

Effect of pH and Salt Concentration on Protein Solubility of Slaughtered and Non-Slaughtered Broiler Chicken Meat

(Kesan pH dan Kepekatan Garam ke atas Keterlarutan Protein Ayam Daging yang Disembelih dan tidak Disembelih)

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ABSTRACT

This study examined the influence of pH and salt concentration on the protein solubility of slaughtered and non-slaughtered broiler chicken meat. Three types of salt (NaCl , Na_2SO_4 , and $(\text{NH}_4)_2\text{SO}_4$), five different pH levels (5.0, 6.0, 7.0, 8.0 and 9.0) and five salt concentrations (0.4, 0.8, 1.2, 1.6, and 2.0 M) were examined. Each type of salt showed distinctive activities for slaughtered and non-slaughtered meat protein solubility. Soluble protein concentration increased as pH increased ($p < 0.05$) from pH 5.0 to 8.0 and decreased from pH 8.0 to 9.0. It was also observed that protein solubility increased as the salt concentration increased. Protein solubility significantly increased ($p < 0.05$) in the non-slaughtered meat compared to the slaughtered meat at pH 8.0 for Na_2SO_4 at 1.2 M.

Keywords: Broiler chicken meat; non-slaughtered; protein solubility; salts; slaughtered

ABSTRAK

Penyelidikan ini mengkaji pengaruh pH dan kepekatan garam pada keterlarutan protein daging ayam yang disembelih dan tidak disembelih. Tiga jenis garam (NaCl , Na_2SO_4 , dan $(\text{NH}_4)_2\text{SO}_4$), lima tahap pH yang berbeza (5.0, 6.0, 7.0, 8.0 dan 9.0) dan lima kepekatan garam (0.4, 0.8, 1.2, 1.6 dan 2.0 M) dikaji. Setiap jenis garam menunjukkan aktiviti yang tersendiri bagi keterlarutan protein daging yang disembelih dan tidak disembelih. Kepekatan protein larut meningkat sebagai pH meningkat ($p < 0.05$) dari pH 5.0-8.0 dan menurun daripada pH 8.0 dan 9.0. Diperhatikan juga bahawa keterlarutan protein meningkat apabila kepekatan garam meningkat. Protein keterlarutan meningkat dengan ketara ($p < 0.05$) dalam daging disembelih berbanding dengan sembelihan pada pH 8.0 untuk Na_2SO_4 1.2 m.

Kata kunci: Ayam daging; disembelih; garam; protein keterlarutan; tidak disembelih

INTRODUCTION

Protein macromolecules play a vital role in pharmaceutical products and the functionality of food, as well as in biological systems. Because proteins are also used as a formulation in foods and other industrial mixtures, there has been an increasing need for proteins to contain ingredients with consistent and specific functional properties (Vojdani 1996). Every biochemical experiment is concerned with the solubility of protein. Protein solubility is a thermodynamic parameter defined as a soluble protein concentration that is in equilibrium with a crystalline solid phase influenced by the conditions of pH, buffer concentration, temperature and various additives (Ries-Kautt & Ducruix 1997). Various interactions, including protein-protein, protein-ion, protein-water and ion-water interactions are determined by protein solubility. The solubility of protein widely varies from almost complete insolubility to values of several hundreds of milligrams per milliliter. Insight into protein solubility could deliver important information on the prospective utilization of proteins and their functionality, such as emulsions, gels and foams (Zayas 1997).

The amount of total protein that goes into a solution under specified conditions refers to protein solubility

(Zayas 1997). Protein structure, amino acid composition, pH, ionic strength, solution composition, temperature, duration of extraction and many other extrinsic factors determine protein solubility. In addition, optimum protein extraction and purification conditions are determined by using protein solubility data. Surface hydrophobic (protein-protein) and hydrophilic (protein-solvent) interactions are related to protein solubility; in regard to food, such a solvent is water and thus, the solubility of protein is classified as a hydrophilic property.

Broiler chicken meat is a major livestock product and many people depend on chicken meat as their main source of protein. Currently, the development of healthy protein sources for the global food market is a major challenge. Slaughtering is an important step in the production process for broiler chicken and in determining the quality of meat. A healthy protein source will contain distinguishing characteristics that differentiate the chicken meat that has been properly halal slaughtered (by cutting the throat) and non-slaughtered (but dead). Proper halal slaughtering is performed by a throat cut to bring the animal to a quick death through severing the carotid arteries, jugular veins, trachea and esophagus, allowing a rapid and complete bleeding out (Grandin & Regenstein 1994). Promoting a

faster exsanguination by cutting the throat (Warriss 2010) has positive health and hygiene implications, as blood is considered an excellent medium for contamination and growth of microorganisms that are harmful to human health (Rosen 2004). Removing the blood as quickly as possible and stopping the delivery of oxygen to the brain is an efficient and humane slaughtering process (Gregory & Grandin 2007). In addition to slaughtering the animal through the loss of blood, the removal of flowing blood is necessary because it is considered an inconsumable impurity from the halal perspective. The execution of halal slaughtering in the poultry industry begins with an incision on the neck at a point just below the glottis (Man & Sazili 2010). Halal meats are increasingly perceived as wholesome and healthy (Nakyinsige et al. 2012). In recent studies, many researchers have differentiated slaughtered and non-slaughtered meat by using different methods in various types of meat, such as chicken (Almur Abdelkreem et al. 2012, 2011), goat (Mohiri et al. 2012; Mohammad et al. 2011) and sheep (Mohammad et al. 2010).

Thus, the focus of this research (the first of its type) was to determine the protein solubility of slaughtered and non-slaughtered broiler chickens, which differentiates properly halal slaughtered and non-slaughtered meat. In addition, researchers have not completely studied slaughtered and non-slaughtered meat, as there has been insufficiently reported data on meat. The purpose of this research was to measure the protein solubility in meat, distinguishing between properly halal slaughtered and non-slaughtered broiler chicken meat. Therefore, the main objective of this work was to evaluate the effect of pH and salt concentration on protein solubility of slaughtered and non-slaughtered broiler chicken meat for three salts (Na_2SO_4 , $(\text{NH}_4)_2\text{SO}_4$ and NaCl) at 25°C (room temperature).

MATERIALS AND METHODS

MATERIALS

All chemicals were purchased from Sigma-Aldrich, Malaysia Branch. Solutions were prepared with analytical grade reagents and deionized water was used in these experiments.

METHODS

SAMPLE COLLECTION

Two broiler chickens were purchased from a large, local commercial market. One chicken had been properly slaughtered according to Islamic Halal rules (by cutting the throat) and the other, non-slaughtered (by hitting the head with a stone to stop the delivery of oxygen to the brain). After 4 h, the postmortem meat was collected. The samples were kept at 0°C and brought to the research laboratory under refrigeration.

PREPARATION OF MEAT SAMPLE

Frozen chicken breast meat was taken from a research laboratory and used for this study. Then the preparation of muscle samples began by removing all epimysial connective tissues and visible fatty tissues. After that the muscle was separated from the frozen meat and cutting it into small pieces. It was then mashed using a blender machine for 2 min to prepare the samples. Mashed samples were subjected to analysis instantly. Mashed samples were immediately subjected to analysis.

PREPARATION OF BUFFER SOLUTION

The buffers added to achieve the desired pH were the following: citrate-citric acid (pH5.0), phosphate-citrate (pH6.0), citric acid-phosphate (pH7.0), phosphate (pH8.0) and Tris-HCl (pH9.0). Pre-set concentrations (0.4, 0.8, 1.2, 1.6 and 2.0 M) were assessed by previously added salts (Na_2SO_4 , $(\text{NH}_4)_2\text{SO}_4$ and NaCl) in each buffer solution (Machado et al. 2007).

PROTEIN CONCENTRATION

The Lowry method was utilized for the protein concentration measurement (Lowry et al. 1951), using a standard calibration curve built by varying bovine serum albumin concentration from 0.05 to 1.0 mg/mL, in aqueous solutions. Using a Thermo Spectronic (GENESYS 20, Sigma Aldrich, USA) spectrophotometer at 660 nm, the absorbance was measured.

SOLUBILITY DETERMINATION

Van Laack et al. (2000) developed a collaborative study and reliable procedure for determining the solubility of meat protein. For the solubility of protein isolates, 3 g of minced meat was homogenized with 60 mL of ice-cooled buffered solution. For the homogenization of meat, 3 g of minced meat was blended with 60 mL of ice-cooled buffered solution using a blender for 2 min. The homogenates were filtered through a Whatman No.1 paper. The homogenate solutions were immediately centrifuged at 10000 g for 30 min at 4°C (Sartorius centrifuge machine, model Sigma 3-18k). After centrifugation, the resulting supernatants were used to determine protein solubility. The solubility of protein (S , g protein in the supernatant/100 g total amount of protein in sample before centrifugation) was calculated using (1) (Machado et al. 2007):

$$S = \left[\frac{PS}{PA} \right] 100, \quad (1)$$

where PS is the protein content (g) in supernatant; and PA is the protein content (g) in the sample. Each experiment was triplicated and the protein soluble content was the mean value of the three replications.

STATISTICAL ANALYSIS

Each experiment and each assay were taken at least three times from the same batch samples to get the average results. The results were carried out as the mean \pm standard deviation. All data were subjected to the Analysis of Variance (ANOVA) using the General Linear Model process in the Minitab16 software. Differences between least square means were determined using HSD Tukey differences, and also significance at the 95% confidence level ($p < 0.05$) was reported.

RESULTS

The protein solubility of NaCl, Na₂SO₄ and (NH₄)₂SO₄ are shown in Figures 1-3, respectively. Initially, protein solubility at each increment significantly increased for all three types of salts. Moreover, these significant increments of protein solubility for NaCl, Na₂SO₄, and (NH₄)₂SO₄ were observed at the concentrations of 1.6, 1.2 and 1.2 M, respectively. After that, the protein solubility gradually declined for both NaCl and (NH₄)₂SO₄ salts while a continuous increase was observed for the Na₂SO₄ salt. The solubility of protein for each salt was re-evaluated between the contiguous lower and upper value of the maximum observed value of the individual salt in 0.4 M increments to obtain a more accurate concentration for each type of salt. Table 1 shows the solubility of the proteins' average values of three replicates, calculated from (1), which indicates that the maximum protein solubilities for NaCl, Na₂SO₄, and (NH₄)₂SO₄ were observed at 1.6, 1.2 and 1.2 M, respectively.

Figures 4 and 5 illustrate the powerful influence of pH variation on the solubility of protein. Protein solubilities of 1.6 M NaCl, 1.2 M Na₂SO₄ and 1.2 M (NH₄)₂SO₄ were examined within the pH ranges of 5.0-9.0. A maximum protein solubility for NaCl, Na₂SO₄ and (NH₄)₂SO₄ at pH8.0 and a minimum protein solubility at pH5.0 were observed (Table 2). However, NaCl, Na₂SO₄ and (NH₄)₂SO₄ salts showed significant differences in their protein solubility within the pH ranges.

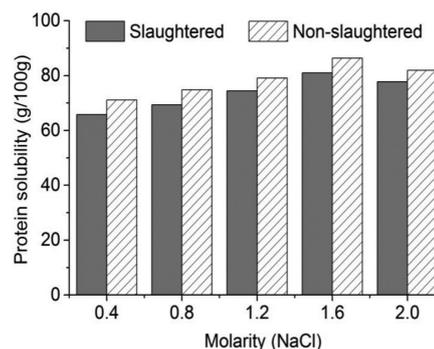


FIGURE 1. Effect of NaCl concentration on protein solubility at pH8.0

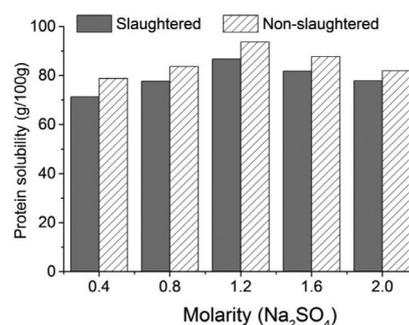


FIGURE 2. Effect of Na₂SO₄ concentration on protein solubility at pH8.0

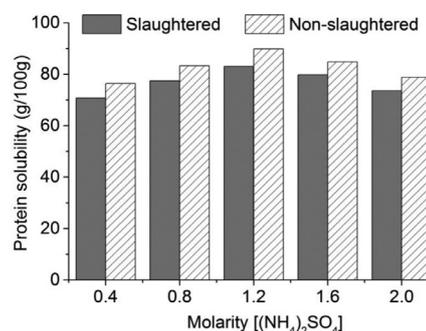


FIGURE 3. Effect of (NH₄)₂SO₄ concentration on protein solubility at pH8.0

TABLE 1. Effect of molar concentration on protein solubility (g/100 g) of NaCl, Na₂SO₄, and (NH₄)₂SO₄ at pH 8.0

Salt		Molarity				
		0.4	0.8	1.2	1.6	2.0
NaCl	Slaughtered	65.81 \pm 0.57 ^a	69.35 \pm 1.03 ^b	74.44 \pm 1.21 ^c	81.02 \pm 0.58 ^c	77.81 \pm 0.49 ^b
	Non-slaughtered	71.15 \pm 0.58 ^a	74.82 \pm 0.35 ^b	79.11 \pm 0.62 ^c	86.34 \pm 0.93 ^c	81.96 \pm 0.57 ^b
Na ₂ SO ₄	Slaughtered	71.34 \pm 0.86 ^a	77.73 \pm 0.42 ^b	86.76 \pm 0.49 ^c	81.85 \pm 0.54 ^c	77.94 \pm 0.47 ^b
	Non-slaughtered	78.80 \pm 0.46 ^a	83.78 \pm 0.43 ^b	93.70 \pm 0.58 ^c	87.81 \pm 0.74 ^c	82.00 \pm 0.68 ^b
(NH ₄) ₂ SO ₄	Slaughtered	70.77 \pm 0.50 ^a	77.48 \pm 0.55 ^b	83.07 \pm 0.60 ^c	79.83 \pm 0.82 ^c	73.64 \pm 0.70 ^b
	Non-slaughtered	76.36 \pm 1.12 ^a	83.35 \pm 1.02 ^b	89.87 \pm 0.58 ^c	84.80 \pm 0.72 ^c	78.81 \pm 0.57 ^b

All values are means \pm SEM of three replicates

^{a-c} Means within a row with different letters are significantly different ($p < 0.05$)

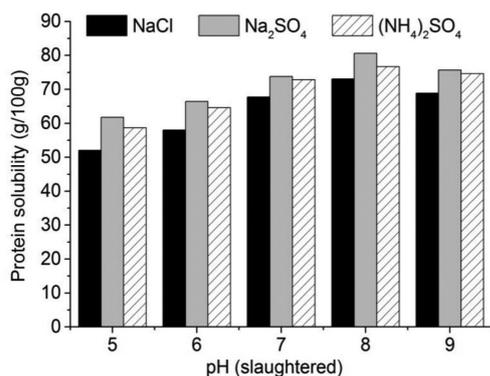


FIGURE 4. Effect of pH on protein solubility (g/100 g) of 1.6 M NaCl, 1.2 M Na₂SO₄ and 1.2 M (NH₄)₂SO₄ (slaughtered meat)

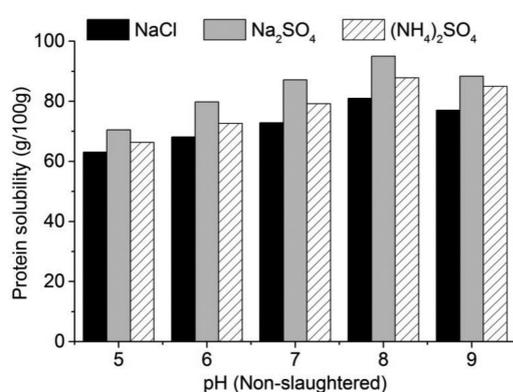


FIGURE 5. Effect of pH on protein solubility (g/100 g) of 1.6 M NaCl, 1.2 M Na₂SO₄ and 1.2 M (NH₄)₂SO₄ (non-slaughtered meat)

Table 3 illustrates the protein solubility of 1.6 M NaCl, 1.2 M Na₂SO₄ and 1.2 M (NH₄)₂SO₄ at pH 8.0 that were compared to choose the best salt for slaughtered and non-slaughtered broiler chicken meat. The maximum solubility of proteins was observed for the Na₂SO₄ salt followed by NaCl, and (NH₄)₂SO₄ salts. From the above data, it is observed that non-slaughtered meat protein solubility is significantly higher than that of slaughtered meat.

DISCUSSION

In the aqueous solution, the solubility of protein is a sensible function of the dissolved salts concentration. This parameter can be used to explain the ionic strength effects that result from the theoretical considerations of the ionic solutions. Nevertheless, solubility of proteins at a given ionic strength diverges with the dissolved ions in the solution. Solubility of protein influenced by various effective ions for different proteins is quite similar and is evidently based on the size and hydration of ions. Protein solubility normally increases with the salt concentration at a low ionic strength. With the increase of the salt concentration in the protein solution, the protein molecules are shielded more effectively by the extra counter ions' multiple ionic charges and based on that increase, the protein's solubility is known as the salting-in phenomenon.

Maximum solubility of protein was found for Na₂SO₄ compared to (NH₄)₂SO₄ and NaCl at 1.6, 1.2 and 1.2 M, respectively; the ammonium cations are less effective than the alkaline metal cations in protein precipitation followed by the Hofmeister series (Leberman 1991) (Table 1). As observed in Table 1, the solubility of protein increased in the order of Na₂SO₄ > (NH₄)₂SO₄ > NaCl. The respective pH changed the solubility of protein according to the content and type of salt present in the solution. The solubility of protein for b-lactoglobulin, lysozyme and bovine serum albumin showed similar results with different types of salt, as reported by Arakawa and Timasheff (1982). Therefore, besides realizing the salts competence in sustaining the structure of protein stability, the preferential protein-salt interaction is an important parameter for measuring salt behavior as an upgrading agent of salting-out or salting-in (Arakawa & Timasheff 1982). The consequence behavior of salting-out effect is also due to the existence of SO₄²⁻ ions.

The maximum solubility of protein data was observed on the slaughtered and non-slaughtered meat at the alkaline pH value (8.0) rather than at an acidic pH. At the alkaline pH, the protein-protein interactions

TABLE 2. Effect of pH on protein solubility (g/100 g) of 1.6 M NaCl, 1.2 M Na₂SO₄ and 1.2 M (NH₄)₂SO₄

Salt	pH					
	5.0	6.0	7.0	8.0	9.0	
NaCl	Slaughtered	52.02±0.28 ^a	58.00±0.81 ^b	67.74±0.35 ^c	73.08±0.65 ^d	68.85±0.60 ^e
	Non-slaughtered	63.12±0.58 ^a	68.12±0.67 ^b	72.85±0.70 ^c	81.02±0.49 ^d	77.07±0.49 ^e
Na ₂ SO ₄	Slaughtered	61.77±0.58 ^a	66.44±1.12 ^b	73.79±0.63 ^c	80.63±0.69 ^d	75.67±0.71 ^e
	Non-slaughtered	70.48±1.06 ^a	79.82±0.33 ^b	87.15±0.63 ^c	95.03±0.39 ^d	88.38±1.13 ^e
(NH ₄) ₂ SO ₄	Slaughtered	58.70±0.46 ^a	64.60±0.54 ^b	72.86±0.57 ^c	76.68±0.67 ^d	74.61±0.67 ^e
	Non-slaughtered	66.33±1.11 ^a	72.67±0.71 ^b	79.22±0.92 ^c	87.81±0.52 ^d	84.96±0.31 ^e

All values are means ± SEM of three replicates

^{a-e} Means within a row with different letters are significantly different ($p < 0.05$)

TABLE 3. Protein solubility (g/100 g) of 1.6 M NaCl, 1.2 M Na₂SO₄ and 1.2 M (NH₄)₂SO₄ at pH8.0

Salt	Slaughtered	Non-slaughtered
NaCl	81.02±0.58 ^{Aa}	86.34±0.93 ^{Ba}
Na ₂ SO ₄	86.76±0.49 ^{Ab}	93.70±0.58 ^{Bb}
(NH ₄) ₂ SO ₄	83.07±0.60 ^{Ac}	89.87±0.58 ^{Bc}

Values are means ± SEM of three replicates

^{A-B, a-c} Means within a row and column with different letters are significantly different ($p < 0.05$)

decreased while the protein-water interactions increased because of the negative net charges (COO⁻) present on the surface of protein molecules, which increased their repulsion (Fennema 1993). Under these conditions, the solubility of protein decreased with the increase of salt concentration. This effect is known as salting-out due to the competition between the salt ions and protein for the water molecules. Therefore, the surrounding water in the protein will be removed, which enhances the protein-protein interaction and accordingly, precipitation contributes to the accumulation of the protein molecules (Fennema 1993).

Amino acid is the major building block of proteins. In solutions, amino acid at a neutral pH exists predominantly as zwitterions (also called dipolar ions). In the zwitterion form, the carboxyl group is deprotonated (-COO⁻) and the amino group is protonated (-NH₃⁺). The pH depends on the ionization state of the amino acid. The carboxyl group is not dissociated (-COOH) and the amino group is protonated (-NH₃⁺) in the acidic solution. First, the carboxylic acid group gives a proton, in as much as its pK_a is near 2 when the pH is raised. The dipolar form persists until the pH approaches 9, when the protonated amino group loses a proton. Proteins usually bear various ionized groups that have a type of pK_s. For each protein at a pH characteristic, the molecules are exactly balanced with positive and negative charges. At this pH, which is the isoelectric point (pI) of the protein, the protein molecule contains no net charge and is therefore immobile on the electric field. Protein's pI depends on the pH variation that increases the protein's net charge. It should be increasingly subject to salting-in because the electrostatic interaction between adjacent molecules, which promotes accumulation and precipitation, should increase as well. The minimum solubility of protein was found at its isoelectric point, where the protein has no net charge with the absence of any added ions. Because protein contains multiple charged groups, its solubility depends on the salt concentration, temperature and pH.

For all tested salts, the protein solubility increased when the pH increased (Table 2). Maximum protein solubility was observed at pH8.0 because in this condition, the protein's positive and negative net charged molecules interact more with water. Protein solubility is lower in acidic pH than in alkaline pH. Minimum solubility values for any salt are at a pH of 5.0; in this condition, the electrostatic forces are the lowest and less water interacts with the protein molecules, which causes the

increase of protein-protein interactions. Indeed, the number of positively charged ions at pH > pI is smaller than the number of negatively charged ions at pH > pI (Fennema 1975). A trend of increasing protein solubility was confirmed with the molarity of salts (NaCl, (NH₄)₂SO₄ and Na₂SO₄) increasing at pH5.0. Solubility of protein may increase for each type of salt concentration ranging from 0.4 to 2.0 M because of the salting-in process in which the salt ions interact with the opposite charged protein groups to form a double layer of ionic groups, after which the electrostatic forces among the protein molecules decrease, thus increasing their solvation (Vojdani 1996).

The protein solubility of slaughtered and non-slaughtered broiler meat registered a minimum at pH5.0 and increased as the pH increased; however, it decreased again from pH8.0 to 9.0 (Table 2). The non-slaughtered meat protein was significantly more soluble than the slaughtered meat at pH 8.0. The protein solubility of 1.6 M NaCl, 1.2 M Na₂SO₄ and 1.2 M (NH₄)₂SO₄ at pH 8.0 were compared in the slaughtered and non-slaughtered broiler chicken meat. The maximum solubility of protein was observed for Na₂SO₄ followed by NaCl and (NH₄)₂SO₄. In addition, the soluble protein content for each type of salt was found to significantly differ from each other. This might be due to the inherent properties of the three salts consisting of three ions resulting in the differences.

CONCLUSION

One of the functional properties of protein solubility is that it can influence other functional properties of protein, such as gelation, foam and emulsion and it could also be a vital component in possible applications of protein components in food. In addition, protein solubility data were used to determine protein extraction and purification conditions. This work measured the effect of pH and salt concentration on the protein solubility of slaughtered and non-slaughtered broiler chicken meat. pH and salt concentration changes influenced the solubility of protein and each type of salt exhibited a particular behavior in the broiler meats. Therefore, the protein solubility determination for slaughtered and non-slaughtered broiler chicken meat has been established. The result indicates that the solubility of protein is significantly higher in non-slaughtered meat than in slaughtered meat; this difference. Based on the data, the results indicate that this difference in protein solubility between slaughtered and non-slaughtered broiler meat

is substantial and can be used to distinguish proper halal slaughtered meat from non-slaughtered meat.

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