2013 International Conference on Agriculture and Biotechnology
IPCBEE vol.60 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V60. 5

Cocoa Husk Extract Administration on Suspected Stress Cattle Prepares Good Meat Quality

Hikmah M Ali[1+], Effendi Abustam [1], Syamsuddin Hasan[1], Salengke[2] and Mawardi A. Asja[3]

[1]Animal Husbandry Faculty, Hasanuddin University
[2]Agriculture Faculty, Hasanuddin University
[3]Animal Husbandry and Fisheries Faculty, West Sulawesi University

Abstract. The aim of this study was to observe the beneficial effects of cocoa husk extract compounds on some meat quality properties. A total of 25 male Bali cattle (*bos sondaicus*) were administrated by stress condition, recover condition after 12 hours, injection with polyphenol, theobromin and their combination from cocoa husk extract. The *M. Longisimus dorsi* were taken from animal after slaughtered at one hour after administrations. The results showed that no significant differences (P>0.05) between pH values changes at the first and the second hour postmortem, the significant changes were showed after 3 hour until 8 hour postmortem. At the 8th hour, the lower pH value (P<0.05) were found at theobromine injection compared with polyphenol injection and its combination with theobromine. Water holding capacity values were significantly higher (P<0.01) on stress (control) animals meat than recovered animals, but no significant different (P>0.05) within all injection treatments, and the same evidences found at cooking loss parameter. Sarcomere lengths of the meat were significantly (P<0.01) longer in recovered animals than in stress animals, but there was no significant different within all injection treatments.

Keywords: cocoa extract, theobromine, injection, pH, Water holding capacity, cooking loss, sarcomere length

1. Introduction

The meat quality was a major problem in meat supplying in Indonesia. Especially in South Sulawesi, almost meat from slaughter house were recognized by darken appearance, dry and tough, and identified as DCB (dark cutting-beef) or DFD (dark, firm, and dry). Abustam [1] reported that DCB occurred about 37%, and mostly on Bali cattle about 14.2%. Stress was identified as the causes [2], some stressors from feed yard, implant treatment, days from final implant to harvest, maximum and minimum daily temperatures, and temperature fluctuations from 2 d before harvest to the day of harvest all contributed (P<05) [3]. It closely related to its pH (*degree of acidity or alkalinity*). Meat with high pH will be very dark red to purplish in colour and is not acceptable to consumers. The dark colour is caused by changes in the physical properties of the meat at higher pH after slaughter or during storage. Slaughtered the animal under stress condition, will cause increase on pH value [4] and some meat quality properties [5].

Most of slaughtered cattle in local slaughter house in South Sulawesi were from long transportation duration (6 – 12 h), and they will be put into the cutting pit after 12 – 24 hours after arrived. Local meat consumers need meat in large number and quickly, in another hand the lack of adequate storage facilities, urging the cutter to hasten the process of slaughter. Normally cattle needs at least 24 hour for rest before slaughter [6]. Buckham Sporer et al. [7] reported that peak of blood cortisol level found at 4.5 h after transportation and then linearly decreased during 14.2 h and back in to normal levels at 24 to 48 h after resting, but in this situation was not able to prevented slaughter under stress condition or let the animal to

[+] Corresponding author. Tel.: +62814773125; fax: 62411587217.
E-mail address: hikmah_unhas@hotmail.com.

recover before slaughter. Thus, it needs to give treatment on cattle to accelerate recovery condition from stress, and expected to give beneficial effect on meat during storages period.

Theobromine is one of cocoa polyphone alkaloid that known has lot benefit potential for health[8] and on physiological metabolism [9], experiment on human show that it was available to increase mood and lowered blood pressure about 3 h after oral administration [10]. In chocolate form, it can lower the leukotriene/ prostacyclin ratio and is shown to have beneficial effects on platelets and possibly inflammation and vessel dilatation, they also inhibit LDL oxidation and rise HDL level in blood [11]. Theobromine were consisted in cocoa bean about 0.19-3.0%, and 0.19 – 2.98% in husks [12]. In South Sulawesi cocoa husk is agricultural industry wastes and have not been utilized yet. Our study aimed to observe the cocoa husks extract injection effect on establish meat quality during storage.

2. Materials and Methods

2.1. Cocoa Husks Extraction

Cocoa husk were oven at 50^{o}C for 1 hour than milling till sieve in 20 meshes. A 20g sample was then extracted in hexane (1:5) for 4 times for fat extraction followed by drying in oven. Sieve 100 mesh powder from oven was extracted their polyphone using 80% acetone, this step was repeated 3 times at 80^{o}C. The solution was evaporated with vacuum evaporation and drying with freeze-dryer [13]. Theobromine extracts were purchased from Rhino-Pharmeutical, US.

2.2. Animal Samples

A total of 25 male Bali cattle with 1.5 – 2 years of age were selected and confirmed with the traders before the animal arrived at Tamangapa Slaughter house (SH), Makassar, South Sulawesi at about one or two days before. Healthy and clinical references appropriate with the healthy card follow with the animals.

2.3. Design and Parameters

The study was designed with one-way ANOVA, and especially for pH value an addition timed sampling required two-way repeated measurement. Independent variables were A) Animal without rest (*negative/stress animal control*), 2) rest for 12 hours (*positive/recovered animal control*), 3) injection of polyphenol extract, and 4) injection of theobtomine and 5) combination of polyphenol extract and theobromine. Objective measured parameters were meat (*M. Longisimus dorsi*) pH value, water holding capacity (WHC) presentation, cooking loss (CL) presentation, and tenderness from sarcomer length. For pH value changes, measurement conducted every 1 hour during 8 hour post mortem,

3. Result and Discussion

3.1. Result

Contras value from Univariate Test showed that there was no significant different ($P>0.05$) between the first and the second hour post-mortem in all treatments (6.61 ± 0.24), the significantly different ($P<0.01$) among the meat samples appeared at 3th (6.35 ± 0.28) hours until to 8th (5.71 ± 0.19) hour. At the 8th hour, meat from stress animals (6.10 ± 0.12) showed the highest pH ($P<0.01$) compared with all treatments. The pH value of meat from recovered animals (5.58 ± 0.07) was significantly lower ($P<0.05$) than the meat injected by theobromine, polyphenol, and its combination (5.67 ± 0.14). The pH meat values from stress animals decreased slightly in comparison to other treatments and the recovered animal meat pH value declined clearly in comparison to the other treatments (Fig. 1).

The percentage of WHC on stress animal ($41.97 \pm 1.81\%$)was significantly higher ($P<0.01$) than the percentage of WHC of meat from recovered animal after 12 hours. However, there was no significant different ($P>0.05$) with all injection administrations. Cocoa extracts administrations resulted a higher WHC values then recovered animals, but no significant differences within the same administration. Same condition was found on cooking loss parameter values. The highest percentage of cooking loss value was found at rested animal's meat, and the value was significantly ($P<0.01$) different with others treatments. Although the lowest cooking loos value was found at meat from stress animal, it had not any difference with injection treatments. The tenderness were indicated from sarcomere length showed that without resting

animals meat had the shortest (P<0.01) sarcomere. The meat from recovered animal had same sarcomere length (P>0.05) with other injection treatments (Table 1).

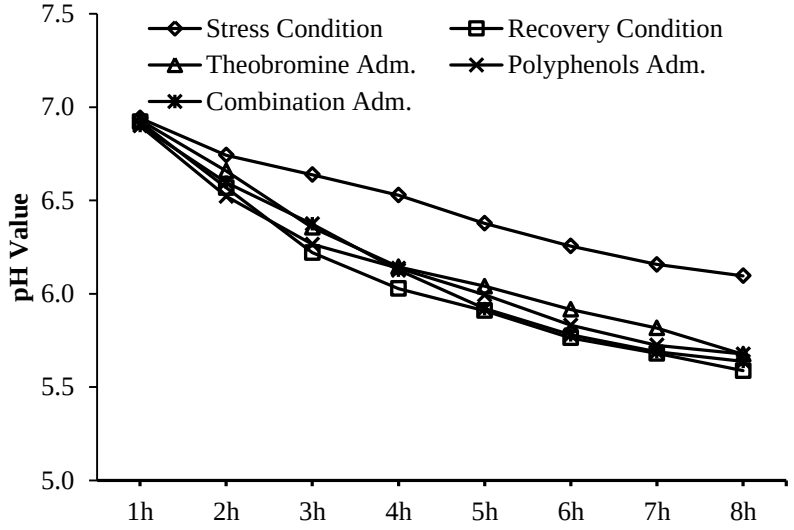

Fig. 1: pH Changes Between 8 hours Post-mortem.

Table 1. Water Holding Capacity, Cooking Loss presentation of meat, and Sarcomer Length Meat Fibers

Treatments	Water Holding Capacity	Cooking Loss	Sarcomere Length
	------- % -------		µm
Stress condition	41.97 ±1.81[a]	24.17 ±2.23[a]	1.68 ±0.07[a]
Rest for 12 hours	39.28 ±2.48[b]	26.43 ±2.74[b]	2.05 ±0.06[b]
Theobromin Adm.	40.68 ±2.89[a]	24.71 ±2.37[a]	1.99 ±0.08[b]
Polyphenol Adm.	40.89 ±2.52[a]	24.52 ±2.44[a]	2.01 ±0.13[b]
Theo + Poly. Adm.	41.28 ±2.22[a]	24.81 ±2.20[a]	2.02 ±0.09[b]

Different superscript in the same column means differences at 1% (P<0.01)

3.2. Discussion

The ultimate pH of the *M. Longisimus dorsi* is generally used to assess carcass quality [14]. In this study, the ultimate pH was measured at 8th hour after slaughter. Animal without rest or suspected as stress animal showed a higher pH than expected ultimate pH value (6.1 ± 0.12 vs 5.58), while recovered animal and injection of cocoa husk extract administrations seemed in normal ranges of ultimate pH (5.58 – 5.68). Stress before slaughter can lead to a depletion of glycogen in the muscles and consequently reduced production of lactic acid postmortem, resulting in elevated pH of the meat. The optimum or normal pH of meat was between 5.60 and 5.80. A pH greater than 5.80 is regarded as unacceptable. Mounier et al. [15] reported that pH ultimate on cattle with 21.3 ng/ml cortisol was 5.71. The dark colour of the meat is closely related to its pH. The dark colour is caused by changes in the physical properties of the meat at higher pH [3]. Polyphenol injection showed the lowest pH value (5.67 ± 0.17) in comparison to its combination with theobromine extract (5.63 ± 0.14). This indicated that polyphenol was able to prevent muscle glycogen pre-slaughter followed the production of lactic acid during rigor period by recovering stress condition on animals.

Recovered animal after 12 hours had the lowest WHC value, and it significantly different (P<0.01) compare to the other treatments. Although stress animal had higher WHC value, but it was not significantly differences (P>0.05) compare with injection administrations. High WHC value indicated a relationship with final ultimate pH value[16]. Water holding capacity of meat products is a very important quality attribute which has an influence on product yield, which in turn has economic implications, but is also important in terms of eating quality. A number of pre-and post-mortem factors influence the water holding capacity

(WHC) of meat. In the immediate pre-slaughter period, stresses on the animal such as fasting, and different stunning methods are likely to influence meat WHC[17]. Accelerated pH decline and low ultimate pH are related to the development of low water-holding capacity and unacceptably high purge loss. Rapid pH decline resulting in ultimate or near ultimate pH while the muscle is still warm causes the denaturation (loss of functionality and water binding ability) of many proteins, including those involved in binding water. The most severe purge or drip loss is often found in PSE (Pale, Soft, and Exudative) product from pigs that have inherited a mutation in the ryanodine receptor/calcium release channel (halothane gene) in the sarcoplasmic reticulum [18].

Cooking loss value was higher significantly on meat of recovered animals, this value is also higher than cooking loss presents at *Longisimus thorachalis* of African beef (Nguni = 25,1; Bonsmara= 23,5) and Angus=24,9 [19], while the lowest cooking loss was found on meat from un-rested animal. High lactic acid production (in expected) as the result of polyphenol and theobromin injections in muscle, denaturize protein myofibrils, made the loss in ability to holding water while cooking. Lactic acid has a responsibility in cooking loss, as the ability to altered dark-cutting beef after post-rigor enhancement [20] and [21].

Sarcomere length has significant positive relationship with tenderness [22]. Rest animal (recovered from stress condition) seems had longer sarcomere length and also on injection treatments meat. This mean that cocoa husk extract injections were able to gave the same effect to increase the tenderness of meat. Short sarcomere length in stress animal meat was related with their pH ultimate level (Fig. 1), early post-mortem muscle pH which varies over a wide range affects tenderness significantly[23]. This short sarcomers and pH decreasing value was consistent with research by [24], it was reported that slow fall of pH value resulted higher sear force values compare with fast and intermediate pH falls.

4. Acknowledgements

The authors are grateful to the Hasanuddin University and Direktorat Jenderal Pendidikan Tinggi Kementrian Pendidikan dan Kebudayaan Republik Indonesia for funding some of the work reviewed in this paper.

5. References

[1] E. Abustam, "The occurrence of dark cutting-beef on meat animals in South Sulawesi," *BIPP IV*, vol. 11, pp. 27 – 34, 1996.

[2] J. Denaburski, T. Bak, and P. Matusevicius, "Relationships between the turnover before slaughter, welfare and the occurrence of defective DFD type meat in cattle.," *Pol. J. Vet. Sci.*, vol. 7, no. 1, pp. 67–73, Jan. 2004.

[3] J. A. Scanga, K. E. Belk, J. D. Tatum, T. Grandin, and G. C. Smith, "Factors contributing to the incidence of dark cutting beef," *J. Anim. Sci.*, vol. 76, no. 8, pp. 2040–2047, Aug. 1998.

[4] D. A. King, C. E. S. Pfeiffer, R. D. Randel, T. H. W. Jr, R. A. Oliphint, B. E. Baird, K. O. C. Jr, R. C. Vann, D. S. Hale, and J. W. Savell, "Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle," *Meat Sci.*, vol. 74, pp. 546–556, 2006.

[5] R. Z. Zhong, H. W. Liu, D. W. Zhou, H. X. Sun, and C. S. Zhao, "The effects of road transportation on physiological responses and meat quality in sheep differing in age," *J. Anim. Sci.* , vol. 89 , no. 11 , pp. 3742–3751, Nov. 2011.

[6] R. Browning and M. L. Leite-Browning, "Comparative stress responses to short transport and related events in Hereford and Brahman steers.," *J. Anim. Sci.*, vol. 91, no. 2, pp. 957–69, Feb. 2013.

[7] K. R. Buckham Sporer, P. S. D. Weber, J. L. Burton, B. Earley, and M. a Crowe, "Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress.," *J. Anim. Sci.*, vol. 86, no. 6, pp. 1325–34, Jun. 2008.

[8] I. Andújar, M. C. Recio, R. M. Giner, and J. L. Ríos, "Cocoa polyphenols and their potential benefits for human health," *Oxid. Med. Cell. Longev.*, vol. 2012, p. 906252, Jan. 2012.

[9] H. H. Cornish and A. A. Christman, "A study of the metabolism of theobromine, theopillyne, and caffeine in man," *J. Biol. Chem.*, vol. 228, pp. 315–323, 1957.

[10] E. S. Mitchell, M. Slettenaar, N. vd Meer, C. Transler, L. Jans, F. Quadt, and M. Berry, "Differential contributions of theobromine and caffeine on mood, psychomotor performance and blood pressure," *Physiol. Behav.*, vol. 104, no. 5, pp. 816–822, Oct. 2011.

[11] R. M. D. Kelishady, "Cacao to cocoa to chocolate: healthy food?," *ARYA J.*, vol. 1, no. 1, pp. 28–34, 2005.

[12] A. H. Ensminger, M. E. Ensminger, J. E. Konlande, and J. R. K. Robson, "The Concise Encyclopedia of Foods and Nutrition," *Philadelphia CRC Press*, pp. 206 – 7, 1995.

[13] Haryadi, B. Rahardjo, and D. W. Marseno, "Antioxidant activity of crude polyphenol extract from microwave roasted cocoa beans," *Food Technol. Ind.*, vol. 17, no. 3, pp. 176 – 182, 2006.

[14] G. Monin, "Recent methods for predicting quality of whole meat.," *Meat Sci.*, vol. 49S1, pp. S231–43, Jan. 1998.

[15] L. Mounier, H. Dubroeucq, S. Andanson, and I. Veissier, "Variations in meat pH of beef bulls in relation to conditions of transfer to slaughter and previous history of the animals1," *J. Anim. Sci.*, vol. 84, no. 6, pp. 1567–1576, Jun. 2006.

[16] E. Huff-Lonergan and S. M. Lonergan, "Mechanisms of water-holding capacity of meat : The role of postmortem biochemical and structural changes," *Meat Sci.*, vol. 71, no. 1, pp. 194–204, Sep. 2005.

[17] Q. Cheng and D.-W. Sun, "Factors affecting the water holding capacity of red meat products: a review of recent research advances.," *Crit. Rev. Food Sci. Nutr.*, vol. 48, no. 2, pp. 137–59, Feb. 2008.

[18] J. Fujii, K. Orsu, F. Zorzato, S. D. E. Leon, V. K. Khanna, J. E. Weiler, P. J. O. Brien, and D. H. Maclennant, "Identification of a Mutation in Porcine Ryanodine Receptor Associated with Malignant Hyperthermia," *Science (80-.).*, vol. 253, pp. 448–451, 1990.

[19] N. Jama, V. Muchenje, M. Chimonyo, P. E. Strydom, K. Dzama, and J. G. Raats, "Cooking loss components of beef from Nguni , Bonsmara and Angus steers," *African J. Agric. Research*, vol. 3, no. June, pp. 416–420, 2008.

[20] J. T. Sawyer, J. K. Apple, Z. B. Johnson, R. T. Baublits, and J. W. S. Yancey, "Fresh and cooked color of dark-cutting beef can be altered by post-rigor enhancement with lactic acid," *Meat Sci.*, vol. 83, no. 2, pp. 263–270, Oct. 2009.

[21] J. T. Sawyer, J. K. Apple, and Z. B. Johnson, "The impact of lactic acid concentration and sodium chloride on pH, water-holding capacity, and cooked color of injection-enhanced dark-cutting beef," *Meat Sci.*, vol. 79, no. 2, pp. 317–325, Jun. 2008.

[22] F. J. Smulders, B. B. Marsh, D. R. Swartz, R. L. Russell, and M. E. Hoenecke, "Beef tenderness and sarcomere length.," *Meat Sci.*, vol. 28, no. 4, pp. 349–63, Jan. 1990.

[23] B. B. Marsh, J. V Lochner, G. Takahashi, and D. D. Kragness, "Effects of early post-mortem pH and temperature on beef tenderness.," *Meat Sci.*, vol. 5, no. 6, pp. 479–83, Nov. 1981.

[24] R. E. Klont, V. M. H. Barnier, A. van Dijk, F. J. M. Smulders, A. H. Hoving-Bolink, B. Hulsegge, and G. Eikolenboom, "Effects of rate of pH fall , time of deboning , aging period , and interaction on veal quality characteristics," *J. Anim. Sci.*, vol. 78, no. 7, pp. 1845–1851, 2000.