

## Amino Acid Profiling of the Leaves of *Senna Obtusifolia* and Biochemical Changes during Fermentation for Production of "Kawal", a Traditional Food Condiment

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### **ABSTRACT**

*Kawal* is made by a natural or uncontrolled fermentation of *Senna obtusifolia* leaves. It is a food condiment, and appreciated because of its particular flavor. It is also used as a meat or fish substitute. *Kawal* is an important source of protein in the diet of low-income families. The present work was done to determine the total amino acid content in raw and fermented *Senna obtusifolia* leaves. The high performance liquid chromatography (HPLC) with The Pico-Tag method was used for the analysis and determination of amino acid profiles. The results obtained showed that the *kawal* protein is rich in essential amino acids and semi-essential for humans, with different concentrations. Leucine gave a maximum value of 208.56 mg/100 g (DM) and 538.29 mg/100 g (DM); valine showed the value of 173.46 mg/100 g (DM) and 476.25 mg/100 g (DM), for lysine this value is 205.58 mg/100 g (DM) and 456.13 mg/100 g (DM). In the background, we have histidine, threonine, tyrosine, isoleucine and phenylalanine, whose best concentrations are between 157.36 mg/100 g and 397.93 mg/100 g (DM) in fermented leaves. For semi-essential amino acids, the fermented leaves have concentrations of between 121.96 mg/100 g and 1260.92 mg/100 g (DM).

### **KEYWORDS**

*Kawal*; *Senna obtusifolia*; Amino acid; HPLC

### **INTRODUCTION**

Leafy vegetables are generally good sources of nutrients, high in dietary fiber, rich in minerals, vitamin and other nutrients. They are an important source of protein and

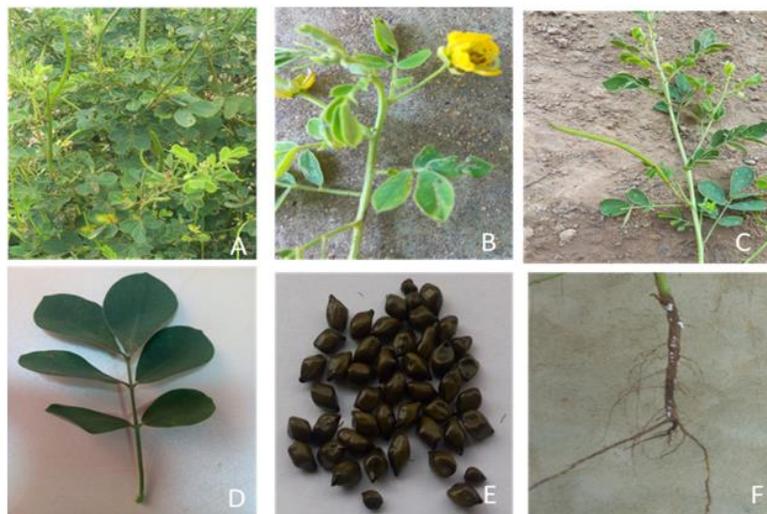
variable amino acid composition [1-5]. Different parts of the world have a valuable heritage of various indigenous leafy vegetables. In Africa, most of these species of vegetables are wild forest vegetables used by local communities living in different agro-ecological zones [6].

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These vegetables are usually consumed in raw form or traditionally processed by fermentation before consumption. Proteins are an indispensable component of nutrition whose nutritional role is to provide amino acids, which are the only source of nitrogen available to humans by providing essential amino acids [7]. The

essential and semi-essential amino acids and their proportions make it possible to compare the nutritional value of the various foods analyzed [8]. Among the protein-rich forest vegetables, there is *Senna obtusifolia* (Figure 1) whose fermented leaves were commonly called *kawal* in Chad.



**Figure 1:** (a) *Senna obtusifolia*, (b) flower, (c) pod, (d) leaf, (e) seeds, (f) root.

*Kawal* is a product obtained by natural fermentation of leaves of *Senna obtusifolia*, and widely consumed in Chad, Sudan and others parts of Central Africa. It is used as a substitute for meat, fish or appetizing in the sauce. The process of transformation of *kawal* is artisanal. They are realized with rudimentary equipment and an uncontrolled fermentation. There are different types of traditional *kawal* production diagrams in Chad with main operations being fermentation and sun drying [9]. *Kawal* contains diverse microorganisms, dominated by *Bacillus* genus that perform an alkaline fermentation, associated with *Lactobacillus* genus [9,10]. Traditional production processes and microorganisms associated with fermentation can have a significant impact on the biochemical composition of the product [11]. *Kawal* is rich in protein and protected many children against kwashiorkor during famine years and there are few studies on *kawal* amino acids.

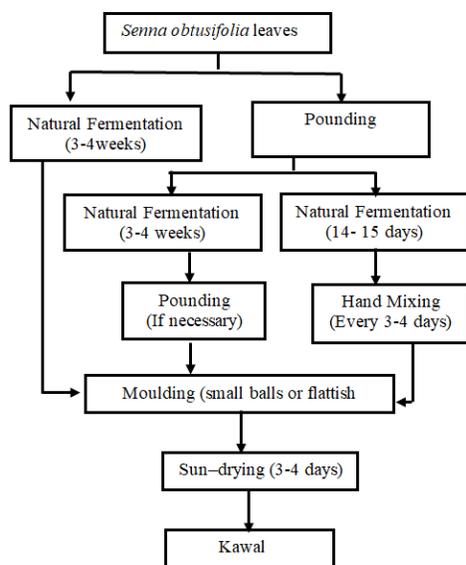
## MATERIAL AND METHODS

### *Sampling*

*Kawal* or *Senna obtusifolia* leaves (Figure 1 and Figure 2) samples were collected in three regions in Chad (East, Central and South) and in the municipalities of N'djamena (capital). The samples were obtained from different technological process at different production sites according to the production diagram described by Abakar et al. [9]. *Kawal* manufacturing process consists of several stages, the main unit operations are fermentation and drying (Figure 3).



**Figure 2:** *Kawal* samples (A and B: fermented leaves forms).



**Figure 3:** Flow general chart for *kawal* production [9].

**Analysis and Determination of Amino Acid Profiles**

Amino acids were analyzed by high performance Liquid Chromatography (HPLC), using Pico-Tag method, first described by Heinrichson and Meredith [12] and recently developed commercially by Waters Associates, under the name of Pico-Tag [13]. The Pico-Tag technique includes a hydrolysis step for proteins or peptide samples. Then pre-column derivatization of the samples under basic conditions, with PITC and analysis by reverse phase HPLC. A reverse phase Pico-Tag column (3.9 × 150 mm) C18 at 40°C and a UV detector at 254 nm were utilized during chromatographic separation on the hydrolyzates. The solvent system consisted of two eluents, A and B and standard.

**A. Preparation of Eluent A**

19 mg of sodium acetate trihydrate was weighted and dissolved in 1000 ml of milli-Q water and mixed with 0.5 ml of triethylamine (TEA). Then adjusted the pH 4.5 with glacial acetic acid and filtered the solution through 0.45 µm membrane filter.

**B. Preparation of Eluent B**

710 mg of disodium hydrogen was mixed with 400 ml of milli-Q water and 600 ml of acetonitrile. Adjusted the pH 7.4 with 10% phosphoric acid.

**Description of standard**

(Thermo Scientific Pierce Amino Acid Standard H): Mixture contains seventeen (17) amino acids (Table 1) for assessment of amino acid composition of peptide hydrolyzates.

Amino Acid	Molar mass (g/ mol)	Dilution : 66 µL(83.5 pmol)
Asp	132.11	0.002752
Glu	147.13	0.003065
Ser	105.09	0.002189
Gly	75.07	0.001563
His	155.16	0.003232
Arg	174.2	0.003629
Thr	119.12	0.002481
Ala	89.1	0.001856
Pro	115.13	0.002398
Tyr	181.19	0.003774
Val	117.15	0.00244
Met	149.21	0.003108
Ile	131.17	0.002732
Leu	131.17	0.002732
Phe	165.19	0.003441
Lys	146.19	0.003045
Cys	121.16	0.001262

**Table 1:** Composition of the standard of amino acids.

**C. Hydrolysis**

After defatting, 0.4 g of each sample was weighed exactly for the amino acid analysis. The protein and peptide of the samples were hydrolyzed in vacuum for 24 hours at 105°C in 15 ml of 6 N HCl solution. The solution is filtered with a 0.45 µm diameter filter and collected in the tubes.

**D. Derivatization**

An aliquot (10 µl) of hydrolyzed was dried under vacuum for 15 minutes. After drying, the sample suspended in 20 µl of solution (methanol, triethylamine, and water), incubated at room temperature for 20 minutes, and dried again under vacuum for 45 minutes. The injection was made by placing 40 µl of each solution with 100 ml of Pico-Tag solvent. This injection operation was performed in duplicate.

**Statistical Analysis**

The analyses were carried out in triplicate and all values are given as the mean ± standard deviation. The SPSS software (SPSS Statistics, 17.0) were performed for statistical analyses. A value of p <0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

Determination of seventeen amino acid concentration of the *kawal* was conducted by HPLC and the results of amino acid profile of different samples and the standard were shown in the chromatograms (Figure 4). The mean of total amino acid contents of the samples from different techniques of production summarized in Table 2. Sixteen amino acids were detected and identified in unfermented and fermented leaves of *Senna obtusifolia*. The result showed the differences in amino acid contents among these samples. The Samples from site of production K4 presented the highest average amounts in amino acids. These results showed a significant difference in the content of amino acids between the production sites. It follows from this table that *kawal* protein is rich in amino acids; it largely covers the essential amino acid requirements (lysine, histidine, threonine, tyrosine, leucine, isoleucine, phenylalanine, and valine) and semi-

essential for humans and limiting in cysteine (semi-essential amino acids). Sulfur amino acids are not strictly limited in the product. Essential amino acids cannot be synthesized de novo by the organism, because the body does not have the enzymes required to produce them and most also have specific functions [14]. The lysine content in the raw leaves of *Senna obtusifolia* and the increase of the concentration in some samples resulting from fermentation is important from the nutrition point of view. Lysine is a truly essential amino acid that has specific functions, facilitates the synthesis of carnitine and the assimilation of other amino acids, and is limited in many plant foods. However, the highest concentration of lysine in the product were 205.58 mg/100 g (DM) for raw leaves and 456.13 mg/100 g (DM) for fermented leaves of *Senna obtusifolia*. Lysine and others essential amino acid, which many plant proteins are deficient in [15,16].

Amino acid	mg/100 g dry weight				
	Raw leaves	K1	K 2	K3	K4
Aspartic acid	444.52 ±0.10	230.27 ±0.50	124.81 ±0.09	249.40 ±0.30	697.75 ±0.01
Glutamic acid	375.02 ±0.01	359.73 ±0.30	222.02 ±0.02	185.81 ±0.10	1163.24 ±0.32
Serine	130.47 ±0.20	115.5 ±0.10	64.81 ±0.03	158.01 ±0.08	340.16 ±0.50
Glycine	135.97 ±0.03	142.55 ±0.50	80.43 ±0.01	74.49 ±0.07	410.18 ±0.40
Histidine	79.66 ±0.02	76.45 ±0.09	47.23 ±0.09	41.84 ±0.09	191.17 ±0.16
Arginine	136.14 ±0.40	191.98 ±0.08	119.34 ±0.04	140.35 ±0.0	121.96 ±0.22
Threonine	137.40 ±0.60	134.93 ±0.04	70.92 ±0.10	208.74 ±0.21	353.72 ±0.16
Alanine	169.12 ±0.20	233.92 ±0.40	95.62 ±0.30	236.16 ±0.41	499.55 ±0.09
Proline	632.43 ±0.10	381.31 ±0.03	280.02 ±0.20	251.68 ±0.12	1260.92 ±0.28
Tyrosine	122.24 ±0.05	119.33 ±0.60	64.38 ±0.30	112.08 ±0.20	273.13 ±0.40
Valine	173.46 ±0.10	235.38 ±0.60	168.32 ±0.10	203.86 ±0.32	476.25 ±0.25
Methionine	73.03 ±0.04	93.11 ±0.08	53.523 ±0.08	84.67 ±0.12	157.36 ±0.39
Cystine	7.88 ±0.07	-	-	62.20 ±0.31	-
Isoleucine	91.56 ±0.50	134.51 ±0.06	97.05 ±0.04	195.39 ±0.20	300.69 ±0.21
Leucine	208.56 ±0.30	262.42 ±0.03	157.12 ±0.06	115.64 ±0.35	538.29 ±0.36
Phenylalanine	149.25 ±0.10	17.131 ±0.20	104.25 ±0.05	96.17 ±0.14	397.93 ±0.17
Lysine	205.58 ±0.30	183.62 ±0.60	45.41 ±0.20	178.97 ±0.30	456.13 ±0.59

Where: K1, K2, K3 and K4, samples from different techniques and different sites of productions.

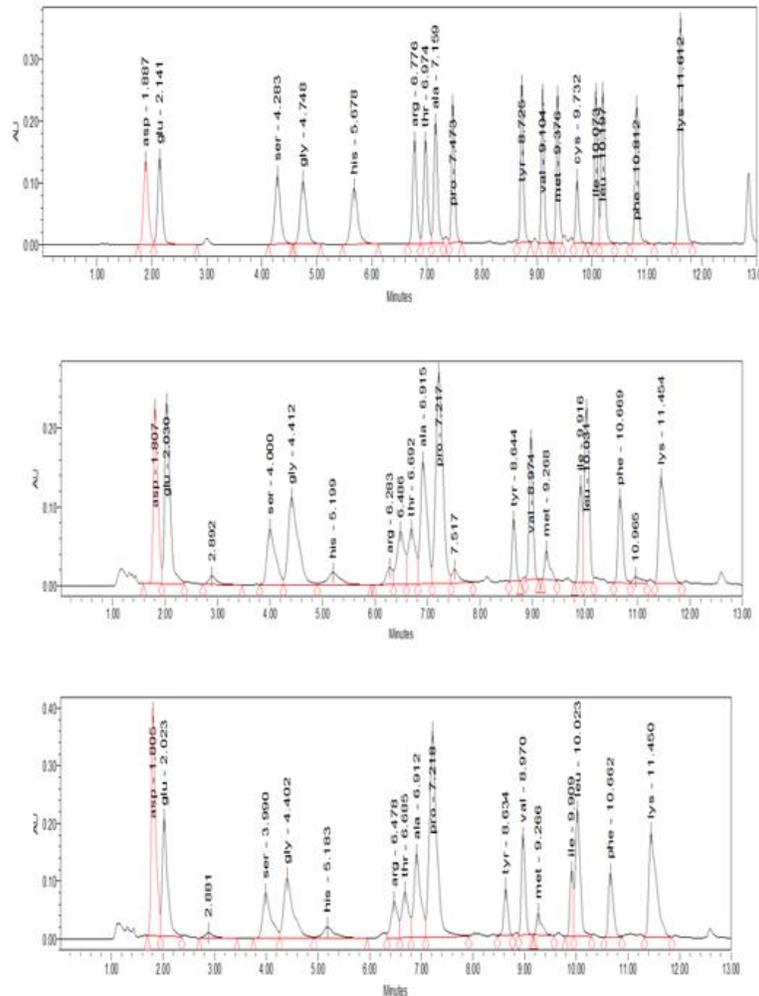
**Table 2:** Amino acid content of unfermented and fermented *Sanna obtusifolia* leaves.

*Senna obtusifolia* fermented leaves or not it is well balanced in essential amino acids, and semi-essential amino acids, as shown by the results of this study. These

concentration of amino acids are sufficient according to the amino acid requirements for different age groups by the FAO (Food and Agriculture Organization) and WHO

(World Health Organization) [17,18]. Leucine remarked to be the highest essential amino acids in all samples analyzed. The concentration of leucine in products is 538.29 mg/100 g (DM) two fold more was found in fermented leaves, and 205.58 mg/100 g (DM) for

unfermented leaves. Leucine has a regulatory role in muscle protein synthesis and the protein balance in the body [19]. Methionine recorded the lowest concentration of amino acid in the unfermented leaves but the values increased in fermented leaves.



**Figure 4:** Chromatograms showing different peaks for the amino acids determined in the product with the HPLC method. A (Standard), B (green leaves) and C (fermented leaves).

The most important amino acids from quantitative point of view proline and glutamic acids are presented in all samples analyzed with highest concentration. Aspartic acid, glycine and glutamic acid are known to play an important role in the process of wound healing [20]. Arginine is an essential amino acid for children growth [21] and it is present in the all samples analyzed. Others amino acids have shown variants concentration in the product and have important physiological role, Histidine is a nutritionally essential amino acid that is also a precursor for several hormones and hemoglobin

[22].When individuals are fed histidine-free diets, the effects on hemoglobin concentrations will be observed [23,24].

The methods of chromatographic separation of amino acids are numerous. The most used technique is high performance liquid chromatography or HPLC. The chromatographic conditions used made it possible to separate the seventeen amino acids with a good separation and a perfect resolution of the peaks corresponding to Aspartic acid, Glutamic acid, Serine,

Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Methionine, Cysteine, Isoleucine, Leucine, Phenylalanine and Lysine (Figure 4).

Results of these studies showed that fermentation caused an increase in the concentration of total amino acids in the fermented leaves. Generally, amino acid composition of *Senna obtusifolia* leaves did not show decrease after processing stage. However, fermenting method used has significant effect to the amino acid concentration of raw leaves. Similar increases of amino acid content in vegetable fermented food, during fermentation of Baobab seeds, *Parkia biglobosa*, *Prosopis africana*, and soybean seeds were also reported by Ouoba et al. [25]; Odibo et al. [15]; Dakwa et al. [26] and Parkouda et al. [11]. Proteolysis has been reported to be the main metabolic activity during the fermentation of these products. This proteolytic activity of the microorganisms responsible for the fermentation degrade the proteins into peptides then into amino acids and has been observed during fermentation of *kawal* [9]. The increase in nutritional value was also observed during the fermentation of legumes; an example is provided by the "tempeh", made of fermented soybeans [27]. These improvements have been attributed to increased digestibility induced by the proteolysis of complex proteins into peptides of various lengths and amino acids [28]. Similar changes were observed during the fermentation of 'idli', an Indian product made by mixing black chickpeas (*Phaseolus mungi*) and rice [27].

Alkaline fermentations cause the hydrolysis of proteins to amino acids and peptides by the action of *Bacillus* species such as *B. subtilis*, *B. licheniformis* and *B. pumilius* has been reported by Blades et al. [29] and Chelule et al. [30]. Previous research has shown that *B. subtilis* B9 and B15 have the best degradation capacity of faba bean proteins, resulting in the release of high concentrations of essential amino acids such as lysine,

tryptophan, leucine, valine, isoleucine, histidine and phenylalanine. The amino acid composition of a given product is likely to vary depending on various factors, mainly variety or species, physiological stage, cropping conditions, treatments. Given these possibilities of variation, our results are generally in agreement with those of the various authors, insofar as they find comparable results between them. However, some differences between the results obtained by the different authors seem to be related to the methods used. Mbaiguinam et al. [31] showed from their results obtained using GC and GC/MC that fermentation of leaves of *Senna obtusifolia* to produce *kawal* results in a loss of protein content of 20.2% to 12.9% in fresh leaves. It has also been reported by these authors that the amino acid content reduced by the same amount. In contrast, our results agree with other authors who confirm that *kawal* fermentation increases nutrient concentration and protein content from 21.87% to 30.20% [32,33]. It is necessary to carefully interpret the studies on fermentation and the nutritional value of products, contradictory results may be due to the different test methods used and the microorganisms concerned.

## **CONCLUSION**

The fermented or unfermented *Senna obtusifolia* leaves appear to be relatively well balanced in amino acids, yet they have all the essential amino acids requirements. The results obtained demonstrated that the concentration of total amino acids fluctuated during fermentation of *Senna obtusifolia* leaves. The *kawal* contains significant profiles and could cover the essential amino acid requirements of adults and children. These values are sufficient according to the recommendations of FAO and WHO. This nutritional value should be fully exploited in the interest of human nutrition.

## **CONFLICT OF INTEREST**

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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