increasing liquid smoke level (Table 2). It indicates that liquid smoke in UCSMB feeds up to 2% has not been able to trigger cathepsin enzymes to digest muscle protein so that the RMSF value even increases. In theory, maturation will increase the tenderness of meat due to the work of cathepsin enzymes. The provision of liquid smoke in the block feed needs to be increased by the amount given by the weight of the cow body, which is likely to have a noticeable effect. As the RMSF increases with increasing levels of liquid smoke suppressed by protein oxidation, as suggested by [1] stated that if the protein undergoes oxidation, it can decrease the tenderness of the flesh. [15] suggest that the protein oxidation will change the WHC and the tenderness of the meat.

The maturation time had a very significant effect ($P < 0.001$) on the RMSF where there was a marked difference between day maturation to zero by day four, day six and day eight (Table 2). The more maturation time the RMSF increases even though there is no real difference between the fourth day with the sixth day and the eighth day. This condition indicates that the level of liquid smoke in the feed shows two groups of RMSF namely the maturation group 0 and two days and the maturation group 4, 6, and eight days. An increase in RMSF in the second group reached an average of 0.48 points from the first group. It concludes that the RMSF increases with increasing maturation time until the fourth day.

In this case liquid smoke, up to 2% in the feed has not been able to improve the tenderness during maturation. Oxidation of proteins may be the cause. [1] stated that if the protein undergoes oxidation, it can decrease the tenderness of the flesh. [15] suggest that the protein oxidation will change the WHC and the tenderness of the meat.

Increasing the level of liquid smoke in UCSMB feed, the WHC values decrease even though there is no real difference between liquid smoke level 0 and 1%. At the 2% level, it lowers the WHC to 6.11 point of the no-smoke liquid. This increase indicates that the provision of liquid smoke in the feed to 2% has not been able to maintain a high WHC when the new cattle finish slaughtering [2,3]. The amount of liquid smoke provided is relatively low compared with the weight of the cow body, and the high activity of rumen microbes may be the cause.

The maturation time has no significant effect on WHC values , but there is a tendency for WHC to increase. The results of this study do not support previous studies where the WHC increased significantly with increasing maturation time [10]. Differences in treatment wherein the previous study using the treatment of differences in smoke concentration of 0, 10, 20% as much as 2% in the diet of block supplements can explain this.

The higher the liquid smoke level increases the TBARS value reaching 65.62% at 2% level (Table 2), indicating that the delivery of liquid smoke in UMSCB feed has not been able to inhibit the increase of TBARS value, although the value of TBARS is still very low. This value is really low when compared with the critical value of 3 mg MDA/kg in which the level of rancidity is detected [16]. [17] point out that the low intensity on fat oxidation during storage occurs if TBARS values remain low in the threshold to detect rancidity but remain high in meat maturation sampling.

Increased maturation time resulted in increased TBARS value reaching 0.93 points on the eighth day compared to day zero. The increase in TBARS values is quite high but is still within the limits of low-fat oxidation rates. [17] point out that the low intensity on fat oxidation during storage occurs if TBARS values remain low in the threshold to detect rancidity but remain high in meat maturation sampling. An addition of vitamin E to the pasture as an antioxidant in cattle increases the vitamin E in muscle and decreases the impact of fat oxidation on beef [18].

Analysis of variance showed a significant interaction ($P < 0.05$) between liquid smoke level and maturation time on TBARS value (Figure 1). Until the fourth day of maturation, the TBARS values are more or less the same at all three levels of liquid smoke. The three levels of liquid smoke though not significantly different, increase the value of TBARS from the fourth day to the eighth day of maturation. At 0% level, the increase of TBARS value until the eighth day of maturation is lowest among the three levels of liquid smoke. At the end of maturation, the value of TBARS at 1% and 2% levels increased significantly with the 0% level.

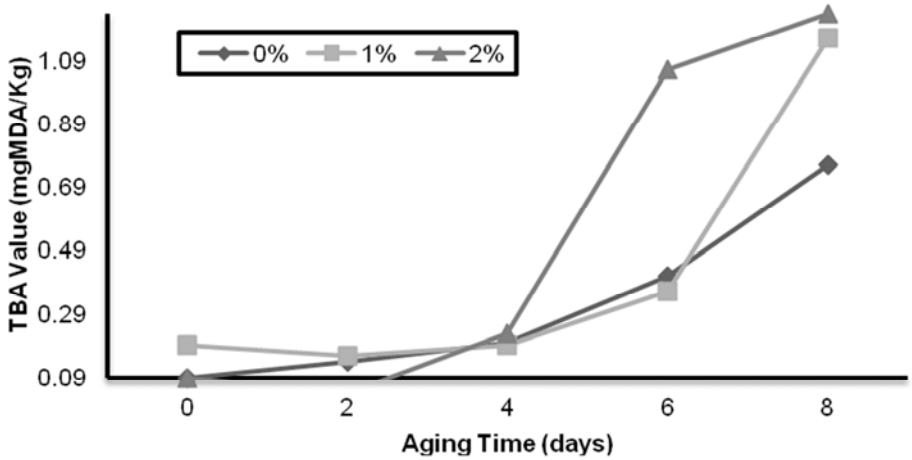

Figure 1. Interaction between levels liquid smoke and time of aging on TBARS value

Analysis of variance showed that liquid smoke level did not significantly influence, while maturation time had a significant effect (P <0.001) on antioxidant activity. There is a tendency for antioxidant activity to decrease with increasing levels of liquid smoke in UCSMB feed. Differences between liquid smoke treatments that did not differ in antioxidant activity may cause the WHC to decrease, the value of TBARS increases and RMSF is approximately equal to the increase of liquid smoke level (Table 2).

Antioxidant activity increased with increasing maturation time reaching 57.20% on the eighth day compared to days zero (Table 2). The high antioxidant activity reached 88.44% on the eighth day of maturation indicating that beef protein is a good source of antioxidant. [19] argued that food of animal origin that contains high protein is a good source of antioxidants. This increase has implications for the RMSF decrease until the second day of maturation, the WHC trend more or less the same during maturation and the increasing TBARS in the sixth day of maturation.

Table 2. Effects of liquid smoke levels in UCSMB and aging time of *Longissimus dorsi* on the functional properties of meat quality of Bali cattle (means and SE)

Treatments	RMSF (kg/cm^2)	WHC (%)	TBARS (mg MDA/kg)	DPPH (%)
LS Levels:	Sig:NS	Sig:0.001	Sig:0.05	Sig:NS
0%	1.20±0.38	30.28±3.80 ^{a}	0.32±0.25 ^{a}	60.42±18.47
1%	1.24±0.32	30.12±4.04 ^{a}	0.41±0.41 ^{ab}	60.72±20.98
2%	1.26±0.33	24.17±3.61 ^{b}	0.53±0.26 ^{b}	57.74±21.36
Aging:	Sig:0.001	Sig:NS	Sig:0.001	Sig:0.001
0 days	1.03±0.24 ^{a}	26.06±2.94	0.12±0.09 ^{a}	31.24±7.39 ^{a}
2 days	0.86±0.18 ^{a}	28.70±5.38	0.11±0.08 ^{a}	58.93±7.17 ^{b}
4 days	1.40±0.20 ^{bc}	29.71±5.88	0.21±0.02 ^{a}	52.44±6.55 ^{c}
6 days	1.45±0.22 ^{bc}	28.37±5.10	0.61±0.50 ^{b}	67.07±7.70 ^{d}
8 days	1.41±0.35 ^{bd}	28.12±4.00	1.05±0.25 ^{c}	88.44±2.56 ^{e}

Description: Numbers with different superscripts in the same column stated a significant difference (P <0.05) and a highly significant difference (P <0.001).
LS Levels: Liquid smoke levels; RMSF, raw meat shear force; WHC, water holding capacity; TBA, thiobarbituric acid reactive substance; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

4. Conclusion

This study revealed that liquid smoke levels lowered the WHC, RMSF more or less the same, increased fat oxidation rate, and antioxidant activity more or less the same. While maturation tends to increase WHC, increase RMSF, fat oxidation rate, and antioxidant activity. It can be concluded that liquid smoke as an antioxidant in the diet of block supplements can maintain the functional properties of Muscle *Longissimus dorsi* of Bali cattle during maturation.

Acknowledgements

The authors would like to thank DRPM Kemenristek Dikti who has funded this research through Competency-Based Research Program FY 2017 with contract No. 005 / SP2H / PPM / DPRM / IV / 2017.

References

[1] Bhattacharya D, Kandeepan G and Vishnuraj M R 2016 Protein oxidation in meat and meat products- A Review. *J. Meat Sci. Technol.* April-June, 2016.**Vol 4** Issue 2 Pages 44

[2] Abustam E 2012 *Meat Science: Aspects of Production, Chemistry, Biochemistry, and Quality.* 1st Ed. Masagena Press. Makassar (Indonesian)

[3] Soeparno 2005 *Meat Science and Technology*. Gajah Mada University Press, Yogyakarta (Indonesian).

[4] Setiadji B A H 2000 Liquid coconut shell. Liquid smoke as the natural preservative safe for humans *(www.asapcair.com)*, PPKT, Jogjakarta (Indonesian)

[5] Abustam E, Yusuf M, Ali HM and Yuliati F N 2015a Effect of muscle types of Bali beef pre and post-rigor on the quality of meatballs during storage. *Pak. J. Nutrition* **14**(3)170

[6] Abustam E, Yusuf M, Ali HM and Yuliati FN 2012 Increased durability and quality of meat and meat products processed through the addition of liquid smoke as an eco-friendly natural preservative. *National strategic research report*. Hasanuddin University (Indonesian)

[7] Budijanto S, Hasbullah R, Prabawati S, Setyadjit S, Sukarno and Zuraida I 2008 Identification and safety test on liquid smoke made from coconut shell for a food product. *J. Pascapanen* **5** (1) 32

[8] Ernawati, Purnomo H and Estiasih T 2012 Antioxidant effect of liquid smoke on the stability of oxidation sausage catfish (*Clarias gariepinus*) dumbo during storage. *J. Tech. Agriculture* **13** (2)119

[9] Budaraga I K 2017 Effect of combination treatment of concentrated liquid smoke, immersion duration, packaging and old type storage different levels of protein Nila fish fillet (*Oreochromis niloticus*). *International J. ChemTech Res.* **10**(3)1

[10] Abustam E, Said MI, Yusuf M, Nahariah 2016 Effect of liquid smoke concentration in feed supplement and maturation time M. *Longissimus dorsi* against the quality of Bali beef. Prosiding Nasional conference on *Optimalisasi Teknologi dan Agribisnis Peternakan Dalam Rangka Pemenuhan Protein Hewan Asal Ternak* ISBN 978-602-1004-42-5 642

[11] Abustam E, Yusuf M, Said MI and Mide MZ 2015b IbM Showroom Bali cattle in district Tanete Riaja of Barru Regency. *Reports of PPM DP2M Dikti*, Faculty of Animal Science Hasanuddin University (Indonesian)

[12] Apriyantono A, Fardiaz D, Puspitasari N.I, Sedarnawati, Budiyanto S 1989 *Food Analysis*. IPB Press, Bogor (Indonesian)

[13] Pajak P, Socha R, Galkowska D, Roznowski J and Fortuna T 2013 Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chem*., **143** 300

[14] Steel RGD and Torrie JH 1991.*Principles and Procedures of Statistics.* McGraw-Hill, Book Co. Inc, New York

[15] Rowe L, Maddock J, Lonergan KRSM, Huff-Lonergan E 2004 Influence of early post-mortem protein oxidation on beef quality**.** *J. Anim. Sci*., **82**(3) 785

[16] Wong JW, Hashimoto K, Shibamoto T 1995 Antioxidant activities of rosemary and sage extracts and vitamin E in a model meat system. *J. Agric. Food Chem*., **43**(10) 2707

[17] Popova T, Marinova P, Vasileva V, Gorinov Y, Krasimira L 2009 Oxidative changes in lipids and proteins in beef during storage. *Archiva Zootechnica* 12:3, 30

[18] Gatellier P, Mercier J, Juin H, Renerre M 2005 Effect of finishing mode (pasture -or mixed diet) on lipid composition, color stability and lipid oxidation in meat from Charolais cattle. *Meat Sci*., **69**(1) 175

[19] Hernandez L, Zermeno B, Arino, Blasco 2003 Antioxidant, lipolytic and proteolytic enzyme activities in pork meat from different genotypes. *Meat Sci*., **66**: 525.