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Effects of processing on amino acids composition of *Leucaena leucocephala* (Lam De Wit) leaf meal

AGBO, A. N.

National Open University of Nigeria, Faculty of Agricultural Sciences Kaduna.

Abstract

The high cost of animal protein sources has made it necessary to evaluate the use of alternative plant protein sources especially unconventional plant proteins. *Leucaena leucocephala* (white lead tree) is a leguminous plant, the leaves of which have the potential of being used as a plant protein source. *Leucaena* leaf has been analysed to have a crude protein value of 15.2-34.3%. The plant leaves were collected from the forage section of the College of Agriculture and Animal Sciences (CAAS) Mando, Kaduna. The first group of the leaves was divided into three and sun-dried for 24 hours, 48 hours and 72 hours respectively. The second group was further divided into three subgroups which were soaked in water for 24 hours, 48 hours and 72 hours then sun-dried. The two groups of the leaves were ground into leaf powder. The effects of sun-drying and soaking on amino acid composition of *L. leucocephala* leaves was investigated. The amino acid analysis was done by ion-exchange chromatography (IEC) using the Technicon Sequential Multisample Amino Acid Analyser (TSM) Technicon Instruments Corporation, New York. The essential amino acids detected in *L. leucocephala* leaf were arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine. Alanine, cysteine, tyrosine, aspartic acid, glutamic acid, glycine, serine and proline were the non-essential amino acids detected. Sun-drying and soaking led to significant (P≤0.05) losses in the concentration of all the amino acids in the leaf samples (Sundried for 24 hours - SD_1, Sundried for 48 hours - SD_2, Sundried for 72 hours - SD_3, soaked in water for 24 hours – SW_1, soaked in water for 48 hours - SW_2, soaked in water for 72 hours - SW_3) when compared with the raw leaf samples (SU). Soaking caused higher reduction in the amino acid levels when compared to sun-drying in the test leaf meals which was significant at P≤0.05. The amino acid scores revealed that lysine and methionine were the limiting amino acids in *L. leucocephala* leaves relative to WHO/FAO/UNU standard for preschool aged children. Therefore, processed *L. leucocephala* leaves should be supplemented with other feed ingredients rich in these amino acids for animal feed production.

Keywords: *Leucaena leucocephala* leaf, sun-drying, soaking, amino acid composition.
Corresponding author: ronkeagbo@gmail.com

Introduction

The high cost of traditional protein-rich ingredients has compelled animal nutritionists to explore the use of other unconventional protein-rich feed ingredients. The research into some of these feed ingredients stems from their rich nutrients such as essential amino acids, vitamins and minerals (Aye, 2013). However, the presence of anti-nutritional factors and imbalance of amino acids in plants has limited the usage of these feed ingredients especially plant proteins (Francis *et al.*, 2001). It is well established that amino acids, as nutrients, are building blocks of protein which play an essential role in determining the quality

and bioavailability of a proteinous ingredients (Robinson and Menghe, 2007). Amino acids can be grouped into essential and non-essential amino acids. The level and proportion of essential amino acids in proteins is important because there must be proper balance for the amino acids to be useful (Robinson and Menghe, 2007).

Plant proteins require some form of processing to reduce antinutritional factors and make their nutrients available (Onimisi *et al*., 2017). Some of the processing methods employed are cooking, roasting, soaking, boiling, autoclaving, radiation, sun-drying, dehulling, germination, toasting, extrusion, blanching, malting and fermentation (Francis *et al*., 2001). Simple processing methods such as soaking and sun-drying can be easily used by farmers. The effects of some of these processing methods on amino acid composition has been reported (Chinyere and Obasi, 2011; Augustine *et al*. 2018).

Leucaena leucocephala (white lead) tree is a fast growing evergreen multipurpose legume which is native to Mexico but has found its way to many tropical and sub-tropical countries (Heuze and Tranc, 2014). *L. leucocephala* belongs to the family *Fabaceae*, sub-family *Mimosoidae* and genus *Leucaena* ; the tree has bipinnate leaves which are 15-20cm long, lanceolate leaflets, flat brown pods containing small seeds and white flowers (Heuze and Tranc, 2014). *L. leucocephala* trees have been used as wind breakers, ruminants feed, and for agroforestation, nitrogen fixation, paper and timber production (Hetrampf and Piedad-pascual, 2003). The leaves have crude protein values of 15.2-34.3% dry matter (Foroughbakhch *et al*., 2007; Monoj and Bandyopadhay, 2007; Ayssiwede *et al*., 2011, Adedeji *et al*., 2013). *L. leucocephala* leaf also contains vitamin A, Vitamin B, carotene and the 10 essential amino acids (Hetramphf and Piedda-Pascual, 2003; Monoj and Bandyopadhyay, 2007). The objective of this study is to evaluate the effect of sun-drying and soaking on the amino acid composition of *Leucaena* leaf meal for use in fish nutrition.

Materials and methods

Collection of L. leucocephala leaves

L. leucocephala leaves were collected from trees planted at the forage section of the College of Agriculture and Animal Sciences (CAAS) Mando, Kaduna, Nigeria. The leaves were identified at the Department of Pasture and Range Management, College of Agriculture and Animal Sciences (CAAS) Mando, Kaduna after which the leaves were separated from the long leaf stalk and some unwanted materials removed.

Processing of L. leucocephala leaves

Soaking: approximately 500g of *L. leucocephala* leaves was soaked in water at room temperature (30˚ C). Ten litres of water was measured into 3 different rubber bowls after which 500g of the leaf sample was soaked in one bowl for 24 hours (SW_1), another 500g in the second bowl for 48 hours (SW_2) and the third bowl for 72 hours (SW_3). The leaves were removed from the water and allowed to dry homogeneously in the sun until they became crispy (Amisah *et al*., 2009).

Sun-drying: about 500g of the leaf samples was spread on a concrete slab outside and the first portion was sundried for 24 hours (SD_1), the second portion for 48 hours (SD_2) while the third portion was for 72 hours (SD_3) as described by Ayssiwede *et al.* (2011).

All the dried leaf samples were ground into fine powder and separately packed in labelled polythene bags. Representative samples of the leaf meal were taken to the laboratory for amino acid analysis. All analyses were done in triplicates.

Determination of Amino Acid Profile

The amino acids composition of the leaf sample was analysed as described by Benitez (1989). Two grams of each sample was defatted with chloroform-methanol (2:1) using soxhlet extraction apparatus as described by AOAC (1990). The extraction was carried out for 15 hours. A defatted sample of 30 to 35 mg was weighed into a glass ampoule. Approximately 7ml of 6 N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids, such as methionine and cysteine, during hydrolysis). A bunsen burner flame was used to seal the glass ampoule, after which it was put in an oven preset at 105˚C for 22hours. The ampoule was allowed to cool , then the tip was opened and the content was filtered to remove humins. The filtrate was then evaporated to

dryness at 40° C under vacuum in a rotary evaporator. Five (5) ml of acetate buffer (pH 2.0) was used to dissolve the residue, then stored frozen in a plastic specimen bottle .

Loading of the hydrolysate into TSM analyzer

The amount loaded was between 5 to 10 microlitres. This was dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Method of Calculating Amino Acid Values from the Chromatogram Peaks

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and the width of the peak on the half height was accurately measured and recorded. Approximately, the area of each peak was then obtained by multiplying the height with the width at half-height.

The norcleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$\text{NE} = \frac{Area\ of\ Norcleucine\ Peak}{Area\ of\ each\ amino\ acid}$$

A constant S was calculated for each amino acid in the standard mixture using the equation

$$S_{std} = NE_{std} \times Molecular\ weight \times uMAA_{std}$$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formular:

$$Concentration\ (C)\ (g/100g\ protein) = NH \times W@\frac{NH}{2 \times S_{std} \times C}$$

Where $C = \frac{Dilution \times 16 + NH \times W(nlcu)}{Sample\ Wt(g) \times N\% \times 10 \times vol.loaded}$

NH= Net height

W = Width at half height

nlcu = Norleucine

Amino acid score

The following formula by WHO/FAO/UNU, (2007) was used to calculate the amino acid score.

$$Amino\ acid\ score = \frac{mg\ of\ amino\ acid\ per\ g\ N\ in\ test\ protein}{mg\ of\ amino\ acid\ per\ g\ N\ in\ reference\ protein}$$

Estimation of leaf Samples Protein Quality

The reference value for good quality protein as described by WHO/FAO/UNU (2007) for children of preschool age was adopted. To estimate the quality of dietary protein in the leaf samples the following parameters were calculated: Total Essential Amino Acid (TEAA), Total Non-Essential Amino Acid (TNEAA) and Total Sulphur Amino Acid (TSAA), as described by Dalibard *et al.* (2014).

Statistical Analysis:

The experiment was carried out using a completely randomized design. The data collected was subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

Results

Table 1 shows the amino acid composition of raw, sun-dried and soaked *L. leucocephala* leaf meals. Lysine, histidine, arginine, valine, methionine, isoleucine, leucine, threonine and phenylalanine were the essential amino acids identified. While the non-essential amino acids were aspartic acid, serine, glutamic acid, proline, glycine, alanine, cysteine and tyrosine. Leucine was the most predominant essential amino acid which ranged from 7.48-9.72 (g/100g protein) while methionine was least abundant with a range of 1.35-1.77 (g/100g protein).

Table 1: Amino acid composition of raw, sun-dried and soaked L. *leucocephala* leaf meal

Amino acid (g/100g protein)	SU	SD1	SD2	Treatments SD3	SW1	SW2	SW3	WHO/FAO/UNU Ref value [1]
Essential amino acid								
Lysine	6.05±0.01 [a]	5.50±0.5 [b]	5.62±0.01 [b]	5.73±0.01 [b]	4.86±0.01 [d]	4.97±0.01 [cd]	5.79±0.01 [c]	5.70
Histidine	2.97±0.02 [a]	2.74±0.1 [b]	2.37±0.03 [e]	2.59±0.01 [c]	2.21±0.01 [g]	2.27±0.01 [fg]	2.32±0.02 [ef]	2.00
Arginine	6.37±0.02 [a]	6.11±0.1 [b]	5.87±0.01 [c]	5.87±0.01 [c]	5.18±0.01 [f]	5.35±0.01 [e]	5.70±0.02 [d]	-
Valine	6.02±0.02 [a]	5.97±0.02 [b]	5.33±0.01 [c]	5.27±0.01 [d]	4.52±0.02 [g]	4.63±0.01 [f]	4.80±0.01 [e]	4.30
Methionine	1.77±0.02 [a]	1.67±0.01 [b]	1.55±0.01 [d]	1.62±0.01 [c]	1.35±0.01 [g]	1.41±0.01 [f]	1.46±0.01 [e]	2.70
Isoleucine	5.91±0.03 [a]	5.64±0.01 [b]	4.76±0.01 [d]	4.88±0.01 [c]	4.19±0.01 [f]	4.31±0.01 [e]	4.31±0.01 [e]	3.20
Leucine	9.72±0.02 [a]	9.26±0.06 [b]	8.58±0.02 [d]	8.90±0.01 [c]	7.48±0.01 [g]	7.56±0.03 [f]	7.91±0.01 [e]	6.60
Threonine	5.72±0.01 [a]	5.08±0.1 [c]	5.08±0.02 [c]	5.40±0.01 [b]	3.49±0.01 [g]	4.16±0.01 [f]	4.44±0.01 [e]	3.10
Phenylalanine	6.66±0.05 [a]	6.07±0,01 [b]	5.75±0.02 [c]	5.74±0.02 [c]	4.72±0.02 [g]	4.89±0.01 [f]	5.06±0.01 [e]	5.20
Non-essential amino acid								
Aspartic acid	9.87±0.01 [a]	9.75±0.22 [a]	9.44±0.01 [b]	9.44±0.01 [b]	8.62±0.01 [d]	8.67±0.01 [d]	8.93±0.02 [c]	
Serine	4.99±0.02 [a]	4.95±0.01 [b]	4.28±0.01 [d]	4.47±0.01 [c]	3.60±0.01 [g]	3.85±0.01 [f]	3.88±0.01 [e]	
Glutamic acid	12.72±0.52 [a]	12.50±0.02 [b]	11.97±0.02 [d]	12.42±0.02 [c]	11.32±0.06 [g]	11.52±0.02 [f]	11.66±0.01 [e]	
Proline	4.47±0.04 [a]	4.03±0.03 [b]	3.47±0.01 [c]	3.36±0.02 [d]	3.05±0.03 [f]	3.25±0.04 [e]	3.27±0.01 [e]	
Glycine	5.91±0.01 [a]	5.67±0.01 [b]	5.13±0.06 [d]	5.29±0.01 [c]	4.37±0.10 [f]	4.43±0.02 [f]	4.53±0.01 [e]	
Alanine	6.30±0.01 [a]	5.70±0.02 [b]	5.01±0.01 [d]	5.39±0.01 [c]	4.19±0.01 [f]	4.23±0.10 [f]	4.40±0.10 [e]	
Tyrosine	5.72±0.01 [a]	5.08±0.00 [c]	5.08±0.01 [c]	5.40±0.01 [b]	3.49±0.01 [f]	4.16±0.15 [e]	4.44±0.03 [d]	
Cysteine	1.32±0.01 [a]	1.06±0.01 [b]	0.93±0.01 [c]	0.93±0.01 [c]	0.66±0.02 [f]	0.79±0.02 [e]	0.86±0.02 [d]	

Means with the same superscript within the same row were not significantly different (P>0.05)
SU – raw
SW1 –Soaking in water at room temperature for 24 hours
SW2 –Soaking in water at room temperature for 48 hours
SW3 –Soaking in water at room temperature for 72 hours

SD1 – Sundried for 24 hours
SD2 – Sundried for 48 hours
SD3 – Sundried for 72 hours
1 – WHO/FAO/UNU (2007)

Tryptophan was not detected in the amino acids profile assay . Glutamic acid was the most predominant non-essential amino acid with a range of 11.32-12.72 (g/100g protein) followed by aspartic acid (8.62- 9.87 g/100g protein) while cysteine was the least abundant (0.66-1.32 g/100g protein). There was a significant (P≤0.05) reduction between the raw and all the processed leaf meal samples. Soaking led to a higher percentage reduction in all the detected amino acids when compared to the sun-drying method. The values for all the amino acids were significantly different (P≤0.05) between the soaked and sun-dried samples. The reduction effect was observed more in some of the amino acids (Table 1). A reduction of 13.87% (4.76 – 5.64 g/100g protein) was observed in isoleucine and 6.59% (5.87 – 6.11 g/100g protein) in arginine for the sun-drying method (Table 1). In the soaking method, isoleucine had a reduction of 29.44% (4.19 – 4.31 g/100g protein), while lysine a reduction of 14.04% (4.79 –4.97 g/100g protein), then phenylalanine a reduction of 26.58% (4.72 –5.06 g/100g protein).

Table 2 shows the amino acid scores of the sun-dried and soaked *L. leucocephala* leaf meals. Amino acid scores of the sun-dried and soaked *L. leucocephala* leaf meals when compared to the reference standard revealed that lysine (98.6%), methionine and cysteine (95.5%) ere the limiting amino acids for the sun-dried samples while lysine (91.2%) methionine and cysteine (80.7%) were the limiting amino acids for the soaked samples.

Table 2: Amino acid scores for the raw, sun-dried and soaked *L. leucocephala* leaf meals

Essential Amino acid	Amino acids concentration (g/100g protein) raw	Amino acids concentration (g/100g protein) sundried	Amino acid score (%)	Amino acids concentration (g/100g protein) soaked	Amino acid score (%)
Lysine	6.05	5.62	98.5	5.2	91.2
Histidine	2.97	2.57	128.5	2.27	113.5
Valine	6.02	5.52	128.3	4.65	108.1
Methionine and Cysteine	3.07	2.58	95.5	2.18	80.7
Isoleucine	5.91	5.09	184.3	4.17	130.3
Leucine	9.72	8.91	135	7.65	116
Threonine	5.72	5.19	167.4	4.03	130
Phenylalanine and Tyrosine	12.38	11.03	212.1	8.92	171.5

The concentrations of the various amino acids detected in the raw, sun-dried and soaked *L. leucocephala* leaf meals are presented in Table 3 with the aim of providing data on protein quality. The concentration of the total essential amino acids (TEAA) of the raw *L. leucocephala* leaf meal was 51.19 g /100g protein which was higher than that of the sun-dried (46.00-48.04 g /100g protein) and soaked (38.00-41.79 g /100g protein) samples. The levels of total non-essential amino acids (TNEAA) for the *L. leucocephala* leaf meal samples followed the same pattern : sun-

dried (46.70-48.81 g /100g protein) and soaked (39.3-41.97 g /100g protein) which were lower than the raw (50.61 g /100g protein) sample. The total non-essential amino acids (TNEAA) were generally higher in concentration compared to the total essential amino acids (TEAA) for the processed samples.

Table 3: Concentrations of the amino acids from the raw, sun-dried and soaked *L. leucocephala* leaf meals

Amino acid (g/100g protein)	Treatments						
	SU	SD1	SD2	SD3	SW1	SW2	SW3
TEAA (Total Essential Amino Acids)	51.19	48.04	44.91	46.00	38.00	39.55	41.79
TNEAA (Total Non-Essential Amino Acids)	50.61	48.81	45.31	46.7	39.3	40.9	41.97
TSAA (Total sulfur-containing amino acids)	3.09	2.73	2.48	2.55	2.01	2.20	2.32

SU – raw
SW1 –Soaking in water at room temperature for 24 hours
SW2 –Soaking in water at room temperature for 48 hours
SW3 –Soaking in water at room temperature for 72 hours
SD1 – Sundried for 24 hours
SD2 – Sundried for 48 hours
SD3 – Sundried for 72 hours

Discussion

This study corroborated the work of Chinyere and Obasi (2011) on the predominance of leucine as the most abundant essential amino acid in selected vegetables. Igwe *et al.* (2012) and Oluwasola and Dairo (2016) gave similar results for legumes and *Tithonia diversifolia* leaves, respectively. Methionine being the most abundant essential amino acid is in line with the work of Mirinda *et al*. (2012) on fresh *L. leucocephala leaves* and Okereke and Ucheje (2014) working on leaf, root and seed of *Moringa oleifera*. Chinyere and Obasi (2011) stated that legume leaf vegetables are low in sulphur-containing amino acids (methionine and cysteine). The absence of tryptophan might be a result of acid hydrolysis employed in the determination of amino acid composition, tryptophan requires alkaline hydrolysis (Nwosu *et al*., 2008). The result also reveals that processing by soaking in water led to a significant reduction (P≤ 0.05) in all the essential amino acids considered in this study. Madalla *et al*. (2013) reported a high reduction in value of sulphur- containing amino acids (methionine and cysteine) when Moringa leaves were soaked in water overnight. The lower amino acids content of the soaked *Leucaena* leaf meal when compared with the sun-dried one may be attributed to loss of soluble nitrogenous compounds and free water-soluble amino acids through leaching. This study supports the observation of Augustine *et al*. (2018) that there was a decrease in amino acids composition of *Senna occidentalis* as soaking time increased. The essential amino acid composition of the processed *Leucaena* leaf meals in this study was similar to those reported by earlier workers (Hetrampf and Piedad-pascual, 2003; Minrida *et al*., 2012).

The findings of this experiment revealed that most of the essential amino acids in all the processing methods scored above 100% of the WHO/FAO/UNU standard for preschool age children. This shows that the processed *Leuceana* leaf meals are a rich source of essential amino acids. The limiting amino acid being lysine and sulpur-containing amino acids has also been reported by Chinyere and Obasi (2011) for some Nigerian vegetables (*Veronica amygdaline, Gnetum africana, Gongronema latifolium and Ocimum gratissimum)*. The higher level of TNEAA (Total Non-Essential Amino Acids) when compared to TEAA (Total Essential Amino Acids) followed the pattern reported by Igwe *et al*. (2012) for processed seeds of *Prosopis africana* and *Ricinus communis*. However, the values for TNEAA (Total Non-Essential Amino Acids) and TEAA (Total Essential Amino Acids) were close to

the value reported by Minrida *et al.* (2012) for *Leucaena* leaf.

Conclusion

Soaking and sun-drying processing methods caused different degrees of significant reduction in all amino acids evaluated in this study. However, *L. leucocephala* leaf contains appreciable quantities of essential and non-essential amino acids comparable with some animal proteins and other common legumes. This study indicated sun-drying followed by soaking as being desirable in reducing amino acid losses. The amino acids scores also revealed that lysine, methionine and cysteine were the limiting amino acids in the processed *Leucaena* leaf meals when compared with WHO/FAO/UNU standard for preschool age children. Therefore, processed *Leucaena* should be supplemented with other feed ingredients rich in these amino acids for fish nutrition.

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