



# Estimation of Sulphur Containing Amino Acids in Soybean Products in Nigeria

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**Abstract** Five different samples of soybean products (Raw Soybeans Seed (RSS), Local Soybean Powdered Milk (LSPM), Local Soybean Liquid Milk (LSLM), Vita-milk and Chi-soymilk) were used for the analysis of sulphur containing amino acids (methionine and cysteine) contents. Qualitative analysis using thin layer chromatography technique was carried out on the soybean products to detect the presence of sulphur containing amino acids while spectrophotometric method involving methionine and cysteine standards were used to quantitatively estimate the two amino acids in the soybean products. The RF values (Retardation Factor) of 0.70 and 0.90 for methionine and cysteine standards respectively were used as control values. The presence of methionine in these five soybean products was detected with RF values of 0.69, 0.70, 0.70, 0.70 and 0.71 for RSS, LSPM, LSLM, Vita-milk and Chi-soymilk, respectively. The presence of cysteine in these five samples of soybean products was detected with RF values 0.91, 0.92, 0.90, 0.90 and 0.92 for the five samples. The result of qualitative analysis confirms the presence of methionine and cysteine in all the samples. From the result of the spectrophotometric analysis of the products, methionine concentration in (g/100ml) present in Vita-milk ( $0.90 \pm 0.70$ ) and Chi-soymilk ( $0.64 \pm 0.02$ ) is significantly higher ( $p < 0.05$ ) than in the control RSS ( $0.47 \pm 0.02$ ). Methionine concentration (g/100ml) present in Local Soybean Powdered Milk (LSPM), ( $0.22 \pm 0.02$ ) is significantly lower than RSS ( $0.47 \pm 0.02$ ) but not for Local Soybean Liquid Milk (LSLM) ( $0.50 \pm 0.02$ ). Cysteine concentration (g/100ml) present in Vita-milk ( $1.130 \pm 0.27$ ), Chi-soymilk ( $0.590 \pm 0.06$ ) and Local Soybean Liquid Milk ( $0.610 \pm 0.03$ ) is significantly higher ( $p < 0.05$ ) than the RSS control ( $0.130 \pm 0.03$ ). Also cysteine concentration (g/100ml) present in Local Soybean Powdered Milk ( $0.126 \pm 0.02$ ) is significantly lower ( $p < 0.05$ ) than the RSS control ( $0.130 \pm 0.03$ ). An increase in both methionine and cysteine concentrations for Chi-soymilk and Vita-milk suggests that these soybean products were enriched with these amino acids during processing, hence can effectively replenish the lost sulphur amino acids in the

body due to the action of trypsin inhibitor present in soybeans.

**Keywords** Soybean Products, Amino Acids, Sulphur Amino Acids

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## 1. Introduction

Soybean (*Glycine Max*), which belongs to the botanical family Leguminosae, is one of the important food resources that can combat diseases ascribed to mal- and under nutrition in developing countries [10]. On the other hand, raw soybean meals in the diet have been reported to cause growth retardation, impaired nutrient utilization, increased pancreatic secretion and pancreas enlargement in some monogastric animals and humans [4]. Serine proteinase inhibitors are considered to account for a significant part of these effects of raw soybeans [6]. Trypsin is one of the three principal digestive proteinases in the digestive tract of animals and humans [5]. In the digestive process, trypsin acts with the other proteinases to break down dietary protein molecules to their component peptides and amino acids. Kunitz Soybean Trypsin Inhibitor (KSTI) and Bowman-Birk Inhibitor (BBI) are the two major trypsin inhibitors in soybeans. These inhibitors are large, tightly folded proteins that are not completely deactivated during ordinary cooking [3]. Trypsin inhibitor present in soybean products inhibit the action of trypsin enzyme in the intestine leading to more synthesis of this enzyme and its subsequent loss from the body. Soybean processors have worked hard to get antinutrients (e.g. trypsin inhibitors) out of the finished product, particularly soybean protein isolate (SPI) which is the key ingredient in most soybean foods that imitate meat and dairy products, including baby formulas and some brands of soybean milk. Much of the trypsin inhibitor content can be removed through high-temperature processing, but not all. High temperature processing

performed to inhibit or denature the inhibitor activity has unfortunate side-effect of denaturing the other proteins in soybean. [8]. Since sulphur containing amino acids (cysteine and methionine) are the major amino acids involved in the synthesis of trypsin in the body, standard soybean products are supposed to be enriched with these two amino acids. This will enable the body to replenish the lost sulphur containing amino acids as a result of the action of the trypsin inhibitor present in the soybean product. The main part of this study is devoted to the estimation of sulphur containing amino acids present in soybean products in Nigerian market. The results of this study will be used to ascertain the quality of the soybean products in the market and to which extent the standard production procedures are followed.

## 2. Materials and Method

### 2.1. Materials

Raw Soybean Seed (RSS), Local Soybean Powdered Milk (LSPM), Local Soybean Liquid milk (LSLM), Vita-milk, Chi-soymilk, were purchased directly from producers in Enugu State Nigeria.

### 2.2. Chemicals and Reagents

The following chemicals were obtained from Sigma Aldrich Chemical Company. Phenol, ninhydrin, cysteine amino acid, methionine amino acid, petroleum ether, sodium chloride, distilled water, sodium hydroxide, acetone, acetic acid, phosphoric acid, concentrated hydrochloric acid (Conc. HCl) aluminium foil and 95% ethanol. All chemicals were of highest purity and of analytical grade.

### 2.3. Equipment

Ultraviolet spectrophotometer (CE Voltage 220Volts, frequency 50Hetz, power 40W, Model NO 722510154, B. Bran scientific instrument and scientific company England), water bath (model DK-420 power 220Volts50Hetz).

## 3. Methods

### 3.1. Thin-layer Chromatography Analysis

This method was used to detect the presence of the sulphur containing amino acids in the soybean products. Standard method for Thin-layer chromatographic procedure was used [9]. Briefly, the starting line, approximately 1 cm from the edge of the Thin-layer chromatographic paper was marked. The spots of individual amino acids and sample solutions were applied to the paper using a dry glass capillary. A mixture of n-butanol, acetic acid distilled water in volume ratio 5:1:5 were stirred for 10 minutes and allowed to

separate into layers. The upper layer was used as the solvent. The paper was inserted into the eluent chamber, the solvent front allowed to travel up the plate until 7-10 mm from the lid. The solvent front was marked with pencil, the paper dried in the oven and sprayed with ninhydrin solution to identify the solutes. The retardation factor (Rf) values for each of the solutes present in each soybean sample were measured using the formula below. The Rf values of methionine and cysteine were used as reference or control to detect their presence in each of the test sample. See Table 1 and 2.

$$RF = \frac{SF}{sf}$$
 Where *SF* is the distance travelled by the solute (solute front), *sf* is the distance travelled by the solvent (solvent front).

### 3.2. Sample Hydrolysis

The method used by Aishah Bujang & Nurul Akmal Taib [1] was adopted for sample hydrolysis. 5 ml of the soy sample was pipetted into a beaker and 0.5 ml of the acid ninhydrin was added. The mixture was capped with an aluminum foil, heated in a water bath for 10 min at a temperature of 37°C and cooled in a tap water for 5 min. 5 ml of 95% ethanol was added into the mixture. A reagent blank was also prepared at the same condition.

### 3.3. Quantification of Cysteine and Methionine by Spectrophotometric Method

Cystein and Methionine concentrations were determined by methods of Gaitonde [11] and Ferrel [12] respectively. An acid ninhydrin reacts with the samples to form a pink product upon hydrolysis, with maximum absorption at 560nm with cysteine and 510nm with methionine. The amount of cystein and methionine present in the soy samples were calculated by reference to a calibration curve (figure 1 and 2) obtained under similar conditions for known amounts of cysteine and methionine.

### 3.4. Statistical Analysis

The data were analyzed using the student's *t* test. The results were expressed as mean ±SEM at 95% confidence level. The significant level was taken at (*p* < 0.05).

## 4. Results

**Table 1.** Retardation factor (Rf) values for methionine amino acid

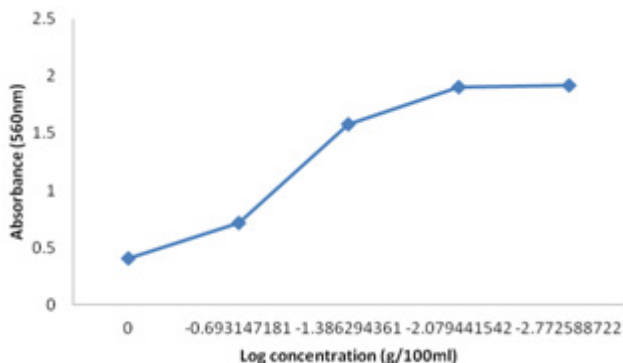
Soybean products	Retardation factor (Rf)
Methionine (control)	0.70
Raw Soybean Seed	0.29, 0.51, 0.59, 0.69, 0.32
Local Soybean Powdered Milk	0.32, 0.35, 0.70, 0.84, 0.80, 0.98
Local Soybean Liquid Milk	0.70, 0.95
Vita-milk	0.46, 0.69, 0.70, 0.89
Chi-soymilk	0.65, 0.71, 0.91

From Table 1.0 above, the presence of methionine amino acid was detected in all the soybean products. An Rf value of 0.70 for methionine was used as control. The five soybean products have Rf values close to the control value 0.69, 0.70, 0.70, 0.70, 0.71, respectively for Raw Soybean Seed, Local Soybean Powdered Milk, Local Soybean Liquid Milk, Vita-milk and , Chi-soymilk.

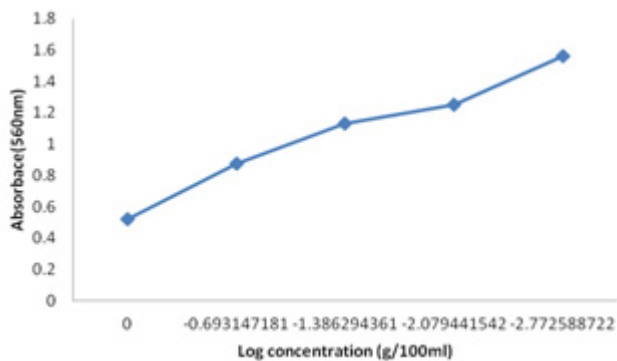
**Table 2.** Retardation factor (Rf) values for cysteine amino acid

Soybean products	Retardation factor (Rf) values
Cysteine (control)	0.90
Raw Soybean Seed	0.91, 0.51
Local Soybean Powdered Milk	0.24, 0.43, 0.92
Local Soybean Liquid Milk	0.26, 0.66, 0.72, 0.80, 0.90, 0.94
Vita-milk	0.45, 0.63, 0.72, 0.90
Chi-soymilk	0.11, 0.211, 0.611, 0.75, 0.92

From Table 2 above, the presence of cysteine amino acid was detected in all the soybean products. Rf values for 0.90 for cysteine was used as control. The five soybean products have Rf values close to the control value 0.91, 0.92, 0.90, 0.90, 0.92, respectively for Raw Soybean Seed, Local Soybean Powdered Milk, Local Soybean Liquid Milk, Vita-milk and Chi-soymilk.



**Figure 1.** Graph of Absorbance against Methionine concentration



**Figure 2.** Graph of Absorbance against Cystein concentration

Figure 1 and 2 above show the calibration curves of both methionine and cystein standards. The concentrations of both amino acids in the soybean products were read from the graph.

**Table 3.** Concentrations of cysteine in soybean products

Soybean products	Cysteine concentrations (g/100ml)
Raw Soybean Seed( control)	0.13±0.03
Local Soybean Milk Powdered	0.13 <sup>b</sup> ±0.02
Local Soybean Liquid Milk	0.61 <sup>a</sup> ±0.03
Vita-milk	1.13 <sup>a</sup> ±0.27
Chi-soymilk	0.59 <sup>a</sup> ±0.06

Result = mean ±SEM <sup>a</sup> significantly higher compared to control. <sup>b</sup>significantly lower than control.

As shown in table 3 above cysteine concentration (g/100 ml) present in Vita-milk (1.13± 0.27) Chi-soymilk(0.59±0.06) and Local Soybean Liquid Milk (0.61±0.03) is significantly higher ( $p < 0.05$ ) than the one of Raw Soybean Seed that was used as control (0.13± 0.03). Also, cysteine concentration (g/100 ml) present in Local Soybean Powdered Milk (0.13 ± 0.02) is significantly lower ( $p < 0.05$ ) than that of the Raw Soybean Seed control (0.13 ±0.03).

**Table 4.** Concentration of methionine in soybean product

Soybean products	Methionine concentration (g/100 ml)
Raw Soybean Seed (control)	0.47 ± 0.02
Local Soybean Powdered Milk	0.22 <sup>b</sup> ± 0.02
Local Soybean Liquid Milk	0.50 <sup>c</sup> ±0.02
Vita-milk	0.90 <sup>a</sup> ± 0.07
Chi-soymilk	0.64 <sup>a</sup> ± 0.02

Result = mean ±SEM <sup>a</sup>significantly higher compared to control. <sup>b</sup>significantly lower than control. <sup>c</sup>No significant difference compared to control.

As shown in Table 4 above, methionine concentration in (g/100 ml) present in Vita-milk (0.90±0.07) and Chi-soymilk (0.06±0.01) is significantly higher ( $p < 0.05$ ) than the raw soybean seed (0.47±0.02). Also methionine concentration (g/100 ml) present in Local Soybean Powdered Milk (0.22±0.02) is significantly lower than the raw soybean seed (0.47±0.02).The Local Soybean Liquid Milk (0.50±0.02) shows no significant difference in methionine concentration compared to that of the Raw Soybean Seed (0.47 ± 0.02).

## 5. Discussion

The presence of methionine and cystein as detected in the soybean products indicate their essential nature in proteins and enzymes synthesis. The lowest concentrations of methionine and cystein amino acids recorded in Raw Soybean Seeds agrees with Randy [7] according to which soybean seeds are almost deficient in sulphur containing amino acids, hence a standard soybean product should contain the sulphur containing amino acids which are cysteine and methionine to replenish these deficiencies [2]. This is because these are the major amino acids that are used to synthesize trypsin enzymes in the body and helps to replenish the lost sulphur amino acids in the body due to the

action of trypsin inhibitors present in soybean product [3]. Chi-soymilk and Vita-milk contain higher sulphur amino acids (cysteine and methionine) when compared to the raw soybean control as seen in Table 3 and 4. This could be because they were enriched with these amino acids during production process. The Local Soybean Powdered Milk contains lower concentrations of these two amino acids. This could be as a result of the intensive dry heating involved during production. In Local Soybean Liquid Milk, methionine concentration remained unchanged while cysteine concentration showed a significant increase compared to control. The reason for these might be related to certain additives normally added to flavor the milk.

## 6. Conclusions

The presence of sulphur containing amino acids above control concentrations in Chi-soymilk and Vita-milk suggest that both soybean products have adequate nutritional values which may reduce the anti-nutritional effects of trypsin inhibitors in soybeans. Hence, the consumption of these soybean products poses no health challenge. The consumption of the locally produced soybean products should be done with care as they are not fortified with these amino acids which could result to continuous loss of trypsin enzymes involved in protein digestion. Further investigations on the Local Soybean Liquid Milk are needed decipher the reason for varying changes in the concentrations of the amino acids.

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