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EFFECTS OF PREPARATION METHODS ON PROTEIN AND AMINO ACID CONTENTS OF VARIOUS EGGS AVAILABLE IN MALAYSIAN LOCAL MARKETS

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ABSTRACT

Background. The effect of preparation methods (raw, half-boiled and hard-boiled) on protein and amino acid contents, as well as the protein quality (amino acid score) of regular, *kampung* and nutrient enriched Malaysian eggs was investigated.
Methods. The protein content was determined using a semi-micro Kjeldahl method whereas the amino acid composition was determined using HPLC.
Results. The protein content of raw regular, *kampung* and nutrient enriched eggs were 49.9 ±0.2%, 55.8 ±0.2% and 56.5 ±0.5%, respectively. The protein content of hard-boiled eggs of regular, *kampung* and nutrient enriched eggs was 56.8 ±0.1%, 54.7 ±0.1%, and 53.7 ±0.5%, while that for half-boiled eggs of regular, *kampung* and nutrient enriched eggs was 54.7 ±0.6%, 53.4 ±0.4%, and 55.1 ±0.7%, respectively. There were significant differences ($p < 0.05$) in protein and amino acid contents of half-boiled, hard-boiled as compared with raw samples, and valine was found as the limiting amino acid. It was found that there were significant differences ($p < 0.05$) of total amino score in regular, *kampung* and nutrient enriched eggs after heat treatments. Furthermore, hard-boiling (100°C) for 10 minutes and half-boiling (100°C) for 5 minutes affects the total amino score, which in turn alter the protein quality of the egg.

Key words: amino acids, amino acid score, half-boiled eggs, hard-boiled eggs, *kampung*, nutrient enriched, protein

INTRODUCTION

Egg traditionally has been used for breakfast, home meal preparation, and baking and it is highly nutritious. It supplies a large amount of complete, high quality protein and provides significant amounts of several vitamins and minerals [Gutierrez et al. 1996, Lifschitz 2012]. Egg is an important animal protein, it contains all the essential amino acids needed for human body [Sakanaka et al. 2000, Friedman 1996] and it is suitable for persons for all ages [Cook and Briggs 1977]. Based on Malaysia Food Balance Sheet [FAO 2001], egg is a common food among Malaysians, whose consumption was quite high reaching up to 3.8 g protein a day per capita supply. In Malaysia, many cooking methods are used in preparing meals with egg such

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as boiling, half-boiling, frying, and steaming etc. Although eggs contain about 74% water, they are a rich source of protein needed for human growth. The egg protein is of high quality so it is commonly used as a standard for measuring the nutritional quality of other food proteins [Cook and Briggs 1997, Sakanaka et al. 2000]. Egg protein has the 'perfect' amino acid composition, and was used as the reference protein for biological evaluation and assessment of amino acid patterns [Southgate 2000].

The main proteins in albumin and yolk are ovalbumin, ovotransferin, lysozyme, ovomucoid, ovomucin and immunoglobulin Y, the ovalbumin is the most abundant of these proteins, which accounts for more than half of the protein in egg white [Mc Williams 2001]. According to Sosulski and Imafidon [1990] the highest individual amino acid content in egg was lysine (509 mg/g) whereas the lowest was cysteine (128 mg/g). In addition, there were some individual amino acids namely arginine, serine, cysteine and isoleucine in egg showed values higher than that of other animal products. The essential amino acids in different protein sources including casein, beef, egg white, soy protein and wheat flour have been studied [Sarwar et al. 1983]. Among the essential amino acids in egg white, the highest content of amino acid was the combination of phenylalanine and tyrosine whereas tryptophan showed the lowest value. As reported by Sakanaka et al. [2000], the highest and lowest essential amino acid composition in egg were the combination of phenylanaline and tyrosine (85 mg/g protein), and trytophon (15 mg/g protein), respectively. According to Mahan and Escott-Stump [2004], vegetable protein is less well digested than animal protein. In addition, Baudoin and Maquet [1999] found that protein quality in leguminous seeds does not reach the same level as in animal products. So, since egg is one of the animal proteins, it serves as high protein quality food and is well digested than other vegetable protein foods.

Protein sources are treated with heat, oxidizing agents, organic solvents, alkalis and acids during food processing for a variety of reasons [Friedman et al. 1984, Schwass and Finley 198]. Such treatments may cause modification of the nutritional value of proteins, decreasing the amino acid content through desulfuration, deamination or isomerization; reactions with lysine, methionine, cystine and tryptophan are the most susceptible to damage [Cheftel et al. 1989].

Since egg is usually not consumed raw, the effects of various preparation methods (boiling and frying) employed in preparing egg for consumption are very important. These processes could have varying effects on egg protein and amino acid content. There is a general lack of information on the effect of boiling on the protein and amino acid composition of eggs particularly for the eggs under discussion and there is a need to study how these boiling methods affect the protein and amino acid content, considering that eggs are consumed mainly for their protein content.

Therefore the aim of this study was to elucidate the effects of preparation methods (half-boiling, hard-boiling) on protein and amino acid contents of three Malaysian local eggs (regular, *kampung* and nutrient enriched eggs).

MATERIAL AND METHODS

Chemicals

Sulfuric acid (95-97%), sodium hydroxide and hydrochloric acid were purchased from Merck, Darmstadt, Germany. Boric acid, methyl red and bromocrisol green were purchased from BDH Chemicals Ltd., Poole England. Triethylamine (TEA) and phenylisothiocyanate (PITC) were purchased from Schuchardt, Germany whereas a-amino butyric acid (AABA) and amino acid standards were purchased from Sigma (St. Louis, MO, USA).

Materials

Three types of eggs were used, namely regular eggs (RE), *kampung* eggs (*KE*) and nutrient enriched eggs (NEE). Both the RE and NEE were purchased from local supermarket at Batu Caves, Malaysia whereas *KE* were purchased from a hawker, Batu Caves, Malaysia. The NEE used in this study were fortified with omega-3 fatty acid, vitamin E and selenium. All of the eggs were prepared by means or raw, hard-boiling and half-boiling. All of them were undergoing freeze-drying (Model Virtis, Gardiner, New York) followed by delipidation by soaking in *n*-hexane. Total nitrogen and the protein content were determined based on a semi-micro Kjeldahl method [AOAC 1984] using the conversion factor of 6.25. Meanwhile, Pico-tag method was used in determining amino acids composition of those samples [Toran et al. 1996].

Sample preparation

Raw eggs. Shells of three different types of eggs namely regular, *kampung* and nutrient enriched were broken and the contents were placed separately into clean containers. After that, they were frozen at –80°C.

Hard-boiled eggs. Eggs were boiled in tap water at water boiling point 100°C for 10 minutes in order to obtain hard-boiled eggs. The water used (1.0 L) should cover the eggs in the pot. Each type of eggs was boiled separately. After boiling, shell eggs were taken out and the eggs were cut into small portions.

Half-boiled eggs. Eggs were placed in a half-boiled bowl prior to the addition of boiled water. Boiled water (1.0 L) was added into the bowl until covering all the eggs. Then the water was poured through a tiny hole under the bowl into another container. The half-boiled eggs were ready after 5 minutes.

Freeze-drying of samples

The whole egg of RE, KE and NEE using hard and half-boiling method were freeze-dried with liquid nitrogen. The purpose of using liquid nitrogen was to freeze-dry the water molecules in the samples. They were then placed in a clean container separately and sealed with parafilm prior to undergoing freeze-drying (Model Virtis, Gardiner, New York) for three days. The obtained freeze-dried samples were then ground using mortar. For samples of raw and half-boiling methods, additional step was needed before undergoing the same procedures as hard-boiling method that was undergoing freezing at –80°C.

Delipidation of samples

Removal of lipid from the samples was done by soaking them with liquid *n*-hexane. The egg samples (20.0 g) were weighed into baker and followed by the addition of 100 ml liquid *n*-hexane. This procedure was carried out for 3 days. After that, they were filtered using filter paper Whatman no 4. The *n*-hexane residues were evaporated in fume hood for one day.

Amino acids analysis

The amino acids content of the samples were analysed and quantitatively determined using the HPLC (Waters System Interface module 501, Hewlett Packard, California, USA) as described by Pico-tag method [Toran et al. 1996], with slight modification. About 0.12 ±0.10 g (40 mg protein) of egg powder was weighed into a medium wall Pyrex test tube and 15 ml of 6 M hydrochloric acid was added. The tube was quickly sealed and hydrolysed under nitrogen environment in an oven at 110°C for 24 h. After hydrolysis, the mixture was allowed to cool at room temperature, transferred to 50 ml volumetric flask and 10 ml α-aminobutyric acid (AABA) was added as an internal standard. The volume was made to 50 ml with demonized water. The mixture was filtered through a Whatman filter paper No. 1, and then, through a Whatman filter paper No. 42 before the derivatization process. About 10 µl of filtered sample were transferred into derivatization tube, and evaporated to dryness under vacuum (100-200 mtorr) for 30 min. Subsequently, a re-drying reagent (a mixture of methanol-triethylamine and deionised water (2:1:2, w/w/w)) was added and redried for another 30 min. After the final drying, a derivatization reagent (methanol – phenyltiocyanate [PITC] – triethylamine and deionised water (7:1:1:1, v/v/v/v)) was added. To ensure that the coupling reaction with PITC was completed, the derivatization process was set for 20 min. It was then evaporated to dryness for another 20 min. About 100 µl of sample diluent (a mixture of disodium hydrogen phosphate, deionised water, 10% orthophosphoric acid and acetonitrile) were added to the sample. Of the prepared sample, 20 µl of aliquot and 8 µl of blank solution were injected into Pico-tag column (C18, 3.9×150 mm, Waters, Medford, MA). All determinations were performed in duplicates. The quantification of each amino acid was determined from a standard calibration. Tryptophan was destroyed by acid hydrolysis [White and Hart 1992] and thus, was not determined.

Quality of the amino acids

Amino acid score. Amino acid ratios (mg of an essential amino acid in 1.0 g of test protein/mg of the same amino acid in 1.0 g of reference protein × 100) for 9 essential amino acids calculated by using the FAO/WHO [1991].

STATISTICAL ANALYSIS

All the samples were carried out at least in duplicate. Mean and standard deviation were calculated using Statistical Package for Social Sciences (SPSS) (version 12.0 for Windows). Data obtained was analysed using analysis of variance (one-way ANOVA) to see the differences in protein and amino acid content as well as the amino acid score in raw, hard-boiled and half-boiled regular, *kampung* and nutrient enriched eggs. Pearson's correlation coefficients were performed to compare the data and the significant level for all test was set at $p < 0.05$.

RESULTS AND DISCUSSION

Protein content in egg samples

The protein content of raw, hard-boiled and half boiled three egg samples is shown in Table 1. From this table the protein content of raw, hard-boiled and half-boiled from RE, *KE* and NEE samples were in the range of 49.9-56.5%, 53.7-56.8% and 53.4-55.1%, respectively. The highest protein content among the raw egg samples was found in nutrient enriched egg (56.5%); followed by *KE* (55.8%) and RE (49.9%). Based on these results there were significant differences ($p < 0.05$) of protein contents among the egg samples after heat treatment. From Table 1 the protein content of raw, hard-boiled and half-boiled of RE was 49.9 ±0.2, 56.8 ±0.1 and 54.7 ±0.6%, respectively, there was a significant difference ($p < 0.05$) of protein content after preparation methods (hard-boiling and half boiling). In other words, the preparation methods have an impact on the protein content and it increased the protein content. For *kampung* eggs, the protein content of raw, hard-boiled and half-boiled eggs were 55.8 ±0.2, 54.7 ±0.1 and 53.5 ±0.4%, respectively. There was a significant difference ($p < 0.05$) of protein content among raw, hard-boiled and half-boiled *KE* (Table 1). The concentration of protein in raw, hard-boiled and half-boiled NEE was 56.5 ±0.5, 53.7 ±0.5 and 55.1 ±0.7%, respectively. In contrast and depending on different methods of processing, Caboni et al. [2005] reported that, drying treatments (spray-drying for whole egg and pasteurization of liquid egg) caused substantial chemical modifications in processed egg, but did not change the protein content.

Table 1. Protein content (% in sample) and essential amino acid composition (mg/g protein) of three egg samples prepared by different methods*

Essential amino acid	Regular egg			*Kampung* egg			Nutrient enriched egg		
	raw	hard-boiled	half-boiled	raw	hard-boiled	half-boiled	raw	hard-boiled	half-boiled
Protein content	49.9 ±0.2	56.8 ±0.2	54.7 ±0.6	55.8 ±0.2	54.7 ±0.1	53.5 ±0.4	56.5 ±0.5	53.7 ±0.5	55.1 ±0.7
Histidine	9.2 ±0.2	13.1 ±0.1	14.4 ±0.1	11.4 ±0	12.6 ±0.1	13.9 ±0	10.3 ±0.9	11.3 ±0.9	13.1 ±0.3
Threonine	19.5 ±0.2	ND	ND	ND	ND	ND	ND	44.4 ±0.0	ND
Valine	15.8 ±0.2	29.6 ±0.1	36.2 ±0.1	24.2 ±0.1	27.2 ±0.1	29.6 ±0.2	21.2 ±1.8	27.1 ±0.1	29.8 ±0
Methionine	9.9 ±0.2	20.8 ±0.2	21.1 ±0.2	13.6 ±0.1	18.1 ±0	20.1 ±0	14.1 ±1.1	19.6 ±0.3	20.8 ±0.2
Isoleucine	13.4 ±0.1	23.8 ±0.5	25.1 ±0.3	19.8 ±0.3	21.9 ±0.4	24.0 ±0.4	17.7 ±1.6	21.4 ±0.1	23.8 ±0.3
Leucine	25.2 ±0.0	43.5 ±1.1	46.6 ±1.5	37.7 ±0	42.4 ±1.1	45.8 ±1.1	33.6 ±2.7	40.9 ±1.2	44.5 ±1.2
Phenylalanine	14.4 ±0.0	31.9 ±0.5	31.0 ±0.7	22.9 ±0.5	26.9 ±0.8	33.8 ±4.0	23.2 ±2.2	28.6 ±0.6	32.8 ±0.1
Lysine	27.3 ±0.4	43.3 ±0.5	47.4 ±1.1	41.1 ±1.3	41.6 ±0.1	48.7 ±1.1	36.5 ±2.9	40.3 ±0.2	44.0 ±0.7
Total	115.1 ±0.1	206.0 ±2.5	221.8 ±3.2	170.6 ±1.5	190.6 ±2.1	215 ±4.5	156.6 ±13.3	189.2 ±0.8	208.8 ±2.9

*Means ±standard deviation of three determinations.
ND – not detected.

The differences of protein content among egg samples can be influenced by many factors. As reported by Cook and Briggs [1977], nutrient concentrations in eggs can be directly influenced by genetic makeup, feed of hens, environment, storage time and different cooking methods. Tee et al. [1997] reported less protein in whole egg (12.9%), and that might be due to the sample preparation, in which the egg samples was freeze-dried to remove the water. The protein content of egg found in Sosulski and Imafidon [1990] was 44.18% which was less than this study (49.9-56.5%). This difference was mostly due to genetic, environment, and feeding of hens.

On the other hand, the protein content of RE increased significantly ($p < 0.05$) after cooking (boiling) as compared with raw eggs. Deman [1999] reported that the denaturation due to the effect of heat could be reversible in certain cases. This probably because of the reactivation of certain enzymes activities and thus lead to the formation of some protein structures.

Amino acid profile

In this study, Waters HPLC was used to detect 17 amino acids namely aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, praline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine and lysine in three Malaysian egg samples. However, tryptophan, glutamine and asparagines were not determined because the first is very labile in an acid environment and is completely destroyed by acid hydrolysis (HCI 6N) [Friedman and Cuq 1988, Fountoulakis and Lahm 1998, Wathelet 1999], while the second and third were not determined to prevent the interferences of actual glutamic acid and aspartic acid content.

Essential amino acid content in egg samples

The essential amino acids cannot be manufactured in human bodies, but can be obtained from food. Eight amino acids are generally regarded as essential for humans: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine and lysine [Young 1994]. Additionally, cysteine (or sulphur-containing amino acids), tyrosine (or aromatic amino acids), histidine and arginine are required by infants and growing children [Imura and Okada 1998]. Deficiency in these amino acids may lead to some health problems.

The essential amino acid composition (mg/g protein) of the three egg samples (RE, *KE*, and NEE) prepared by different methods (hard and half boiling) results are presented in Table 1. All the essential amino acids were present in the egg samples investigated except tryptophan which was not determined. A significant difference ($p < 0.05$) was observed between RE, *KE*, and NEE. The study indicated that lysine, leucine, phenylalanine, and valine gave the highest amino acid content. All essential amino acids were significantly ($p < 0.05$) increased after hard and half boiling treatment. The valine content in raw, hard-boiled and half boiled RE was 15.8 ±0.2, 29.6 ±0.1 and 36.2 ±0.1 mg/g protein, respectively. For *kampung* eggs raw, hard-boiled and half-boiled, valine content was 24.2 ±0.1, 27.2 ±0.1 and 29.6 ±0.2 mg/g protein whereas in NEE raw, hard-boiled and half-boiled was 21.1 ±1.8, 27.1 ±0.1 and 29.8 ±0.0 mg/g protein, respectively (Table 1). These results were in good agreement with Alajaji and El-Adaw [2006] study of chickpea seed proteins, those authors reported that boiling and microwave cooking caused a slight increase in total essential amino acids, but they were not influenced by autoclaving. And that cooking treatments decreased the concentration of lysine, tryptophan, and total aromatic and sulfur amino acids.

Raw egg from the three Malaysian egg samples can only meet the adults requirement while hard-boiled and half-boiled egg sample can fulfill the need for children aged 10-12 years old as well as adult [FAO/WHO 1991] (Table 2). The valine content in raw egg samples of RE, *KE* and NEE was in the range of 15.8--24.2 mg/g proteins. Valine main function is for human metabolism and muscle building. It is also required in the precursors of cholesterol [Young 2004].

The statistical analysis showed a significant difference ($p < 0.05$) in leucine content in RE, *KE*, and NEE after hard and half-boiling when compared with raw eggs (Table 1). In regular egg, the leucine content was found as 25.2 ±0.0, 43.5 ±1.1 and 46.6 ±1.5 mg/g protein, for raw, hard-boiled and half-boiled, respectively. While for raw, hard-boiled and half-boiled *kampung* eggs, the leucine content was 37.7 ±0, 42.4 ±1.1 and 45.8 ±1.1 mg/g protein, respectively, whereas the leucine content in raw, hard-boiled and half-boiled NEE were 33.6 ±2.7, 40.9 ±1.2 and 44.5 ±1.2 mg/g protein, respectively. From Table 1 it was clear that preparation

Table 2. Essential amino acid composition (mg/g protein)* of three egg samples prepared by different methods and FAO/WHO recommended pattern of human requirement

Essential amino acid	Regular egg			*Kampung* egg			Nutrient enriched egg			FAO/WHO [1991]			
	raw	hard-boiled	half-boiled	raw	hard-boiled	half-boiled	raw	hard-boiled	half-boiled	1 year old	2-5 years old	10-12 years old	adult
Methionine + cysteine	15.6	31.3	35.0	24.2	27.8	32.9	22.6	29.8	31.8	42	25	22	17
Valine	15.8	29.6	36.2	24.2	27.2	29.6	21.2	27.1	29.8	55	35	25	13
Isoleucine	13.4	23.8	25.1	19.8	21.9	24.0	17.7	21.4	23.8	46	28	28	13
Leucine	25.2	43.5	46.6	37.7	42.4	45.8	33.6	40.9	44.5	93	66	44	19
Phenylalanine + tyrosine	28.7	55.2	55.1	42.2	48.6	59.2	41.7	49.2	57.4	72	63	22	19
Histidine	9.2	13.1	14.4	11.4	12.6	13.9	10.3	11.3	13.1	26	19	19	16
Lysine	27.3	43.3	47.4	41.1	41.6	48.7	36.5	40.3	44.0	66	58	44	16

*Results obtained were based on three determinations.

methods (hard and half boiling) were significantly ($p < 0.05$) affect leucine content of RE, *KE* and NEE eggs. The main function of leucine is to release energy during muscle work and acts as a precursor of cholesterol [Young 2004]. Therefore, the intake of leucine should be enough since it cannot be synthesized in human body. As compared with the FAO/WHO [1991] recommendation, the leucine in all egg samples met the recommended adult requirement that was 19 mg/g protein (Table 2).

From the statistical analysis in Table 1. It was clear that there were significant differences ($p < 0.05$) of phenylalanine and lysine content in raw, hard-boiled and half-boiled RE, *KE*, and NEE eggs. Lysine is an essential, naturally occurring amino acid that is needed for optimal growth in infants and for maintenance of nitrogen equilibrium in adults. It also promotes bone growth in infants as well as stimulates secretion of gastric juices. It can be found abundantly in muscle tissue, connective tissue and collagen [Young 2004]. Since lysine cannot be synthesized in body and should be taken from diet, the lysine contents in all egg samples with different preparation methods in this study can fulfill the recommended adult requirement by FAO/WHO [1991], which was 16 mg/g protein (Table 2). Raw egg samples can only meet the adults requirement while hard-boiled and half-boiled egg sample can fulfill the need for children aged 10-12 years old, as well as adult [FAO/WHO 1991] (Table 2).

As shown in Table 2 the combination of phenylalanine and tyrosine for all raw, hard-boiled and half-boiled egg samples met the requirement for both children aged 10-12 years old and adult. Phenylalanine is an essential amino acid that should be taken from diet because it acts as a precursor of tyrosine, and together lead to the formation of thyroxine and epinephrine [Mahan and Escott-Stump 1996]. In addition, it is also shown to be useful in management of certain types of depression. Deficiency of phenylalanine may result in stunted growth, heart damage, fatigue and lethargic.

From Table 1, histidine, methionine and isoleucine acids showed the lowest content of essential amino acids in all egg samples. Histidine is essential for optimal growth in infants and the synthesis of histamine, which causes vasodilatation in the circulatory system [Mahan and Escott-Stump 1996]. The histidine content of raw egg samples in this study was in the range of 9.2-11.4 mg/g proteins, this was lower than that reported by Woo et al. [1996], which was 19.1 mg/g protein. This might be due to the different egg species. *KE* showed the highest histidine content (11.4 ±0.0 mg/g protein), followed by NEE (10.3 ±0.9 mg/g protein) and RE (9.2 ±0.2 mg/g protein). On the basis of preparation methods, the histidine content of hard-boiled regular, *kampung*, and NEE were 13.1 ±0.1, 12.6 ±0.1 and 11.3 ±0.1 mg/g protein, respectively. Meanwhile, the histidine content of half-boiled eggs in the same sequence was 14.4 ±0.1, 13.9 ±0.0 and 13.1 ±0.3 mg/g protein, respectively (Table 1). There were significant differences ($p < 0.05$) of histidine content in raw, hard-boiled and half-boiled regular and *kampung* eggs. In addition, the histidine content of raw, hard-boiled and half-boiled regular, *kampung* and NEE did not meet the requirement recommended by FAO/WHO [1991] (Tables 3, 4).

Accordingly, the methionine content in raw regular, *kampung* and NEE were 9.9 ±0.2, 13.6 ±0.1 and 14.1 ±1.1 mg/g protein whereas Woo et al. [1996] reported 15.8 mg/g protein. This difference could be due to the different species studied and there may be has some lost occurred because of the oxidation of methionine [Kivi 2000, Friedman 1996]. The methionine content along with cystiene obtained in this study met the adult requirement recommended by the FAO/WHO [1991] for all the samples except for the raw (Table 2), which was slightly lower than the recommended value. Since lysine cannot be synthesized in body and should be taken from diet, the lysine contents in all egg samples with different preparation methods in this study can fulfill the recommended adult requirement by FAO/WHO [1991], which was 16 mg/g protein (Table 2).

The threonine content of egg samples cannot be determined except for raw RE and hard-boiled nutrient eggs, which were 19.5 ±0.2 and 44.4 ± mg/g proteins, respectively. As reported by Friedman [1996], threonine is easily oxidized when exposed to oxygen and it is partially hydrolyzed [Fountoulakis and Lahm 1998] and lost when the acid hydrolysis conditions are harsh [Kivi 2000, White and Hart 1992].

Non-essential amino acid content in egg samples

Table 3 represents the content of non-essential amino acids in the studied egg samples. The total content of all amino acids was significantly different among different egg samples, and that preparation method affects significantly on individual and total amino acids of egg samples. The total content of all amino acids as well as the individual amino acid was significantly ($p < 0.005$) increased as result of hard and half-boiling methods. From this table aspartic, glumatic and serine acids showed higher amino acid content. Aspartic acid content in raw, hard-boiled and half-boiled RE were

Table 3. Non-essential amino acid composition (mg/g protein) of three egg samples prepared by different methods*

Non-essential amino acid	Regular egg			*Kampung* egg			Nutrient enriched egg		
	raw	hard-boiled	half-boiled	raw	hard-boiled	half-boiled	raw	hard-boiled	half-boiled
Aspartic acid	47.3 ±0	87.5 ±1.2	89.5 ±0.3	67.3 ±0.7	77.8 ±1.8	87.1 ±0.7	60.4 ±6.8	76.9 ±0.7	84.7 ±0.2
Glutamic acid	31.8 ±0.7	58.6 ±0.6	61.1 ±0.2	47.9 ±1.0	53.6 ±0.2	58.9 ±0.8	42.3 ±3.1	53.6 ±0.2	58.2 ±0.5
Serine	27.4 ±0	45.2 ±0.1	46.7 ±0.3	38.6 ±0	42.9 ±0.3	47.3 ±0.5	35.6 ±2.7	41.1 ±0	46.5 ±0.3
Glycine	8.8 ±0	17.7 ±0	17.4 ±0.1	13.5 ±0.1	15.4 ±0.1	17.6 ±0.2	12.8 ±1.1	15.1 ±0.1	17.4 ±0.2
Arginine	29.7 ±0	53.0 ±0.1	53.7 ±0.1	42.9 ±0.2	51.3 ±0.3	56.6 ±0.1	40.3 ±3.4	47.8 ±0.6	55.4 ±0.5
Alanine	5.5 ±0.1	33.5 ±1.0	33.6 ±0.1	26.2 ±0.3	30.7 ±0.1	32.7 ±0.2	25.6 ±2.0	30.5 ±0	34.0 ±1.2
Proline	11.6 ±0.1	19.0 ±0.1	19.8 ±0.1	15.9 ±0.2	18.2 ±0.1	19.7 ±0.2	14.7 ±0.9	17.2 ±0.1	19.6 ±0.4
Tyrosine	14.3 ±0.2	23.3 ±0.1	24.1 ±0.1	19.4 ±0.1	21.7 ±0	25.4 ±0	18.5 ±1.6	20.7 ±0.1	24.6 ±0
Cysteine	5.7 ±0.1	10.5 ±0.5	13.9 ±0.2	10.6 ±0.4	9.7 ±0.9	12.8 ±0.8	8.4 ±0.9	10.2 ±0.5	11.0 ±0.6
Total	182.2 ±0.1	348.3 ±1.1	359.6 ±0.6	282.2 ±0.2	321.3 ±0.8	385.2 ±0.1	258.7 ±22.5	313.0 ±0.1	351.3 ±3.5

*Means ±standard deviation of three determinations.

Table 4. Amino acid score (%)* of three Malaysian egg samples prepared by two different methods

Amino acid	Regular egg			*Kampung* egg			Nutrient enriched egg			Standard FAO/ WHO [1991] g/100 g protein
	raw	hard-boiled	half-boiled	raw	hard-boiled	half-boiled	raw	hard-boiled	half-boiled	
Valine	45.1	84.6	103.4	69.1	75.5	84.6	60.6	77.4	85.1	3.5
Histidine	48.4	68.9	75.8	60.0	66.3	73.2	54.2	59.5	68.9	1.9
Methionine + Cysteine	62.4	125.2	140	96.8	111.2	131.6	90.4	119.2	127.2	2.5
Isoleucine	47.8	85.0	89.6	70.7	78.2	85.7	63.2	76.4	85.0	2.8
Leucine	38.2	65.9	70.6	57.1	64.2	69.4	50.9	61.9	67.4	6.6
Phenylalanine + tyrosine	45.6	87.6	87.5	66.9	77.1	93.9	66.2	78.1	91.1	6.3
Lysine	47.0	74.6	81.7	70.8	71.7	83.9	62.9	69.5	75.9	5.8

*Means ±standard deviation of three determinations.

47.3 ±0.4, 87.5 ±1.2 and 89.5 ±0.3 mg/g protein, respectively, and in raw, hard-boiled and half-boiled *KE* were 67.3 ±0.7, 77.8 ±1.8 and 87.1 ±0.7 mg/g protein. The aspartic acid content of NEE in the same sequence was 60.4 ±6.8, 76.9 ±0.7 and 84.7 ±0.2 mg/g protein, respectively. From the results of analysis obtained, it was found that there were significant differences ($p < 0.005$) of aspartic, glumatic and serine acids content in regular, *kampung* and nutrient enriched eggs after preparation methods (hard and half-boiling) as compared with raw egg samples. From Table 3 glycine, arginine, alanine, proline, tyrosine, cysteine non-essential amino acids showed less amino acid content with cysteine as the lowest in the range of 5.7-13.9 mg/g protein. The glycine content of raw egg samples was in range of 8.8-13.5 mg/g proteins. Raw *KE* showed the highest glycine content that was 13.5 ±0.1, followed by nutrient enriched and regular eggs, which were 12.8 ±1.1 and 8.8 ±0 mg/g protein, respectively. Although there is numerous studies on amino acid composition in food have been studied [Alajaji and El-Adaw 2006, Sosulski and Imafidon 1990, Woo et al. 1996, Sarwar et al. 1983, Heems et al. 1998], for best of our knowledge there has been limited studies conducted on the protein quality of egg especially on the basis of effects of food processing or preparation methods. From the results obtained, it was found that there was a tendency of increment of amino acid content when the samples undergoing preparation methods (hard-boling and half-boiling) as compared with the raw egg samples. These findings were in good agreement with Steiner-Asiedu et al. [1991] study, in which there was an increase of amino acid content after undergoing processing methods. In such study, the effect of cooking, frying and smoking on the amino acid composition was studied on three different fishes. As mentioned, there is a limited study conducted on the effect of preparation methods on egg, therefore the findings of Steiner-Asiedu et al. [1991] study were used because fish is also another animal protein source that contains high amount of protein and essential amino acids. Furthermore, as reported by Powrie [1984], mild heat treatments in the presence of water can significantly improve the protein's nutritional value in some cases. In other words, hard-boiling and half-boiling in mild heat condition can increase the amino acid composition since the amount of amino acid present is one of the determinants of the protein quality.

According to the analysis obtained, in regular and *kampung* eggs, there were significant differences ($p < 0.05$) of essential amino acids content (histidine, isoleucine and leucine) and non-essential amino acids content (serine, glycine, arginine, alanine,proline and tyrosine) in hard-boiled and half-boiled eggs as compared with raw egg samples. Besides, for nutrient enriched eggs, there were significant difference

($p < 0.05$) of essential amino acids content (histidine, isoleucine and leucine) and non-essential amino acids content (serine, glycine, argine, alanine proline and tyrosine) between raw and half-boiled eggs but no significant differences between raw and hard-boiled eggs.

From the data analysis obtained, there were significant differences ($p < 0.05$) of essential amino acids content (valine and methionine) and non-essential amino acids content (aspartic acid and glumatic acid) in hard-boiled and half-boiled regular, *kampung* and NEE as compared with raw egg samples. In other words, there were significant effects of preparation methods (hard-boiling and half-boiling) on such amino acids content.

Amino acid score

A number of methods have been used to determine the quality of protein foods. One of these methods is by calculating the amino acid score, which is also referred to as chemical score. Although egg is often used as reference protein in calculating the amino acid score to measure the protein quality of food [Sakanaka et al. 2000], the effect of heat treatments (hard boiling and half boiling) on the protein quality was investigated in this study. The amino acid with the lowest score is designated as the limiting amino acid [Mahan and Escott-Stump 1996]. The amino acid scores of the various egg samples are presented in Table 4. From this table the limiting amino acid is leucine and the amino acid score for leucine in raw, hard-boiled and half-boiled RE were 38.2, 65.9 and 70.6, respectively. In *kampung* eggs, the amino acid score for leucine in raw, hard-boiled and half-boiled eggs were 57.1, 64.2 and 69.4, respectively, while in NEE the amino acid score for leucine in raw, hard-boiled and half-boiled eggs were 50.9, 61.9 and 67.4, respectively.

From the results obtained, the amino acid score for histidine in raw, hard-boiled and half-boiled RE were 48.4 ±0.0, 68.9 ±0.0 and 75.8 ±0.0, respectively. In *kampung* eggs, the amino acid score for histidine in raw, hard-boiled and half-boiled eggs were 60.0 ±0.0, 66.3 ±0.0 and 73.2 ±0.0 whereas the amino acid score for histidine in NEE in the same sequence were 54.2 ±0.0, 59.5 ±0.0 and 68.9 ±0.0, respectively (Table 4). Some samples have an amino acid score of 100 or even more (Met + Cys), since all values greater than 100 are considered to be 100 [Harper and Yoshimura 1993], histidine is considered the second limiting amino acid. The amino acid score for valine in raw, hard-boiled and half-boiled RE were 45.1, 84.6 and 103.4, respectively. In *kampung* eggs, the amino acid score for valine in raw, hard-boiled and half-boiled eggs were 69.1, 75.5 and 84.6 whereas the amino acid score for valine in NEE in the same sequence were 60.6, 77.4 and 85.1, respectively (Table 4). In the case of methionine plus cysteine, the amino acid score in raw, hard-boiled and half-boiled RE were 62.4, 125.2 and 140.0 whereas KE in the same sequence were 96.8, 111.2 and 1131.6, respectively. While the amino acids score for methionine plus cysteine in NEE in the same sequence were 90.4, 119.2 and 127.2, respectively (Table 4). The amino acid score for isoleucine in raw, hard-boiled and half-boiled RE were 47.8, 85.0 and 89.6, respectively. In *kampung* and nutrient enriched eggs, the amino acid score for isoleucine in raw, hard-boiled and half-boiled eggs were the same, which were 70.7, 78.2 and 85.7, respectively (Table 4).

Besides, the amino acid score for leucine in raw, hard-boiled and half-boiled RE were 38.2, 65.9 and 70.6, respectively. In *kampung* eggs, the amino acid score for leucine in raw, hard-boiled and half-boiled eggs were 57.1, 64.2 and 69.4 whereas the amino acid score for leucine in NEE in the same sequence were 50.9, 61.9 and 67.4, respectively (Table 4). In the case of phenylalanine plus tyrosine, the amino acid score in raw, hard-boiled and half-boiled RE were 45.6, 87.6 and 7.5, respectively. In *kampung* and nutrient enriched eggs, the amino acid score for phenylalanine plus tyrosine in raw, hard-boiled and half-boiled eggs were similar, which were 66.9, 77.1 and 93.9, respectively (Table 4). On the other hand, the amino acid score for lysine in raw, hard-boiled half-boiled RE were 47.0, 74.6 and 81.7, respectively. In *kampung* eggs, the amino acid score for lysine in raw, hard-bolied and half-boiled eggs were 70.8, 71.7 and 83.9 whereas the amino acid scores for lysine in NEE in the same sequence were 62.9, 69.5 and 75.9, respectively (Table 4).

From literature effect of different traditional and microwave processing methods on other foods were found. In-vitro protein digestibility, protein efficiency ratio and essential amino acid index of chickpea (*Cicer arietinum* L.) seeds were improved by all cooking treatments. The chemical score and limiting amino acid of chickpeas subjected to the various cooking treatments varied considerably, depending on the type of treatment [Alajaji and El-Adaw 2006]. Data presented in Table 4 show boiling (hard and half boiling) caused a significant increase in amino acid score. According to the statistical analysis (ANOVA), there were significant differences ($p < 0.05$) of amino acid score in regular, *kampung* and NEE after undergoing hard-boiling and half-boiling as compared with raw egg samples. These findings were in disagreement with Alajaji and El-Adaw [2006], who reported that boiling, auto-calve and microwave treatments slightly decreased amino acid scores of chickpea seeds. Hence, the preparation methods or heat treatments affect the amino acid score, which in turn affect the protein quality of the egg samples. This may be due to the mild heat treatments in the presence of water which significantly improve the protein's nutritional value [Powrie 1984].

CONCLUSIONS

The effects of hard-boiling and half-boling on the protein and amino acid composition, as well as the protein quality (amino acid score) of three different types of eggs available in Malaysian local markets were studied. The eggs studied include regular, *kampung* and nutrient enriched eggs. There were significant differences ($p < 0.05$) of protein content in raw, hard-boiled and half-boiled regular and *kampung* eggs. In nutrient enriched eggs, there was a significant difference ($p < 0.05$) of protein content between raw and hard-boiled eggs but no significant difference between raw and half-boiled eggs. In the of case amino acid composition, lysine showed the highest content but histidine showed the lowest content among the essential amino acids in all egg samples. On the other hand, aspartic acid and cysteine showed the highest and lowest value among the non-essential amino acids, respectively. Boiling treatments significantly ($p < 0.05$) increased the total and individual amino acid, as well as amino acid score in RE, *KE*, and NEE.

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