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Changes on Amino Acids Content in Soybean, Garbanzo Bean and Groundnut during Pre-treatments and *Tempe* Making

(Perubahan pada Kandungan Asid Amino di dalam Kacang Soya, Kacang Kuda dan Kacang Tanah semasa Proses Pra-olahan dan Pembuatan Tempe)

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ABSTRACT

Fermentation of legumes is a well-recognised preserving method that not only diversifies the sensory attributes of the legume-based foods but also enhances its nutritional value. This study aimed to evaluate the changes in amino acids content of soybean, garbanzo bean and groundnut upon pre-treatments (soaking and steaming) and fermentation times (18, 24 and 30 h). The amino acids analysis was performed with a HPLC system using AccQTag method. The results obtained showed that raw soybean contain the highest amount of essential and non-essential amino acids followed by raw garbanzo bean and groundnut. After the soaking process, the amount of amino acids obtained was significantly increased in all three legumes. However, the steaming process was found to cause significant decrease in the amino acids content. After the steaming process, the legumes were inoculated with Rhizopus oligosporus *and incubated at 30°C and 65% RH. At 18 h of fermentation time, the amount of total amino acids were found to increase by 26% in fermented groundnut and 16% in both soybean and garbanzo bean compared to after steaming. Further fermentation time of 24 h shows further enhancement in the amino acids content in all three legumes. Groundnut shows the highest increased of 71% in total amino acids content followed by 63% increased in garbanzo bean and 53% increase in soybean. However, prolong fermentation time of 30 h shows a decrease in total amino acids content in all three legumes which indicates the slow process of mould degradation have occurred. Therefore, fermented legumes must be consumed at the appropriate time of fermentation in order to get the benefit from the highest accumulation of amino acids content.*

Keywords: Amino acids; fermentation; legumes; pre-treatments

ABSTRAK

Fermentasi kekacang merupakan kaedah pengawetan yang diketahui bukan sahaja dapat mempelbagaikan sifat sensori bagi makanan berasaskan kekacang tetapi juga meningkatkan nilai nutrisinya. Kajian ini bertujuan untuk menilai perubahan pada kandungan asid amino kacang soya, kacang kuda dan kacang tanah ketika pra-olahan (merendam dan mengukus) dan pada masa fermentasi (18, 24 dan 30 jam). Analisis asid amino telah dilaksanakan dengan sistem HPLC menggunakan kaedah AccQ·Tag. Keputusan yang diperoleh menunjukkan bahawa kacang soya mentah mengandungi jumlah tertinggi asid amino perlu dan tidak perlu, diikuti oleh kacang kuda mentah dan kacang tanah. Selepas proses merendam, jumlah asid amino yang diperoleh telah meningkat dengan ketara pada ketiga-tiga kekacang. Walau bagaimanapun, proses mengukus didapati telah menyebabkan penurunan ketara dalam kandungan asid amino. Selepas proses mengukus, kekacang telah diinokulasi dengan Rhizopus oligosporus *dan diinkubasi pada suhu 30°C dan kelembapan udara 65%. Pada masa fermentasi 18 jam, jumlah kandungan asid amino didapati meningkat sebanyak 26% pada kacang tanah yang telah difermentasi dan 16% dalam kedua-dua kacang soya dan kacang kuda berbanding selepas pengukusan. Fermentasi seterusnya selama 24 jam menunjukkan peningkatan dalam kandungan asid amino di dalam ketiga-tiga kekacang. Kacang tanah menunjukkan peningkatan yang tertinggi sebanyak 71% dalam jumlah kandungan asid amino diikuti dengan peningkatan 63% dalam kacang kuda dan peningkatan 53% dalam kacang soya. Walau bagaimanapun, masa fermentasi lanjut selama 30 jam menunjukkan penurunan dalam jumlah kandungan asid amino yang menunjukkan proses degradasi kulat telah berlaku. Oleh itu, kekacang yang difermentasi mestilah dimakan pada masa fermentasi yang tertentu bagi mendapat manfaat daripada pengumpulan tertinggi dalam kandungan asid amino.*

Kata kunci: Asid amino; fermentasi; kekacang; proses pra-olahan

INTRODUCTION

Food legumes are derived from Leguminosae family, also called Fabacae and classified as vegetables. Legumes play an important role in human nutrition since they are rich sources of carbohydrate, protein, minerals (zinc, calcium and magnesium) and vitamins (vitamin E, niacin, riboflavin and thiamine). They have been reported to contain adequate amounts of lysine, except for the sulphur-containing amino acids, which are methionine and cystine (Iqbal et al. 2006; Pisulewska & Pisulewski 2000).

Soybean (*Glycine max* (L.) Merr.) is a major source of protein, energy, poly-unsaturated fats, fiber, vitamins, minerals and other nutrients for humans and livestock. It contains an average of 36-38% protein (Brumm & Hurburgh 2002; Krishnan 2000, 2005; Nielsen et al. 1997; Zarkadas et al. 1993, 1999) and high in glutamic acid, aspartic acid and leusine content (Song et al. 2008). Garbanzo bean (*Cicer arietinum* L.) is the third most important legume in the world and it's protein content ranges from 23 to 25% (Abu-Salem & Abou-Arab 2011). Groundnut (*Arachis hypogaea* L.) is also an important source of vegetable protein with protein content of 26 to 30% (Singh et al. 2008).

Legumes are mostly subjected to boiling, frying, roasting or baking before consumption. In Asia, soybeans are commonly consumed as soymilk or processed into fermented products such as soy sauce and *tempe*. Garbanzo bean is commonly being eaten after boil, stew and become snack while groundnut is eaten after frying besides being an ingredient in other food products.

Home practices such as soaking, dehulling and cooking are some of the effective ways to improve the nutritional values of legumes. In the production of *tempe*, pre-treatments such as soaking, dehulling and cooking are steps to prepare the substrate for enzymes breakdown by mould (Egounlety & Aworh 2003). Cooking also destroys the heat-labile anti-nutritional factors (Chau et al. 1997), but it may cause changes in the composition of numerous chemical constituents such as amino acids, vitamins and minerals depending on the temperature and time of thermal treatment used (Lisiewska et al. 2008; Slupski 2010).

Fermentation process represents an alternative technique for improving the nutrient values of legumes and cereals besides maintaining the acceptability of sensory properties (Cuevas-Rodriguez et al. 2005; Osman et al. 2010). Fermentation has been reported to enhance taste, aroma and texture, to prolong the shelf life, converts insoluble proteins to soluble components and increases the level of lysine and vitamin B and C (Steinkraus 2002). According to Sarkar et al. (1997) Bacillus fermented soybean led to an increase in free amino acids and ammonia by 60- and 40-fold, respectively.

This work was aimed to study the changes on amino acids content of soybean (*Glycine max* (L.) Merr.), garbanzo bean (*Cicer arietinum* L.) and groundnut (*Arachis hypogaea* L.) of raw, after pre-treatments (soaking and steaming) and fermentation time.

Materials and Methods

Materials

Soybean (*Glycine max* (L.) Merr.), garbanzo bean (*Cicer arietinum* L.) and groundnut (*Arachis hypogaea* L.) were purchased at local Mydin supermarket, Subang Jaya, Selangor, Malaysia. The species were confirmed by Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia. *Tempeh* inoculum in powder form (*Rhizopus oligosporus*) was bought from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. The amino acids standard was purchased from Thermo Scientific, Rockford, U.S.A. while the chemicals of HPLC grade were bought from Chemolab Supplies, Selangor, Malaysia.

Production of *Tempe*

Tempe was produced in a clean and controlled environment. Soybean, garbanzo bean and groundnut were weighed and pre-wash before being soaked in tap water for 18 h (1:3 v/v). After soaking and manual dehulling, the legumes were steamed in hot steamer (Food Steamer R6781, Cornell, Malaysia) for 90 min, followed by draining and cooling to room temperature. The legumes were then inoculated with *tempe* mould (*Rhizopus oligosporus*) (2 g/kg dry legume). After that, the inoculated legumes were packed into perforated polyethylene bags 17.64 × 25.20 cm and incubated in climatic chamber (Model KBF 115, Binder GmbH, Tuttlingen, Germany) at 30°C and 65% RH.

Sample Preparation

Prior to analysis, raw soybean, garbanzo bean and groundnut were ground to a powder form while for the pre-treatments samples (soaked and steamed legumes) they were first dried in a cabinet drier at 50°C for 24 h before grinding. For fermented legumes (*tempe*) sample preparation, the fermentation process was stopped by submerging the *tempe* into liquid nitrogen before grinding. The powdered samples obtained were sieved through a 250 µm sieve shaker (AS300, Retsch GmbH, Tuttlingen Germany), tightly sealed in plastic container and stored at 5°C until further used.

Amino Acids Analysis

Amino acids content were analysed using AccQTag method and performed by High Performance Liquid Chromatography (HPLC) as described by Seo (2005). The pre-weigh sample was hydrolysed with 5 mL of 6 N HCl in an oven at 110°C for 24 h. Hydrolysis is important to destroy fat and carbohydrate. Then, the hydrolysate obtained was cooled and filtered using filter paper (Whatman, 41 µm). A 4 mL stock of AABA was added and made up to 100 mL with deionised water and the hydrolysate sample was filtered again through 0.2 µm membrane filter cellulose acetate. Sample mixtures of amino acids standard and blank were prepared by adding 10 µL of them with 70 µL borate buffers and 20 µL AccQ reagent. A 5 µL of the samples, standard and blank were injected for analysis by HPLC. In this study, tryptophan was not analysed due to their sensitivity on acidic hydrolysis.

The HPLC Waters 1525 system (Waters Corp., MA USA) was equipped with Waters 1525 binary HPLC pump and a Waters 2475 multi λ fluorescent detector. The amino acids were separated using AccQTag column 3.9 × 150 mm (Waters Corp., MA USA) using a 25 µL micro syringe (SGE Analytical Science, Australia). The multiple steps gradient

elution started from 100% solvent A (1:10; AccQTag Eluent A and deionised water) and change to 100% solvent B at min 34 (60% acetonitrile). After that, it was ended with 100% solvent A at min 38. The mobile phase was filtered using a 0.45 μm nylon filter membrane and the flow rate was maintained at 1 mL/min. The column temperature was controlled at 36°C and the detector temperature was set at 40°C with excitation and emission wavelengths of 250 and 395 nm, respectively.

The data was collected and analysed using Breeze software on a PC Dell Vostro computer connected to HPLC apparatus. The concentration for every amino acid was calculated using the average peak areas compared with the standard and expressed as g/100 g of samples.

STATISTICAL ANALYSIS

The data obtained were analysed by ANOVA, posthoc tukey test were used by employing Statistical Packages for the Social Sciences (SPSS 15) for windows XP. All experiments were carried out in triplicate and means were considered with a significance level of 5%.

RESULTS AND DISCUSSION

AMINO ACIDS COMPOSITION

The importance of essential amino acids is well known and their intake is crucial in our diet since human bodies are unable to synthesise it. The non-essential amino acids are amino acids that can be produced in our body and they are equally important as the essential amino acids in development of our growth. For examples, alanine helps in the metabolism of glucose and organic acids while arginine helps to maintain a proper nitrogen balance and aid in the excretion of excess nitrogen. Cysteine and proline aids in the production of collagen and promotes proper elasticity and texture of the skin, besides reducing the loss of collagen through the aging process (Brennan et al. 2002; McConell 2007).

In this study, 8 types of essential amino acids were determined which were histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine. Another 9 types of non-essential amino acids, which were alanine, arginine, aspartic acid, cystiene, glutamic acid, glysine, proline, serine and tyrosine were also measured. Tables 1, 2 and 3 show the essential and non-essential amino acids content of raw, after soaking and steaming also at different fermentation times of soybean, garbanzo bean and groundnut, respectively.

On overall, the results showed that soybean contain the highest amount of total amino acids content in raw, after pre-treatments and during fermentation followed by garbanzo bean and then groundnut. Glutamic acid was the highest contributor in the total amount of amino acids in all three legumes. Raw soybean contains 8.88±0.10 g/100 g of glutamic acid followed by aspartic acid (5.20±0.02 g/100 g). Similarly, raw

TABLE 1. Amino acids content in raw, after pre-treatments and fern ation time of soybean (g/100 g)

Amino acids	Pre-treatments			Fermentation time		
	Raw	Soaking	Steaming	18 h	24 h	30 h
Histidine	0.83±0.02^{d}	0.96±0.02^{c}	0.68±0.01^{e}	0.96±0.05^{c}	1.55±0.02^{a}	1.42±0.01^{b}
Isoleucine	1.62±0.04^{e}	1.76±0.01^{d}	1.54±0.03^{f}	1.97±0.01^{c}	3.01±0.01^{a}	2.90±0.01^{b}
Leusine	2.81±0.02^{d}	2.97±0.05^{c}	2.72±0.01^{e}	2.98±0.02^{c}	4.85±0.01^{a}	4.75±0.01^{b}
Lysine	2.03±0.02^{e}	2.15±0.05^{d}	1.92±0.08^{f}	2.32±0.05^{c}	3.67±0.03^{a}	3.29±0.04^{b}
Methionine	0.45±0.01^{d}	0.55±0.03^{c}	0.41±0.01^{e}	0.54±0.01^{c}	1.13±0.01^{a}	1.04±0.03^{b}
Phenylalanine	2.06±0.03^{e}	2.17±0.03^{d}	1.99±0.01^{f}	2.34±0.02^{c}	3.78±0.01^{a}	3.54±0.01^{b}
Threonine	0.59±0.03^{e}	0.63±0.01^{d}	0.52±0.01^{f}	0.76±0.01^{c}	0.92±0.01^{a}	0.87±0.01^{b}
Valine	2.16±0.27^{f}	2.66±0.04^{d}	2.29±0.01^{e}	2.87±0.01^{c}	3.44±0.02^{a}	3.33±0.01^{b}
Essential amino acids	*12.55±0.30^{e}*	*13.85±0.13^{d}*	*12.07±0.19^{f}*	*14.74±0.42^{c}*	*22.35±0.35^{a}*	*21.14±0.13^{b}*
Alanine	1.55±0.02^{d}	1.74±0.04^{c}	1.52±0.02^{e}	1.75±0.01^{c}	2.06±0.04^{a}	1.82±0.01^{b}
Arginine	0.22±0.03^{d}	0.25±0.01^{d}	0.21±0.04^{d}	0.34±0.01^{c}	0.69±0.01^{a}	0.57±0.02^{b}
Aspartic acid	5.20±0.02^{e}	5.41±0.02^{c}	5.08±0.01^{f}	5.36±0.01^{d}	5.86±0.05^{a}	5.71±0.01^{b}
Cystiene	0.69±0.03^{e}	0.84±0.01^{d}	0.61±0.01^{f}	0.97±0.01^{c}	1.44±0.01^{a}	1.32±0.01^{b}
Glutamic acid	8.88±0.10^{bc}	9.30±0.38^{b}	8.61±0.14^{d}	9.00±0.01^{bc}	9.88±0.02^{a}	9.76±0.02^{b}
Glysine	2.04±0.05^{e}	2.40±0.04^{c}	1.89±0.09^{f}	2.28±0.05^{d}	2.75±0.15^{a}	2.55±0.02^{b}
Proline	1.77±0.03^{e}	1.85±0.02^{d}	1.62±0.02^{f}	1.97±0.02^{c}	2.50±0.05^{a}	2.37±0.01^{b}
Serine	0.23±0.02^{e}	0.37±0.01^{c}	0.18±0.01^{f}	0.32±0.01^{d}	0.55±0.01^{a}	0.51±0.01^{b}
Tyrosine	1.51±0.07^{e}	1.65±0.03^{d}	1.35±0.04^{f}	1.76±0.01^{c}	2.39±0.02^{a}	2.21±0.01^{b}
Non-essential amino acids	*22.09±0.22^{e}*	*23.81±0.47^{d}*	*21.07±0.28^{f}*	*23.75±0.26^{c}*	*28.12±0.21^{a}*	*26.82±0.12^{b}*
Total amino acids content	34.64±0.26	37.66±0.30	33.14±0.24	38.49±0.34	50.47±0.28	47.96±0.13

Data are mean ±SD (standard deviation) of n=3
Means with different letter for the same row are significantly different (p<0.05)

TABLE 2. Amino acids content in raw, after pre-treatments and fermentation time of garbanzo bean (g/100 g)

Amino acids	Pre-treatments			Fermentation time		
	Raw	Soaking	Steaming	18 h	24 h	30 h
Histidine	0.79±0.01^{e}	0.83±0.01^{d}	0.73±0.01^{f}	0.88±0.02^{c}	1.33±0.01^{a}	1.29±0.02^{b}
Isoleucine	1.31±0.10^{cd}	1.29±0.01^{d}	1.20±0.01^{e}	1.32±0.01^{c}	2.11±0.01^{a}	2.04±0.04^{b}
Leusine	1.89±0.09^{e}	2.02±0.01^{d}	1.54±0.02^{g}	1.68±0.06^{f}	2.33±0.01^{a}	2.17±0.03^{b}
Lysine	0.99±0.13^{cd}	0.97±0.02^{d}	0.88±0.01^{d}	1.11±0.01^{c}	1.73±0.03^{a}	1.64±0.01^{b}
Methionine	0.74±0.10^{d}	0.76±0.01^{d}	0.75±0.01^{d}	0.79±0.02^{c}	1.28±0.01^{a}	1.20±0.01^{b}
Phenylalanine	1.78±0.02^{d}	1.78±0.01^{d}	1.69±0.01^{e}	1.87±0.02^{c}	2.30±0.02^{a}	2.17±0.03^{b}
Threonine	0.77±0.02^{d}	0.91±0.04^{c}	0.65±0.01^{e}	0.86±0.02^{c}	1.32±0.01^{a}	1.24±0.02^{b}
Valine	0.84±0.06^{d}	0.93±0.01^{c}	0.79±0.12^{d}	0.95±0.03^{c}	1.36±0.01^{a}	1.19±0.01^{b}
Essential amino acids	*9.11±0.11^{d}*	*9.49±0.26^{c}*	*8.23±0.24^{e}*	*9.46±0.10^{c}*	*13.76±0.16^{a}*	*12.94±0.14^{b}*
Alanine	0.71±0.02^{e}	0.74±0.02^{d}	0.65±0.01^{f}	0.93±0.02^{c}	1.38±0.01^{a}	1.16±0.03^{b}
Arginine	1.72±0.08^{cd}	1.76±0.02^{c}	1.22±0.01^{e}	1.78±0.02^{c}	2.43±0.01^{a}	2.18±0.01^{b}
Aspartic acid	4.28±0.03^{e}	4.34±0.01^{d}	4.18±0.02^{f}	4.59±0.05^{c}	6.22±0.01^{a}	6.07±0.05^{b}
Cystiene	0.44±0.03^{de}	0.46±0.01^{d}	0.41±0.01^{e}	0.58±0.05^{c}	0.76±0.01^{a}	0.65±0.01^{b}
Glutamic acid	6.30±0.36^{cde}	6.43±0.12^{c}	6.15±0.13^{e}	6.35±0.03^{cd}	8.87±0.09^{a}	8.05±0.01^{b}
Glysine	0.75±0.03^{e}	0.88±0.02^{c}	0.62±0.05^{f}	0.83±0.01^{d}	1.24±0.01^{a}	1.17±0.01^{b}
Proline	0.88±0.02^{d}	0.96±0.03^{c}	0.88±0.01^{d}	0.95±0.03^{c}	1.47±0.03^{a}	1.28±0.01^{b}
Serine	0.92±0.06^{cd}	0.98±0.01^{c}	0.86±0.12^{d}	1.20±0.08^{b}	1.44±0.01^{a}	1.19±0.01^{b}
Tyrosine	0.54±0.11^{e}	0.67±0.01^{c}	0.40±0.02^{e}	0.62±0.01^{d}	1.00±0.01^{a}	0.81±0.04^{b}
Non-essential amino acids	*16.54±0.23^{e}*	*17.22±0.17^{d}*	*15.37±0.13^{f}*	*17.83±0.48^{c}*	*24.81±0.36^{a}*	*22.56±0.15^{b}*
Total amino acids content	25.65±0.17	26.71±0.22	23.60±0.19	27.29±0.29	38.57±0.26	35.50±0.15

Data are mean ±SD (standard deviation) of n=3
Means with different letter for the same row are significantly different (p<0.05)

TABLE 3. Amino acids content in raw, after pre-treatments and fermentation time of groundnut (g/100 g)

Amino acids	Pre-treatments			Fermentation time		
	Raw	Soaking	Steaming	18 h	24 h	30 h
Histidine	0.93±0.07^{de}	0.98±0.02^{d}	0.92±0.01^{e}	1.04±0.01^{c}	1.50±0.04^{a}	1.24±0.02^{b}
Isoleucine	0.84±0.05^{de}	0.89±0.01^{d}	0.81±0.01^{e}	1.01±0.02^{c}	1.36±0.04^{a}	1.28±0.05^{ab}
Leusine	1.57±0.02^{e}	1.67±0.03^{d}	1.26±0.04^{f}	1.74±0.04^{c}	2.80±0.07^{a}	2.55±0.01^{b}
Lysine	0.82±0.02^{e}	0.92±0.01^{cd}	0.75±0.02^{f}	0.96±0.03^{c}	1.47±0.03^{a}	1.31±0.08^{b}
Methionine	0.36±0.03^{d}	0.48±0.02^{c}	0.22±0.01^{e}	0.55±0.03^{b}	0.60±0.03^{a}	0.54±0.05^{ab}
Phenylalanine	1.55±0.04^{e}	1.76±0.02^{d}	1.25±0.02^{f}	1.94±0.04^{c}	2.94±0.04^{a}	2.70±0.05^{b}
Threonine	0.73±0.03^{e}	0.75±0.01^{d}	0.73±0.01^{e}	0.95±0.03^{c}	1.23±0.03^{a}	1.09±0.03^{b}
Valine	1.00±0.13^{d}	1.25±0.06^{c}	0.96±0.01^{d}	1.60±0.06^{b}	1.86±0.03^{a}	1.58±0.06^{b}
Essential amino acids	*7.80±0.06^{e}*	*8.70±0.23^{d}*	*6.90±0.26^{f}*	*9.79±0.10^{c}*	*13.76±0.13^{a}*	*12.29±0.11^{b}*
Alanine	0.93±0.02^{e}	0.98±0.02^{d}	0.94±0.03^{e}	1.02±0.02^{c}	1.47±0.03^{a}	1.37±0.03^{b}
Arginine	2.89±0.06^{d}	2.98±0.01^{c}	2.67±0.03^{e}	3.12±0.10^{b}	3.96±0.01^{a}	3.04±0.05^{b}
Aspartic acid	2.87±0.03^{d}	2.94±0.04^{d}	2.70±0.04^{e}	3.01±0.02^{c}	3.58±0.06^{a}	3.34±0.07^{b}
Cystiene	0.27±0.05^{d}	0.35±0.03^{c}	0.21±0.01^{e}	0.51±0.01^{a}	0.43±0.03^{b}	0.40±0.01^{b}
Glutamic acid	4.85±0.15^{d}	4.99±0.01^{d}	4.34±0.03^{e}	5.06±0.03^{c}	6.57±0.32^{a}	5.77±0.32^{b}
Glysine	0.56±0.03^{d}	0.57±0.02^{d}	0.48±0.03^{e}	0.67±0.07^{c}	1.37±0.04^{a}	1.24±0.09^{b}
Proline	0.73±0.01^{e}	0.83±0.02^{d}	0.71±0.02^{e}	0.92±0.01^{c}	1.21±0.01^{a}	1.05±0.03^{b}
Serine	1.22±0.01^{e}	1.28±0.02^{d}	1.02±0.04^{f}	1.57±0.03^{c}	2.04±0.03^{a}	1.98±0.01^{b}
Tyrosine	1.08±0.01^{d}	1.17±0.05^{c}	0.98±0.02^{e}	1.19±0.06^{c}	2.02±0.01^{a}	1.95±0.02^{b}
Non-essential amino acids	*15.40±0.04^{e}*	*16.09±0.07^{d}*	*14.45±0.43^{f}*	*17.07±0.16^{c}*	*22.65±0.32^{a}*	*20.14±0.39^{b}*
Total amino acids content	23.20±0.05	24.79±0.15	21.35±0.35	26.86±0.13	36.41±0.23	32.43±0.25

Data are mean ±SD (standard deviation) of n=3
Means with different letter for the same row are significantly different (p<0.05)

garbanzo bean shows the highest glutamic acid content of 6.30±0.36 g/100 g followed by 4.28±0.03 g/100 g of aspartic acid. Although glutamic acid was also the highest in raw groundnut (4.85±0.15 g/100 g) but the second highest amino acid was shown by arginine (2.89±0.06 g/100 g).

Among all essential amino acids, leusine was found to be of the highest content while threonine and methionine were the two lowest essential amino acids in all three legumes. Leusine contribute 20-22% from the total of essential amino acids in raw soybean, garbanzo bean and groundnut. The amounts of another 5 essential amino acids were found to vary in the legumes. In raw soybean, the decreasing order of essential amino acids after leusine was valine > phenylalanine > lysine > isoleusine > histidine. In raw garbanzo bean, the decreasing order was phenylalanine > isoleusine > lysine > valine > histidine. In raw groundnut, the decreasing order was phenylalanine > valine > histidine > isolesine > lysine. Similarly, the amount of remaining non-essential amino acids analysed were found to vary in the three legumes with the lowest amount shown by cysteine in garbanzo bean and groundnut however in soybean, arginine was found to be the lowest. Iqbal et al. (2006) have reported that legumes are deficient in sulphur-containing amino acids (methionine, cystine and cysteine). Similarly in this study, sulphur-containing amino acids were found to be in low amount.

Soaking is one of the common home practices conducted before the actual cooking of the legume took place. In this study, the studied legumes were subjected to soaking for 18 h and the amino acids were measured. The results obtained showed that soaking increases the amino acids content. Significant increment of 8.7% in total amino acids content was observed in soybean, 6.9% in groundnut and 4.1% in garbanzo bean due to soaking. The hydrolytic breakdown of the nutrient components during soaking may have caused the increase in amino acids content observed. Study by Ferial and Esmat (2011) also showed that soaking for 12 h increase the essential amino acids content in chickpea seeds by 8.76%.

In contrast to soaking, steaming causes decrease in the amino acids content of the three legumes. On overall, the total amino acids content in groundnut was the most reduced (-13.9%) due to denaturation of protein during steaming followed by soybean (-12.0%) and garbanzo bean (-11.6%). The highest reduction of essential amino acids was observed in steamed groundnut (-20.7%) then garbanzo bean (-13.3%) and the least affected was soybean (-12.9%). For non-essential amino acids content, the highest reduction was observed in soybean (-11.5%) followed by garbanzo bean (-10.7%) and groundnut (-10.2%).

Other researchers have also reported similar observations on the reduction effects of amino acids content due to steaming (Alajaji & El-Adawy 2006; Ferial & Esmat 2011; Pedrosa et al. 2011). It is well known that severe heating can limit protein digestibility and amino acid availability caused by denaturation of protein. However, according to Siddhuraju and Becker (2001), heating may cause formation of insoluble complexes between protein and phytate.

After fermentation of 18 h, the amount of all amino acids in all three legumes were found to increase significantly. The enzymatic breakdown of proteins by *R. oligosporus* increases essential amino acids in soybean by 22% and non-essential amino acids by 16% compared with after steaming. In groundnut, tremendous increase of 42% was observed in essential amino acids but in non-essential amino acids the increase obtained was by 18%. In garbanzo bean both essential and non-essential amino acids were increase by 15 and 16%, respectively.

Further fermentation at 24 h shows greater increase in amino acids in all three legumes studied. The increase of 99% in essential amino acids was observed in groundnut, 85% increase in soybean and 67% increase in garbanzo bean compared with after steaming. For non-essential amino acids, garbanzo bean shows the highest increase of 61% followed by groundnut (57%) and soybean (33%).

Prolong fermentation up to 30 h showed significant decrease in amino acids in all three legumes studied compared with fermentation at 24 h. Groundnut shows similar percentage of 11% decrease in both essential and non-essential amino acids while soybean shows 5% decrease in both essential and non-essential amino acids. However in garbanzo bean, 9% decrease was observed in non-essential amino acids and 6% decrease in essential amino acids.

Other researchers have also reported similar finding as in this study. Baumann and Bisping (1995) found that certain *Rhizopus* strains with high proteolytic activity were able to release nearly 5 times more amino acids than other strains. Ferial and Esmat (2011) observed 21.8% increases in essential amino acids in fermented chickpea with *Rhizopus* at 48 h of fermentation time. Similarly, Angulo-Bejarano et al. (2008) also studied the nutritional value of chickpea fermented with *R. oligosporus* at 34.9°C for 51.3 h. They reported an increase of 37, 41, 107 and 39 g/kg protein in essential amino acids, sulphur-containing amino acids, total aromatic amino acids and threonine, respectively.

Further fermentation until 30 h however limits the growth of mould due to depletion in food supply, oxygen, RH, a_w and increase in surrounding temperature caused by respiration of the microorganisms. According to Baumann and Bisping (1995), a biochemical mechanism such as transamination took place during fermentation that causes the high amount of amino acids release. Decomposition of proteins to amino acids will decrease when degradation of the mould life starts to occur (Okpokwasili & Nweke 2005). Different observation was obtained with fermentation of soybean using different microorganism. Sarkar et al. (1997) found an increase in free amino acids and ammonia by 60- and 40-fold in Bacillus fermented soybean. Song et al. (2008) reported that fermentation of soybean with *L. plantarum* and *B. lactis* caused increase in amino acids content while fermentation with *S. cerevisae* showed the opposite results.

CONCLUSION

The results in this study showed that among the three legumes studied, raw soybean contain the highest amount of amino acids followed by garbanzo bean and finally groundnut. In all three legumes, leusine was found to be the highest essential amino acids while glutamic acid was the highest of the non-essential amino acids. Soaking of legumes was found to be a beneficial home practice that increases the amount of amino acids through the hydrolytic process. However, steaming of legumes greatly reduces the amount of amino acids due to protein denaturation. Fermentation with *R. oligosporus* greatly enhanced the amount of all amino acids in the legumes. Drastic increase in amino acids content was observed at 18 h of fermentation time compared with after steaming but the greatest increment up to 99% was observed at 24 h of fermentation time in fermented groundnut. Further fermentation to 30 h however shows reduction between 5 and 11% of amino acids in the three legumes.

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