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The Effectiveness of Soluble Protein Extractability Under the Effect of pH, Molarity and Type of Buffers of Three Different Major Skeletal Muscles in Cattle

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Abstract: The present study was conducted in an attempt to study the effects of the type, pH and molarity of extraction buffer on protein extractability from beef *Longissimus dorsi* (LD), *Supraspinatus* (SS) and *Semitendinosus* (ST) muscles. All muscle samples were dissected out from carcass immediately after slaughter and subjected to extractions using freshly prepared buffers of different buffer type (Tris Base and Tris HCl), pH (8.3 and 7.5) and molarity (100 and 20 mM). Following extraction, the total extractable protein concentration was determined by Bradford assay. The results exhibited significant ($p<0.05$) effects of muscle type X buffer interaction on extracted total protein concentration. The statistical analysis also revealed interaction between type of buffer, pH and molarity significantly ($p<0.01$), affected the extracted protein concentration. Based on the results, optimal buffer suggested to use for muscle protein extraction is Tris-Base with pH 8.3 and 100 mM. The present study demonstrated that the extractability of skeletal muscle protein was significantly influenced by the type, molarities and pH of the extraction buffers used.

Key words: Protein extraction, buffers, pH, molarity

INTRODUCTION

Cells must be lysed in order to extract soluble protein which, the portion of crude protein that goes into the solution when mixed in a buffered solution in laboratory conditions. Several techniques can be used for the extraction of protein and these include enzyme digestion, osmotic shock lysis, hand homogenization, blade homogenization and French press (Hafiz, 2005). Success of the cell disruption depends on a number of factors such as choice of buffer, presence of protein inhibitors and the osmolarity of the resuspension buffer (Hafiz, 2005).

A buffer is a substance which by its presence in solution increases the amount of acid or alkali that must be added to cause unit change in pH. Proteins are extremely heterogeneous biological macromolecules. Their properties can be severely affected by small changes in hydrogen ion concentration. Some proteins will start to precipitate when , the pH approaches their isoelectric point (pI) (Veiseth and Koohmaraei, 2001). Therefore, in order to

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ensure reproducible results, it is important to maintain the protein solution at the constant pH. It has been observed that partially neutralized solutions of weak acid or weak base are resistant to pH changes on the addition of small amounts of strong acid or strong base. This is known as buffering (Perrin and Dempsey, 1974). Buffer solutions consist of a weak acid and its salt with a strong base, or of a weak base and its salt with a strong acid. Once, an optimal pH has been determined, different buffers within the same pH range may also be examined for specific buffer effects. Buffers at 50 mM concentration are usually non-toxic to cells (Ferguson *et al*., 1980).

At any stage during protein extraction, proteolysis can be a major problem. It may generate degraded proteins. The result may effect in conclusions about the nature of the protein such as activity, size and structure (Hafiz, 2005).

Skeletal muscle is composed of different types of fibers which are the complex of multiple myofibril proteins: Slow Oxidative (SO), fast glycolytic oxidative and fast glycolytic (Takemasa *et al*., 2004; Oe *et al*., 2007). McLoughlin (1968) using Landrace and large white pigs, demonstrated no breed difference in the inherent extractability of the muscle protein before, differences in rate of postmortem glycolysis could induce changes in protein solubility. Different muscles have their own different postmortem glycolytic potential rate and also, ATPase activities and certain other properties as proved by Lebeveda *et al*. (1977). This too may affect the soluble protein extractability.

This study was conducted to study the effects of the type, pH and molarity of extraction buffer on protein extractability from beef *Longissimus dorsi* (LD), *Supraspinatus* (SS) and *Semitendinosus* (ST) muscles. The study aimed to determine the optimal conditions of the extraction buffer for highest protein yields. It was hypothesized that the effect of the different types of extraction buffer on the extractability of protein are influence by the type of muscle.

MATERIALS AND METHODS

Slaughter and Sample Preparation

Four of Malaysian local breed, Kedah-Kelantan bulls at 250-300 kg b.wt. were slaughtered according to the Halal slaughter procedure as outlined in the MS 1500:2004 Halal Food-Production, Preparation, Handling and Storage-General Guidelines (Department of Standards Malaysia, 2004). Immediately, post evisceration, each individual muscle was located and consistently dissected out (50 g) from the left side of each carcass. A total number of 12 samples from the LD, SS and ST muscles were collected from carcasses. The sampling processes were conducted at Slaughter house at Department of Animal Science, Faculty of Agricultural, Universiti Putra Malaysia (UPM) between April to May 2008. The selection of the LD, SS and ST muscles for the study was based on their previously reported metabolite and contractile characteristic as well as fiber type composition (Sazili *et al*., 2005). The different types of muscles were chosen to compare the protein extractability between fast (ST), slow (SS) and medium (LD) types of muscle. Each muscle was divided into three parts, to serve as replications of each sample. All samples were trimmed of any visible fat and connective tissue and blast frozen in liquid nitrogen for 5-10 sec to stop any further biochemical changes. Samples were then stored at -80°C until subsequent analysis.

Extraction

Samples were initially pulvarised using liquid nitrogen and using mortar and pastel crushing process controlled under chilled condition. The extraction was conducted at Biotechnology Lab., at Halal Product Research Institute, UPM. Eight different types of

extraction buffer are used in this study. The buffers were: H^1 (100 mM Tris/HCl, 25 mM EDTA, adjusted with NaOH to pH 7.5), H^2 (20 mM Tris/HCl, 25 mM EDTA, adjusted with NaOH to pH 7.5), H^3 (100 mM Tris/HCl, 25 mM EDTA, adjusted with NaOH to pH 8.3), H^4 (20 mM Tris/HCl, 25 mM EDTA, adjusted with NaOH to pH 8.3), B^1 (100 mM Tris/Base, 25 mM EDTA, adjusted with HCl to pH 7.5), B^2 (20 mM Tris/Base, 25 mM EDTA, adjusted with HCl to pH 7.5), B^3 (100 mM Tris/Base, 25 mM EDTA, adjusted with HCl to pH 8.3), B^4 (20 mM Tris/Base, 25 mM EDTA, adjusted with Hcl to pH 7.5). The protease inhibitor (840 mM of AEBSF) from SIGMA, USA was added into all 8 types of prepared buffers just before the homogenization. Three volumes (v/w) of ice cold buffer were added into 1 g of crushed muscle fiber and the latter homogenized using the Ultra-Turrax homogenizer at 9500 rpm for 10-20 sec. The homogenates were centrifuged at 21,767xg for 15 min. The resulted supernatants were collected for the determination of total protein concentration.

Protein Concentrations

The protein concentrations were determined using Total Protein Kit, Micro from Sigma, USA. This experiment was conducted at Nutrition Lab., at Department of Animal Science, Faculty of Agricultural, UPM. A series of labeled test tube for Blank (0.85% Sodium Cloride), Standards (Human albumin, 0.3 mg mL^{-1}, in saline with 0.1% sodium azide as a preservative) and samples were set up. Samples were diluted to 1:7 with deionized water before adding into working dye which consisted of one volume of mixed Brilliant Blue G (0.35 mg mL^{-1}) in phosphoric acid and methanol with four volume of deionized water. Samples and working dye were mixed thoroughly in disposable plastic test tubes. After approximately 2 min, the solutions were transferred into separate cuvettes. The absorbance (A) of standard and samples were read at 595 nm wavelength. The protein concentrations were calculated based on the following equation:

$$[\text{Protein}]\ (\text{mg mL}^{-1}) = \frac{\text{A(Sample)} \times \text{Concentration of standard}}{\text{A(Standard)}}$$

Statistical Analysis

The data generated from this study were analysed using SAS statistical software (Version 6.12, 1998). The differences between means were analysed using Duncan's Multiple Range Test. Significance of difference were analysed at a confidence level of 95% ($p<0.05$). The interactions between each factor were analysed using the ANOVA procedure.

RESULTS AND DISCUSSION

The extractability of soluble protein from bovine major skeletal muscle was remarkably different for the various extraction buffers used. The results of protein concentration extracted with Tris-HCl were significantly lower than soluble protein that extracted with Tris-Base except for buffer with pH 7.5 and 100 mM (Table 2). The effect of buffer type ($p<0.001$) on protein extractability seems to be influenced by the pH of the extraction buffer (Table 1). In addition, effect of buffer type also influenced by molarity of the buffer itself. The p-values for interaction between type of buffer, pH and molarity is lower than 0.05. It also have been proved that effect of pH of extraction buffer is influenced by concentration of the buffer ($p<0.05$) (Table 1).

Higher concentration of soluble protein extracted with buffer with higher pH than buffer with lower pH. This result also have been reported by Veiseth and Koomaraei (2001), that low

Table 1: Analysis of variance showing the effects of buffer type, pH and molarity of buffer muscle type in protein extractability

Effects	df	p-value	Interactions	df	p-value
Buffer	1	<.0001	Buffer*pH*Molarity*Muscle	2	0.3407
pH	1	<.0001	Buffer*pH*Molarity	1	0.0011
Molarity	1	<.0001	Buffer*Molarity*Muscle	2	0.1511
Muscle	2	0.0003	Buffer*pH*Muscle	2	0.6334
			pH*Molarity*Muscle	2	0.3550
			Buffer*pH	1	<0.0001
			Buffer*Molarity	1	0.5085
			Buffer*Muscle	2	0.0251
			pH*Molarity	1	<0.0001
			pH*Muscle	2	0.4839
			Molarity*Muscle	2	0.7292

Table 2: Mean of soluble protein (μg μL^{-1}) extracted from beef ST, LD and SS

			Muscle			
Buffer	pH	Molarity (mM)	ST	LD	SS	Overall mean*
Tris-Base	8.3	100	7.28	7.45	6.58	7.104^{a}
		20	6.35	6.96	6.75	6.685^{a,b}
	7.5	100	4.27	4.73	5.13	4.711^{c}
		20	6.86	6.73	5.86	6.484^{b}
Tris-HCl	8.3	100	3.75	3.78	4.57	4.029^{d}
		20	4.18	4.42	5.01	4.537^{c}
	7.5	100	3.61	3.90	4.03	3.846^{d}
		20	5.08	4.48	5.47	5.008^{c}

*Means with different superscripts are significantly ($p<0.05$) different.

Table 3: Effect of interactions between types of buffers and muscle soluble protein extractability

Buffer	Muscle	Mean (μg μL^{-1})
TH	SS	4.300^{c,d}
	ST	4.002^{d}
	LD	4.77^{c}
TB	SS	5.877^{b}
	ST	6.342^{a,b}
	LD	6.517^{a}

*Means with different superscripts are significantly ($p<0.05$) different

pH of buffer may cause the soluble protein to precipitate as reported based on reduced of protein activity. This also has been proved by Saffle and Galbreath (1964) when they reported that as pH increased, the amount of protein extracted also increased.

Table 2 showed that, there was a significant different in extractable protein between Tris-Base buffer with higher and lower pH ($p<0.05$) but, there was no significant different for Tris-HCl buffer in the same condition. The extractability of total protein was significantly elevated when, the pH of 20 mM Tris-HCl buffer was pre adjusted to 7.5 prior to the extraction. However, if the extraction buffers same in the type and pH but differ in concentration (pH 7.5, 100 mM), the extractability of soluble protein is lower. Both buffer (pH 7.5, 100 mM and pH 8.3, 100 mM), showed there are no significant different of protein extraction from three types of muscles which are SS, ST and LD. The extractability of protein using buffer Tris-Base with pH 7.5 and 100 mM is lower than using Tris-Base, 20 mM and pH 7.5 (Table 2). Present study showed that highest protein extractability can be achieve by using Tris-Base with pH 8.3 and 100 mM of concentration with the mean of soluble protein concentration is 7.104 μg μL^{-1}.

Type of muscle also influenced the effect of type of extraction buffer in protein extraction ($p<0.05$). Based on Table 3, LD was found to have the highest protein extractability followed by SS and ST for Tris-HCl and ST and SS for buffer Tris-Base.

CONCLUSION

A better extraction buffer for soluble protein in bovine major skeletal buffer has been described in this study. The results of this experiment have been concluded that there are differences in protein extractability based on soluble protein concentration ($\mu g\ \mu L^{-1}$) between type, pH and concentration of buffer and types of muscle. Muscle has effect on protein extractability but it is influenced by type of buffer. Protein extraction is also affected by pH of the buffer depending on the type and concentration used. These findings will help our main project in quantifying the protein interest in further analysis.

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