Protein Quality of Fermented Beef by *Lactobacillus plantarum* 1B1

I. I. Arief, R.R.A. Maheswari, T. Suryati, & N. Kurniawati*

Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor, 16680, Indonesia
**e-mail: irma_isnafia@yahoo.com*

Abstract

This study was conducted to investigate the proteolytic activity and protein characteristics of fermented beef. The effect of different mechanical treatments on beef, (sliced and ground beef) was compared. The contents of amino acid were analyzed using HPLC (high performance liquid chromatography) method. The results indicated that Lactobacillus plantarum 1B1, isolated from beef, had better proteolytic activity on sliced fermented beef if compared to ground fermented beef, and improved crude protein on sliced fermented beef. Sliced and groud fermented beef contained 15 components of amino acids which consisted of threonine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine and lysine as essential amino acid and aspartate, glutamate, serine, histidine, glycine, alanine and arginine as non-essential amino acids. Leucine was the highest among essential amino acids content and glutamate was the highest among non-essential amino acids content. The SDS PAGE profiles showed the beef protein molecule weight range of 94.71 - 13.43 kD. The numbers of protein band of fresh beef, sliced and ground fermented beef were 12, 7 and 7. In conclusion, fermentation process on raw beef by Lactobacillus plantarum could improve amino acid availability because of its proteolytic activity on beef protein.

Key words: fermented beef, Lactobacillus plantarum, protein quality

Introduction

Dark cutting beef is often known as dark, firm and dry (DFD) beef. It occurrs as a result of depletion of muscle glycogen reserves prior to slaughter. Glycogen is used as an energy source for muscle contraction and relaxation. Lactic acid is a by-product of glycogen utilization by the muscle when energy is produced in a stress event. In Indonesia, DFD beef frequently happen in slaughter houses due to unstandardized slaughtering practice procedure. Several studies have shown that quality improvement of DFD beef quality could be improved through fermentation process by lactic acid bacteria, such as *Lactobacillus plantarum (L. plantarum)*. The fermentation might extend the shelf life and appearance of meat products. The addition of starter *L. plantarum* 1B1 in beef fermentation is expected to perform on the proteolytic activity of beef. The aim of this research was to investigate the quality of protein of fermented beef by *L. plantarum* 1B1.

Materials and Methods

L. plantarum 1B1 was obtained from Laboratory of animal product and technology, Faculty of Animal Science, Bogor Agricultural University. The strain was isolated from local beef and was identified using 16S rRNA gene sequencing (Arief, 2011).

The treatment used in this experiment was type of beef fermentation. DFD beef (pH value : 6.2) was used in this experiment. Sliced and grinded beef with inoculated by *L. plantarum* 1B1 for fermentation. Starter culture of 10% w/w *L. plantarum* 1B1 were inoculated into fermentation process. The sliced beef was arranged in a baking dish coated with aluminum foil and covered with plastic top surface. The ground beef was put in a plastic bag, flaked with a thickness of approximately 3 mm, packed and arranged in a baking dish. These treated meat groups were then incubated for 24 h at 37°C, smoked for nine hours at a temperature of 30^{o}C.

Variables observed included the content of crude protein (AOAC, 2005), amino acid composition using HPLC (AOAC, 2005), proteolytic activity of *L. plantarum* 1B1 (Bergmeyer and Gawehn (1983), and SDS-PAGE electrophoresis of protein from fermented beef (Fadda *et al.*, 1998).

The experiment was set up in a completely randomized design with three replication. The data were analyzed using ANOVA, and if found any differences, they were further tested using Tukey test (Steel and Torie, 1995). Amino acid composition and SDS Page electrophoresis were described by descriptive analysis.

Results and Discussion

Crude protein of fermented beef

Proteins in food generally determine the quality of a product primarily derived from meat. Isolates of *L. plantarum* 1B1 used to achieve optimization of beef fermentation with the assumption that these bacteria would be more adaptive to beef. Crude protein and proteolytic activity of of fermented beef is presented in Table 1.

The crude protein of fresh DFD beef was 70.72 % db. If compared with fresh beef, fermentation process could increase crude protein content of fermented beef, because protein from cell wall of *L. plantarum* affected the crude protein content of fermented beef. Crude protein content of sliced fermented beef was higher than

Table 1. Crude protein and proteolytic activity of fermented beef

Parameters	Sliced fermented beef	Ground fermented beef
Crude protein (% db)	81.77 ± 2.46[a]	72.13 ± 2.84[b]
Proteolytic activity (U/g)	0.11 ± 0.04	0.09 ± 0.03

Different superscript in the same line means significantly different (P<0.05)

that of ground fermented beef. Grinding lossed sarcoplasmic protein of beef it could reduce total protein content of ground beef.

Proteolytic activity of fresh beef was not detected (0), it means that natural proteolytic enzyme of beef was not active. Increased proteolytic activity occurred after the fermentation took place in fermented beef. This proved that the isolates of *L. plantarum* 1B1 had proteolytic activity on beef protein. *L. plantarum* 1B1 had extracelullar protease enzyme in the de_man Rogosa Sharp broth 0.041 U/ml. According to Fadda *et al.* (1998) that some strains of *Lactobacillus* bacteria such as *L. casei* and *L. plantarum* can produce proteolytic enzymes in fermented meats.

Molecular weight protein of fermented beef

Fermentation could degrade beef protein, caused by proteolytic activity of L. plantarum 1B1, as shown in Figure 1.

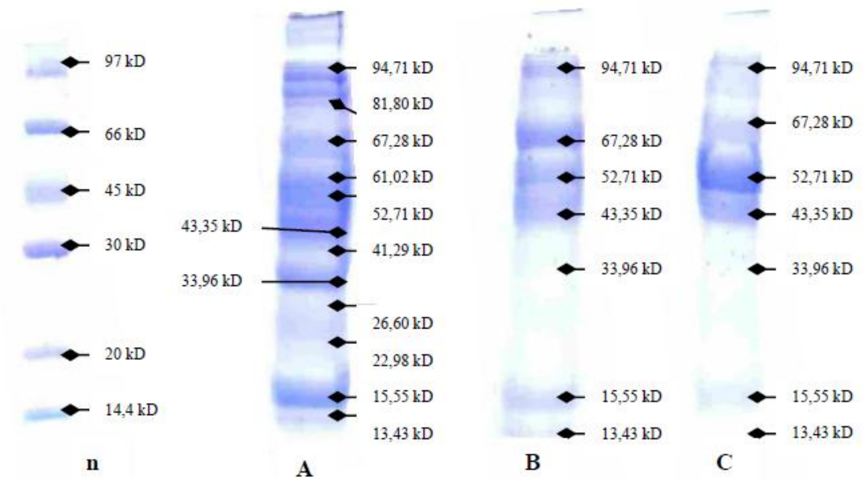

Figure 1. SDS-PAGE electrophoresis bands of beef protein (n= marker; A= fresh DFD beef; B=sliced fermented beef; C=grinded fermented beef).

This type of protein bands detected in fermented beef were closely related to the level of functional protein damage. Reduction in protein occurred in a protein bands with a molecular weight of 81.80 kD, 61.02 kD, 41.29 kD, 26.60 kD and 22.98 kD.

These were due to faulty conformation of the protein after fermentation and protein structure changes into a more simple peptides and amino acids. Fadda *et al.* (1999) showed the degradation of the protein bands because of proteolytic activity of *L. plantarum* that occured in protein with molecular weight 200 kD (myosin), 66 kD and 43 kD (actin). In this research, molecular weight protein bands were (35 to 50 kD) hydrolyzed, whereas myosin and actin partially hydrolyzed after the inoculation of *L. plantarum.* These results further reinforce the fact the degradation of protein beef into simpler form after fermentation.

Amino acid composition of fermented beef

Amino acid composition of sliced fermented beef was different than ground fermented beef by descriptive analysis. Nonessential amino acids were detected by HPLC analysis of the aspartic acid, glutamic acid, serine, histidin, glycine, arginine and alanin, while the essential amino acids detected were treonin, tyrosin, methionin, valin, phenilalanin, isoleusin, leusin and lysin. The quantitative results can be seen in Table 2.

The results of HPLC analysis showed an increase in amino acid content after fermentation. This occurred as a result of the proteolytic enzymes of *L.plantarum*

Table 2. Amino acid composition

Amino acid	Percentage of amino acid content (% w/w)			Increasing of amino acid in fermented beef compared than fresh beef (%)	
	Fresh DFD beef	Sliced fermented beef	Grinded fermented beef	Sliced fermented beef	Grinded fermented beef
Aspartic acid	1.66	2.93	2.56	76.50	54.22
Glutamic acid	3.42	5.64	4.79	64.91	40.06
Serin	0.79	1.31	1.09	65.82	37.97
Histidin	0.70	1.02	0.91	45.71	30.00
Glysin	0.97	1.47	1.41	51.55	45,36
Threonin	0.98	1.58	1.27	61.22	29.59
Arginin	1.57	2.18	1.98	38.85	26.11
Alanin	1.08	1.79	1.60	65.74	48.15
Tyrosin	0.67	0.99	0.79	47.46	17.91
Methionin	0.47	0.72	0.36	53.19	-23.40
Valin	0.90	1.46	1.48	62.22	64.44
Phenilalanin	0.73	1.16	1.07	58.90	46.58
I – leusin	0.90	1.50	1.29	66.67	43.33
Leusin	1.48	2.40	2.09	62.16	41.22
Lysin	1.45	1.90	2.08	31.03	43.45

1B1. Fermentation by *L plantarum* 1B1 could increase amino acid percentage of both fermented beef if compared to fresh DFD beef. Lactic acid produced from the fermentation process caused a decrease in muscle pH beef. This decline continued until the pH reached its optimum value. It then activated proteolytic enzymes of *L.plantarum* 1B1. The enzyme degraded Z-line on myofilamen, eliminating cross-bridge of actomyosin, and amino acid in actomyiosin could be released. In general, amino acid percentages of sliced fermented beef was higher than ground fermented beef. It was supported by crude protein of sliced fermented beef, that was higher than ground fermented beef (Table 1).

Conclusions

Fermentation by *L. plantarum* 1B1 on beef could increase crude protein of beef, while sliced fermented beef had a higher protein content than ground fermented beef. *L. plantarum* 1B1 had proteolytic activity due to fermentation. Fermentation could degrade protein structure, as shown by reduction of protein bands with a molecular weight of 81.80 kD, 61.02 kD, 41.29 kD, 26.60 kD and 22.98 kD. Fermentation by *L plantarum* 1B1 could increase amino acid percentage of both fermented beef. Amino acid percentages of sliced fermented beef was higher than ground fermented beef. In general, sliced fermented beef had better protein quality than ground fermented beef.

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References

AOAC. 2005. Official Methods of Analysis of AOAC International. 18th ed. Assoc. Off. Anal. Chem., Arlington.

Arief, II. 2011. Characterization of Indigenous Lactic Acid Bacteria from Beef as Probiotic and Identification by 16S rRNA gene sequencing. Dissertation. Bogor Agricultural University.

Bergmeyer, H. U. and Karlfield Gawehn. 1983. Methods of Enzymatic Analysis. Second English Edition. Verlag Chemie International. Deerfield Beach, Florida.

Fadda, S., G. Vignolo, A. P. R Holgado and G. Oliver. 1998. Proteolytic activity of *Lactobacillus* strains isolated from dry fermented sausages on muscle sarcoplasmic proteins. J. Meat Science (49) 1:11-18.

Fadda, S., Y. Sanz, G. Vignolo, M. C. Aristoy, G. Oliver and F. Toldrá. 1999.

Characterization of Muscle Sarcoplasmic and Myofibrillar Protein Hydrolysis Caused by *Lactobacillus plantarum*. Applied and Environmental Microbiology. 65: 3540-3546.

Steel, R.G.D. and J. H. Torrie. 1995. Principles and Procedure of Statistics. Mc Graw Hill Book Co. Inc., New York.