J.Food Pharm.Sci. 2 (2014) 46-51

Journal of Food and Pharmaceutical Sciences

Available online at jfoodphamsci.com

Research Article

Protein Profilesof Beef (*Bos indicus*), Pork (*Sus domesticus*),and SausagesBy Using SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) Method

Zilhadia*, Ofa Suzanti Betha, and Charisatul Ummi

SyarifHidayatullah Islamic State University, Jakarta, Indonesia

ARTICLE INFO

Received 18/10/2012
Received in revised form 02/02/2013
Accepted 02/02/2013
Available online 30/07/2013

ABSTRACT

A research has been done to analyze the protein profile in fresh beef, fresh pork, and 10 beef sausage by using SDS PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) with 2 plate gel electrophoresis. From this research, we found several protein bands that become distinctive protein bands. On raw beef protein we found three bands that are not found in pork. They are protein band with molecular weight (MW) of 144,54 kDa, 81,28 kDa and 58,88 kDa respectively. On the raw pork, we found 5 protein bands that are not found in raw beef, namely protein bands with MW 154,88 kDa; 146,55 kDa; 83,18 kDa; 69,18 kDa and 61,66 kDa. There is a band on pork protein found on the second plate on MW gel is 69,18 kDa. Whereas in 10 samples of beef sausages we did not found any specific protein bands. This is presumably due to the difference in manufacturing process performed by the manufacturer.

Keywords : pork, beef, protein, electrophoresis.

1. Introduction

Consumption of ranch products including meat was raising fast in east asia and south east asia for the last ten years, mainly since 1980(FAO, 2009). In Indonesia, meat comsumption raised from 20.07 kkal per day to 44.71 kkal per day since 2002 until 2011 (BPS, 2011). The type of ranch products that were comsumed by Indonesian were varied from beef, lamb, sheep, chicken, horse and pork (BPS, 2011). According to data from Central Agency of Statistics (2011), the most consumable product in Indonesia is beef if compared to lamb, sheep, chicken, horse and pork.

Beef and its refined products have opportunity to be contaminated with other ranch such as pork. For instance, a case that happened in 2009. In this case, it was found that 5 dried beef brands were contaminated by pork and one of the brand was already had halal certification. (Tribune, 2009).

*Corresponding author: zilh60@yahoo.com

Protein was the major component in meat besides water. Meat consists of 19% of protein. The protein component of meat could be one of parameters that used to identify the caracteristic been of the meat. One of the choice methodwhich is easy, cheap and prevail to determine the protein in meat was electrophoresis SDS PAGE (Sodium Dodecy Sulphate Poliacrilamide Gel Elektroforesis). By using this method, we could provide protein profile of the sample according to the molecular weight (MW). This method could be used for refined product of meat (Franks, 1993). Hermanto et al (2009) had conducted the research using electrophoresis method to describe the protein profileof beef sausages, pork and beef. This research had provided the result that there were 3 protein bands that could be the different proteins in fresh beef compared to fresh pork. There were proteins at Rf of 0,29; 0,71 and 0,88 with MW respectively 89,2 kDa, 36,4 kDa and 25,3 kDa respectively. The spesific band for beef, pork and their refined products could be at protein with MW 45,1 kDa

for beef sausages and 69 kDa for pork sausages (Hermanto, 2009).

The similar research also has been conducted by Roswiem *et al* (2010) with different samples. The aim of the research was to find spesific proteins in refined pork products. Roswiem et al 2010, found that refined products showed protein with MW of 85 kDa at Rf of 0.21 (Roswiem *et al*, 2010). In order to develop this study, we conducted the reaserch to identify protein profile of 10 brand sausages compared to beef and pork.

2. Materials and Methods

2.1. Sampling

Samples were collected from 10 different brand sausages from traditional market at Ciputat. Fresh pork and beef were taken from lokal supermarket.

2.2. Protein Quantitative Analysis

Protein quantitative analysis were done by Lowry method (Lowry, 1959).

2.3. Analysis of Protein Profile UsingElectrophoreris (SDS PAGE)

2.3.1. SampelPreparation.

All of sausages, fresh beef and fresh pork were separated manually from unnecessary tissues, such as fat. Ten grams of mince samples and fifty mL of 0,01 M PBS with 0,5M NaCl pH 7,2 were mixed for 5 minutes with blender.The mixtures was homogenated with vortex for 2 minutes, and incubated at 4°C for 2 hours. After 2 hours, it was sentrifugedat 5000 rpm,at 4°C for 30 minutes. The supernatant was separated and kept at -20°C.

Sampel sausages were preparated in 2 different ways. First preparation was stated in firts paraghraph. Second preparation, all of sausages were grinded until being soft and then heated at 100°C for 30 minutes, cooled down into room temperature. Furthermore 2 times quantity of PBS-NaClwas added to the samples. The mixture was homogenized by vortex for 2 minutes, then incubated at 4°C for 2 hours. After being incubated, the mixture was sentrifugedat 5000 rpm,at 4°C for 30 minutes. Supernatant waskept in -20°C (Hsieh *et al*, 2003)

2.3.2. Gel Electrophoresis Preparation

Stacking gel and separating gel were prepared in concentration of 5% and 12%.Running gel was prepared by mixing 3.4 mL of aquabidest, 4 mL of,acrylamide solution 30%, 2.5 mL of tris buffer HCl pH 8.8, 0.1 mL of10% SDS, 0.1 mL of ammonium persulfat10% and 0.01 mL of TEMED. The mixturewas shaked gently to homogenize it. Liquid running gel was poured into gel until mark. Then, aquadest was added to end of the gel print.

After gel was ready, aquadest was replaced by stacking gel. Stacking gel was prepared by mixing 2.85 mL of aquabidest, 0.85 mL 30%acrylamide solution, 1.25 mL tris buffer HCl pH 6.8, 0.05 mL 10%SDS, 0,05 mL 10%ammonium persulphate and 0.005 mL TEMED. The mixture was shake gently to homogenize.The comd was inserted into the liquid stacking gel.

The running gel buffer was trisbuffer (hydroxymethylaminomethane), SDS (sodium dodesilsulfat) andglisin. (Hames, 1998). Before the samples were running, they were mixed (1:1) for fresh pork and beef, (1:2.5) for sausages, with sample buffer by vortex. The sample buffer was consisted of SDS, gliserol 50% , Bromphenol blue 0.1%, and tris-HCl 1 M, in aquadest. The mixtures were heatedat 100°C for 10 minutes, and were directly cooled down with ice. Marker was mixed with sample buffer (1:20). Five microliters of sample were used for electrophoresis, except sausages sample 12 µL. Electrophoresiswas run at 120 volts, 40 mA for about 2 hours.(Hames, 1998).

The gel was stained with the mixture of 100 mL of Coomassie blueR-250, acetic acid, methanol, andaquadest. Then, it was let overnightanddestainedwith the mixture of methanol, acetic acid, aquadest (1:3:6) for 2 hours. Furthermore it was destained once again until the blue bands appeared clearly.(Hames, 1998)

Molecular weight of protein was counted from calibration curveplotting electrophoretic mobility (Rf) against logarithm of molecular weight. Rf was determined from distance of band (cm) divided with distance of sample migration (cm).

3. Result and Discussion

3.1. Protein Quantification.

The result of protein quantification by lowry method can be seenin Table 1.

Table 1. Quantification of protein concentration of freshpork, beef and 10 brand of sausages.

Sample	Protein Concentration (µg/ml).		
	1	2	Average value
Fresh beef	2353	2353	**2353**
Fresh pork	933	1173	**1053**
Sausage brand 1	893	883	**888**
Sausage brand 2	1313	1323	**1318**
Sausage brand 3	783	753	**768**
Sausage brand 4	1303	1223	**1263**
Sausage brand 5	413	393	**403**
Sausage brand 6	1253	1123	**1188**
Sausage brand 7	703	773	**738**
Sausage brand 8	413	613	**513**
Sausage brand 9	1503	1663	**1583**
Sausage brand 10	1053	943	**998**

From the data, we could read that the concentration of protein from each of sausage was varied. This variability may be caused by the process of sausage making, such as crushing, kyuring, cooling, cooking and drying or smoking (Sutaryo, 2004).

3.2. Protein Profiles of Samples.

Electrophoresis analysis of the samples provided result as seen Figure 1.

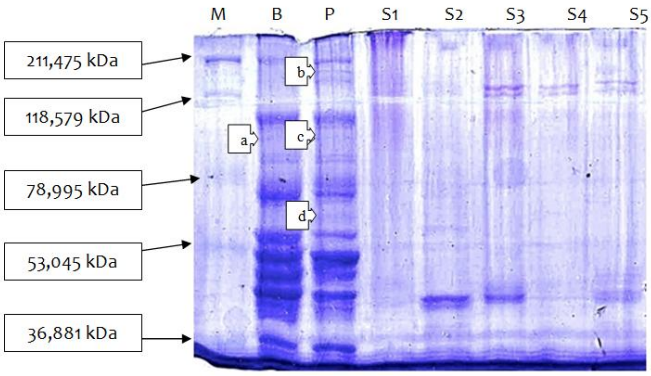

Figure 1.Protein profileof marker and sample 1-5.
M = Marker, B = fresh beef, P = fresh pork, S1= Sausage Brand 1, S2=Sausage Brand 2, S3=Sausage Brand 3, S4=Sausage Brand 4, S5=Sausage Brand 5. Different protein bands : a=81,28 kDa, b=154,88 kDa, c=83,18 kDa, d=69,18 kDa.

From gel 1, it can be determined that the values of Rfand molecular weight (MW). We got 5 protein bandswith MW211,475 kDa, 118,579 kDa, 78,995 kDa, 53,054 kDa and 36,881 kDa. From the logarithm, we can get the Rf value (Table 2).

Tabel2.Values of Log MW and Rfof the marker

No	MW	Log MW	Distance of running (cm)	Band distance (cm)	Rf
1	211.475 kDa	2.33	5	0.4	0.08
2	118.579 kDa	2.07	5	1.0	0.20
3	78.995 kDa	1.90	5	2.2	0.44
4	53.045 kDa	1.82	5	3.3	0.66
5	36.881 kDa	1.57	5	4.7	0.94

From table 2, we can plot the calibration curve (Figure 2).

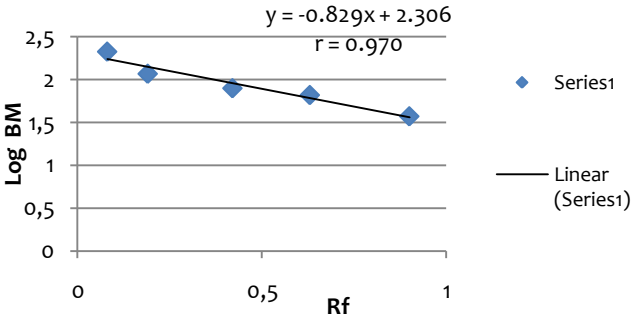

Figure2. Calibration curve gel 1.

From the data of gel 1 (Figure 1) it could be seen that Rf and MW of samples were almost the same. We also could notice that there was one band that couldnot be found in pork, namely at Rf 0.48 with MW 81.28 kDa. In pork, we could recognized 3 bands that couldnot be found in beef, i.eatRf 0.14; 0.46; and 0.56 with MWrespectively 154.88 kDa; 83.18 kDa; and 69.18 kDa respectively.The molecular weight of whole samples could be seen inTables 3.

Table 3.Molecular weight of samples from gel 1

		MW (kDa)						
No	**Band No.**	**Beef**	**Pork**	**Sausage 1**	**Sausage 2**	**Sausage 3**	**Sausage 4**	**Sausage 5**
1	1st Band	173.78	173.78	87.10	151.36	144.54	144.54	151.36
2	2nd Band	154.88	154.88	60.26	144.54	141.25	141.25	144.54
3	3rd Band	125.89	**154.88**	43.65	87.10	87.10	87.10	87.10
4	4th Band	120.23	131.83	36.31	67.61	60.26	57.54	60.26
5	5th Band	112.20	120.23	32.36	60.26	47.86	44.67	52.48
6	6th Band	104.71	112.20	30.90	44.67	46.77	36.31	50.12
7	7th Band	100.00	104.71		43.65	44.67	33.11	47.86
8	8th Band	87.10	100.00		38.90	43.65	32.36	46.77
9	9th Band	**81.28**	87.10		36.31	38.90		44.67
10	10th Band	64.57	**83.18**		33.11	36.31		36.31
11	11th Band	56.23	**69.18**		32.36	33.11		33.11
12	12th Band	50.12	64.57			32.36		32.36
13	13th Band	46.77	56.23					
14	14th Band	43.65	50.12					
15	15th Band	39.81	46.77					
16	16th Band	34.67	43.65					
17	17th Band	33.11	39.81					
18	18th Band		34.67					
19	19th Band		33.11					

Furthermore, the protein profile of branded sausages (S6, S7, S8 and S10) could be seen in gel 2 below (Figure 3).

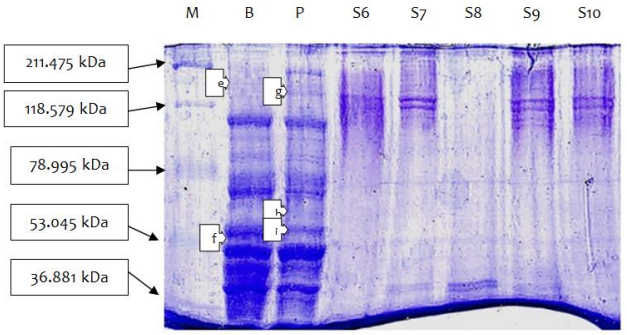

Figure 3.Protein profile of marker and samples
M = Marker, B = Beef, P = Pork, S6 = Sausage Brand 6, S7 = Sausage Brand 7, S8 = Sausage Brand 8, S9 = Sausage Brand 9, S10 = Sausage Brand 10. Different protein bands e = 144.54 kDa, f = 58.88 kDa, g = 146.55 kDa, h = 69.18 kDa, i = 61.66 kDa.

From Gel 2, we heve determined values of Rfand their MW. The result could be seen in Table 4.

Tabel4.Values of Log MW and Rfof marker in gel 2

No	MW	Log MW	Distance of running (cm)	Band distance (cm)	Rf
1	211.475 kDa	2.33	5.2	0.4	0.08
2	118.579 kDa	2.07	5.2	1.1	0.21
3	78.995 kDa	1.9	5.2	2.3	0.44
4	53.045 kDa	1.82	5.2	3.6	0.69
5	36.881 kDa	1.57	5.2	4.8	0.92

From the table 4 we could plot calibration curve as seen below (Figure 4).

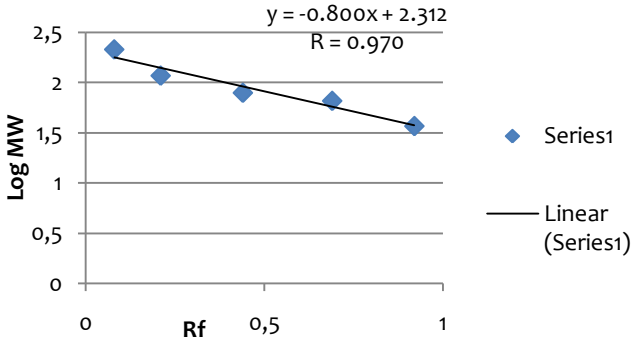

Figure 4. Calibration curve gel 2 deccribing the correlation between Rf and molecular weight

From the calibration curve, we could determine the values of Rf, Log MW, and MW of the sample from gel 2.From gel 2, it could be assumed that majority protein bands from beef and pork hadRf and MW which are relative similar.We could also determine that there were 2 different protein bands between beef and pork. In bands of beef, there were 2 protein bands that couldnot be found in pork. They were atRf 0.19 and 0.67 with MW of 144.54 kDa and 58.88 kDa respectively. In bands of pork, there were 3 protein bandsthat couldnot be found in beef. They were atRf 0.18; 0.60; and 0.65 with MW 146.55 kDa; 69.18 kDa; and 61.66 kDa respectively. The MW of every protein band could be seen in table 5.

Tabel5.Molecular weight of samples from gel 2

No	Band no.	MW (kDa)						
		Beef	Pork	Sausage 6	Sausage 7	Sausage 8	Sausage 9	Sausage 10
1	1st Band	173.78	173,78	144.54	144.54	54.95	144.54	144.54
2	2nd Band	154.88	154,88	138.04	13.,04	43.65	138.04	138.04
3	3rd Band	147.91	147,91	54.95	85.11	39.81	134.90	134.90
4	4th Band	**144.54**	**146.55**		54.95		128.82	128.82
5	5th Band	120.23	120.23		43.65		85.11	85.11
6	6th Band	109.65	109.5		39.81		54.95	54.95
7	7th Band	100,00	100.00				43.65	43.65
8	8th Band	97.72	97.72				39.81	
9	9th Band	85.11	85.11					
10	10th Band	79.43	79.43					
11	11th Band	**58.88**	**69.18**					
12	12th Band	53.70	**61.66**					
13	13th Band	47.86	53.70					
14	14th Band	44.67	47.86					
15	15th Band	41.68	44.67					
16	16th Band	38.9	41.68					
17	17th Band		38.90					

From Gel 1 and Gel 2, we could assume that there was one band, that only could be found in pork band (gel 1 and 2), and it was not found in beef. It was protein with MW 69.18 kDa. This protein band (MW 69.18) wasnot seen in all of the 10 branded sausages. There were a significant protein profile between beef and pork. This differences showed that there was a genetic variety. This could be determined as spesific band for each of the spesies although theyshowed variatively (Nazar, 2007).

CONCLUSION

From protein profile of beef, there were 3 protein bands that could be assumed as different protein bands, because they were not found in pork. They were proteins that hadMW144.54 kDa, 81.28 kDa and 58.88 kDarespectively.

Furthermore from protein profile of pork, there were 5 protein bands that could be assumedas different protein band because they were couldnot be found in beef. They were protein with MW respectively 154.88 kDa; 146.55 kDa; 83.18 kDa; 69.18 kDa and 61.66 kDa.

We couldnot found spesific band in protein profile of 10 branded sausages sausages either beef or pork.

REFERENCES

A.R. Copeland. 1994. *Methods for Protein Analysis*. Chapman and Hall, New York.

Amstrong, F.B. 1995. *Biokimia*. EGC, Jakarta.

Apriyantono, A.,J. Hermiananto, and N. Wahid. 2007. *PedomanProduksiPangan Halal.*Khairul Bayan Press. Jakarta.

BadanPusatStatistik. 2011. *Rata-rata KonsumsiKalori (Kkal) per kabandSehariMenurutKelompokMakanan 1999, 2002-2011.*

Badger MR, Price GD. 1994.The role of carbonic anhydrase in photosynthesis.Annu. Rev. Plant Physiol. *Plant Mol. Bio* vol.45: 369–392.

Basuki, A.A. and Hardjo, 1979.*MinumanBergiziAndBercampurTepungSusuKedelai Dari Jagung*. Balaipenelitian Kimia, Bogor.

Bidlingmeyer,V., Leary, T., and Laskowski, M. 1972. Identity of the Tryptic and ?-Chymotryptic Reactive Sites on Soybean Trypsin Inhibitor (Kunitz).*Biochem.*,v. 11, 3303.

Departemen Agama RI. 2008. *Al-Qur'an and Terjemahnya.* CahayaQur'an :Depok.

Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O. 1995. "A biomarker that identifies senescent human cells in culture and in aging skin in vivo".*Proc. Natl. Acad. Sci. U.S.A.*

Dominiczak M, Baynes J. 2004. *Medical Biochemistry*, 2nd edition.

Dreon M. S., Ituarte S. &Heras H. 2010.*The Role of the Proteinase Inhibitor Ovorubin in Apple Snail Eggs Resembles Plant Embryo Defense against Predation*.

FAO. 2009. *FAOSTAT statistical database*. Rome (available at faostat.fao.org).

Forrest, J.C., E.D. Aberde, H.B. Hendrick, M.D. Judge, R.A. Brandel. 1992. *Principle of Meat Science.* W.H. Freeman and Co. San Fransisco, USA.

Franks, F., 1993.*Protein Biotechnology*. Humana Press. New Jersey.

Habsari, LhoppyYuliaDwi. 2011.*IdentifikasiPolaKhasSpektra Infra Merah Protein Beef AndBabi Rebus MenggunakanMetode Second Derivative (2D.* Tesis.FakultasSains and Teknologi.UIN Malang.

Hammes, B.D. 1998. *Gel Electrophoresis of Proteins*. Oxford University Press. New York.

Hermanto, Sandra. 2009. PerbedaanProfil Protein ProdukOlahan (Sausage) Pork AndSapiHasilAnalisa SDS-PAGE (Sodium DodeclSulphate- Polyacrylamide Gel Electrophoresis).*UIN SyarifHidayatullah*. Jakarta.

Hsieh YH, Sheu SC, Bridgman RC. 1998. Development of Lard in Mixture of body fats of Mutton and Cow by FourierTransform infrared Spectroscopy. *J. Oleo Sci.*, vol 52, No.12, 633-638, 2003.

Hu, H.Y., Du H.N. 2000. Alpha to Beta Structural Transformation of Ovalbumin: Heat and pH Effects.*Journal of Protein Chemistry*.19 (3): 177–183.

Huntington JA, Stein PE (2001) Structure and properties of ovalbumin. *Journal of Chromatography* B 756(1-2): 189-198.

Husni, Elidahanum*et al.* 2007. AnalisaZatPengawetAnd Protein DalamMakananSiapSaji Sausage. FakultasFarmasiUniversitasAndalas. Padang.

Ihsan, Zainoel, Suparno, M. Djono. 2001.*Ensiklopedia Indonesia*.IchtiarBaru – Van Hoeve, Jakarta.

Isgiyanto, Awal. 2009. *TeknikPengambilanSampel*. MitraCendikia Press, Jogjakarta

Jannah, A. 2008.*Tinjauan Kehalalan and AlternatifProduksi.*UIN Press. Malang.

JansonJ.C.. 1998. *Protein Purification*: *Principles, High Resolution Methods, and Application 2nd edition*, John Wiley and Sons Inc. Publication. New York.

Kusnoto. 2003. *Isolasi and karakterisasi protein immunogenik larva stadium II Toxocaracatiisolatlokal.*Tesis. Program PascasarjanaUniversitasAirlangga, Surabaya.

Laemmli, U. 1970. *Cleavage En Structural Proteins During The Assembly Of The Head Of Bacteriophage T_4*. Nature 227:680-685.

Lawrie, R. A. 1991. *Meat Science 4th Edition*. New York: Pergamon Press.

Lawrie, R. A. 1998. *Meat Science 6th Edition*.Terjemahan A. Parakasi and A. Yudha.Universitas Indonesia Press, Jakarta.

Lawrie, R.A. 2003. *IlmuDaging*. Parakkasi, penerjemah.Edisikelima. Jakarta: UI Press. Terjemahandari : Meat Science.

Lowry et al., 1959, *Method of Enzymology*, vol 182, Academic Press, USA.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. & Randall, R.J., 1951. Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem, 193*: 265-275.

Marchello M, Gaerden-Robinson J. 1998. *The Art and Practice of Sausage Making.*North Dakota State University, North Dakota.

Muchtadi, D. 1989. *PetunjukLaboratoriumEvaluasiNilaiGiziPangan.* PusatantarUniversitasPangan and Gizi, IntitutPertanian Bogor, Bogor.

Mudhafier, F. and H. A. F. Wibisono. 2004. *Makanan Halal.* Zakia Press. Jakarta.

Peterson, G.L. 1977. A simplification of the protein assay method of Lowry et.al. which is more generally applicable. *Anal.Biochem*. 83, 346-356.

Purwaningsih, Aning. 2005. Identifikasi Protein Beef and Pork DenganElektroforesis Gel Poliakrilamid-Sodium DodesilSulfat (SDS-PAGE). Thesis Magister IlmuFarmasi. UNAIR Cetral Library.

Revenis ME, Kaliner MA. 1992. Lactoferrin and lysozyme deficiency in airway secretions: association with the development of bronchopulmonary dysplasia.*J.Pediatr.*vol121 (2): 262–70.

Richardson, J.S. 1981. *Anatomy and Taxonomy of Protein Structure.*Advances in Protein Chemistry34: 167–339.

Roswiem, A.P., Kustaman E., Permana G. 2010. *Identification of protein marker of pork in processed meat products.* Biochemistry Department, Bogor Agricultural University. Bogor.

Soeparno. 1998. *Ilmu and TeknologiDaging*. Edisiketiga. Gajah Mada University Press, Yogyakarta.

Yasaguna Stryer L. 1995. *Biokimia terjemahan dari Biochemistry*, oleh Sadikin, M, *et al.*, 2000. Edisi 4. EGC.

Suryaningsih, L. 1997. PengaruhDagingTetelan, Samcan, and PunukSapiBangsa Australian Commercial Cross TerhadapPembentukan Sausage.Thesis.PascaSarjanaPrograme. IPB. Bogor.

Suryaningsih, Lilis. 2006.*PengaruhJenisDaging, Antidenaturan, and NatriumTripolifosfatPadaNikumiTerhadapKarakteristikProdukDagingOlahan.*Disertasi.IntitutPertanian Bogor. Bogor.

Susanto, Edy. 2004. Karakterisasi Fraksi Protein Bakso Babi Dengan Menggunakan SDS-PAGE. Skripsi Program Study Teknologi Hasil Ternak Fakultas Peternakan. UB Central Library.

Sutaryo, Mulyani S. 2004. *Pengetahuan Bahan Olahan Hasil Ternak and Standar Nasional Indonesia (SNI)*. Universitas Diponegoro.

Tribune, Borneo. 2009. *Awas, DendengBabiBerlabel Halal Beredar*. Jakarta.

White, S. L. *et al.* 1998. Myosin heavy isoform in smooth muscle cells in corpus cavernosum penis.*Am J Physiol* 275 (2 Pt 1): C581-9.

Wijaya S.K.S, Rohman L. 2005. Fraksinasi and Karakterisasi Protein UtamaBijiKedelai.Fakultas MIPA UniversitasJember.

Winarno, F.G. 2002. *Kimia Pangan and Gizi*. Cetakan ke-7. PT. Gramedia Pustaka Utama. Jakarta.

Yoshimura K, Toibana A, Nakahama K. 1988. Human lysozyme: sequencing of a cDNA, and expression and secretion by Saccharomyces cerevisiae. *Biochem.Biophys. Res. Commun.*vol150 (2): 794–801.

Yuwono, T. 2005. *BiologiMolekular*. Erlangga. Jakarta.