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Effect of processing on the amino acid profile of the leaf, root and seed of *Moringa Oleifera* grown in Rivers State, Nigeria

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ABSTRACT

The study was carried out to ascertain how processing (by boiling) affects the amino acid profile of the root, leaf and seed of Moringa oleifera grown in Rivers state, Nigeria. The amino acid profiles of the raw and processed samples were analyzed with the HP 6890 gas chromatograph. It was observed that out of the 22 amino acids present in food, seventeen were recorded in both raw and processed root, leaf and seed of Moringa oleifera with Glutamate having the highest concentration range in g/100g protein (12.31-15.14) and Methionine having the least concentration range (0.62-1.15). Statistical comparison of the amino acid profiles of the processed root, leaf and seed of Moringa oleifera to the raw (unprocessed) samples revealed reduction in the level of these amino acids in most case. The present study shows that precaution measures need to be taken as the levels of amino acids in the M. oleifera samples decreases significantly when processed by boiling. Therefore, harsh processing methods are not recommended if the essential amino acids in the root, leaf and seed of M.oleifera must be incorporated into commercial feeds to boost their amino acid contents.

Key words: Processing, Moringa oleifera, Gas chromatograph, Amino acid profile, Commercial feeds.

INTRODUCTION

Moringa oleifera is the most widely cultivated species of genus Moringa in the family Moringaceae [1]. Moringa has long been considered a panacea for improving the nutrition of poor communities in the tropics and subtropics [2].

All part of Moringa oleifera can be used in a variety of ways as food. The leaves especially the young shoots are eaten as greens, in salad, in vegetable curries and as pickles. They are widely used in India, the Philippines, Hawaii and parts of Africa as a highly nutritious vegetable solvent. M. oleifera leaves are considered to offer great potential for those who are nutritionally at risk and may be required as a protein and calcium supplements [3]. The seeds of Moringa oleifera taste like peanuts after frying and can be consumed raw or cooked [4]. The roots of Moringa which have the pungent taste of horse radish (*Armora rusticana*) are used as condiments or garnish after drying and mixing with vinegar [5].

Food processing dates back to prehistoric ages when crude processing incorporated slaughtering, fermenting, sun-drying, preserving with salt and various types of cooking such as roasting, smoking, steaming and oven baking. These tried and tested processing techniques remained essentially the same until the advent of the industrial revolution. It is undeniable that processing of foods have several benefits which include but are not limited to toxin

removal, preservation, easing marketing and distribution task and increasing food consistency [6].

However, any processing of food especially from plant origin can affect its nutritional density; the amount of nutrients lost depends on the food and method of processing. The United States Department of Agriculture conducted a study in 2004, creating a nutrient retention table for several foods [7]. A cursory glance of the table indicates that in majority of foods, processing reduces nutrients by minimal amounts. New research highlighting the importance of human health of a rich microbial environment in the intestine indicates that much food processing endangers that balance [8].

Moringa oleifera is especially promising as a food source in the tropics because the tree is full in leaf at the end of the dry season when other foods are typically scarce [9]. Application of processing to Moringa oleifera root, leaf and seed will give some information, which may increase the utilization of the morphological parts of M. oleifera and enhance their potentials in food formulations.
The objective of this study therefore is to investigate the effects of pretreatment on the amino acid profiles of the Moringa oleifera root, leaf and seed.

MATERIALS AND METHODS

2.1 Collection & Preparation of Raw Test Samples:

The roots, leaves and seeds of Moringa oleifera were collected from Mich farm at Eleme community, Rivers State Nigeria and duly authenticated. The Moringa oleifera seeds, leaves and roots were cleaned and then oven dried at 60^0C in an air circulated oven, ground with mortar and pestle to fine particles and stored in screw capped containers. Chemical analyses were carried out on the ground samples.

2.2 Preparation of the Processed Samples:

The Moringa oleifera leaves, roots and seeds were processed by boiling each in a pot of tap water in the ratio of 1: 10 (w/v) [10]. Boiling was until the water was considerably dried up (about 15 minutes). The boiled samples were then gently pulverized to fine particles in a laboratory mortar. The dried and pulverized samples were made to be free of water by ensuring constant weight for a period of time in the laboratory before they were analyzed.

2.3 Reagents

All reagents used in this study were of analytical grades with high purity.

2.4 Determination of Amino Acid Composition.

2.4.1 Processing.

5.0g of each sample was extracted with petroleum ether (40-60^0C) using Soxhlet extractor for six hours [11]. 30mg of defatted sample was weighed into an extraction thimble of the Soxhlet extraction apparatus and hydrolyzed with deionized water [12]. The amino acid content of each sample was recovered with 30ml of Methylene chloride using the Soxhlet extraction apparatus [12].The extract was then concentrated to 1ml using a rotary evaporator.

2.4.2 Gas Chromatography Analysis.

First the instrument was calibrated by injecting 1ml of the standard mixture of amino acid solution into the column inlet of the HP 6890 Gas chromatograph. The pulsed flame photometric detector (PFPD) in the chromatograph monitors the outlet stream. Thus the retention time of each component (amino acids) is determined. The results from the gas chromatograph are channeled to a data station where an instrument specific software program interprets and translates instrument control and data acquired from the gas chromatography process. This action ascertains the efficiency of the standard mixture of amino acids and calibrates the instrument. The entire procedure is repeated for analysis of 1ml of each of the test samples. The period of analysis lasted for about 60 mins.

2.4.3 Gas Chromatography Conditions.

The gas chromatograph was HP 6890 model. The carrier gas was Hydrogen while the injection temperature was split injection. The split ratio was 10:1. The inlet temperature and column type were 250^0C and HP5 respectively. The column dimensions were 30m X 0.25mm X 0.25 micrometers. The oven program initial temperature was 60^0C. The first ramping was 8^0C per minute maintained for 2 minutes while the second ramping was 12^0C per minute maintained for 2 minutes. The detector was pulsed flame photometric detector. Detector temperature, Hydrogen pressure and Compress air were 320^0C, 20psi and 35psi respectively.

RESULTS

Table 1: Comparison of amino acid profile of raw and processed (Boiled) Moringa oleifera seed and difference (D_1) in g/100g protein for each amino acid present

Amino acid	Raw Seed (X_1)	Processed Seed (Y_1)	$D_1= X_1-Y_1$
Glycine	4.99	4.80	0.19
Alanine	3.23	3.03	0.20
Serine	4.25	4.11	0.14
Proline	2.40	2.12	0.28
Valine	3.09	2.74	0.35
Threonine	3.22	3.02	0.20
Isoleucine	4.35	4.12	0.23
Aspartate	6.14	5.70	0.44
Leucine	5.27	4.47	0.80
Lysine	3.24	3.14	0.10
Glutamate	14.76	13.62	1.14
Methionine	0.97	1.15	-0.18
Phenylalanine	4.53	4.57	-0.04
Histidine	2.01	2.16	-0.15
Arginine	8.06	7.35	0.71
Tyrosine	2.33	1.90	0.43
Cysteine	2.02	1.94	0.03
Total	74.86	69.94	4.92

Table 2: Comparison of the amino acid profile of raw and processed (Boiled) Moringa oleifera leaf in g/100g protein

Amino acid	Raw Leaf (X_2)	Processed Leaf (Y_2)	$D_2=X_2-Y_2$
Glycine	5.15	4.79	0.36
Alanine	3.43	3.08	0.35
Serine	4.20	4.00	0.20
Proline	2.68	2.70	-0.02
Valine	3.36	3.03	0.33
Threonine	4.38	4.18	0.20
Isoleucine	2.33	2.32	0.01
Aspartate	6.88	6.16	0.70
Leucine	5.22	4.95	0.27
Lysine	3.60	3.52	0.08
Glutamate	15.14	14.70	0.44
Methionine	0.95	0.86	0.09
Phenylalanine	4.26	4.06	0.20
Histidine	1.89	1.71	0.18
Arginine	1.88	1.73	0.15
Tyrosine	2.20	2.46	-0.26
Cysteine	2.05	2.26	-0.22
Total	69.59	66.53	3.06

Table 3: Comparison of the amino acid profile of raw and processed (Boiled) Moringa oleifera root in g/100g protein

Amino acid	Raw Root (X_3)	Processed Root (Y_3)	$D_3=X_3-Y_3$
Glycine	4.60	4.08	0.52
Alanine	3.36	3.16	0.20
Serine	3.61	3.50	0.11
Proline	2.73	2.57	0.16
Valine	3.03	2.95	0.08
Threonine	3.94	3.52	0.42
Isoleucine	1.84	1.64	0.20
Aspartate	6.01	5.87	0.14
Leucine	5.02	4.76	0.26
Lysine	3.62	2.83	0.79
Glutamate	13.53	12.32	1.21
Methionine	0.76	0.62	0.14
Phenylalanine	3.98	3.91	0.07
Histidine	1.91	2.10	-0.19
Arginine	1.74	2.16	-0.42
Tyrosine	2.43	2.10	0.33
Cysteine	2.42	2.05	0.37
Total	64.53	60.14	4.39

Table 4: Percentage reduction of amino acids in processed (boiled) Moringa oleifera leaf, root and seed

Amino acid	Seed	Leaf	Root
Glycine	3.81	6.99	11.30
Alanine	6.19	10.20	5.95
Serine	3.29	4.76	3.05
Proline	11.67	-0.75*	5.86
Valine	11.33	9.82	2.64
Threonine	6.21	4.57	10.66
Isoleucine	5.29	0.43	10.87
Aspartate	7.13	10.20	2.33
Leucine	15.18	5.17	5.18
Lysine	3.09	2.22	21.82
Glutamate	7.72	2.91	8.94
Methionine	-18.56*	9.47	18.42
Phenylalanine	-0.88*	4.69	1.76
Histidine	-7.46*	9.52	-9.94
Arginine	8.81	7.98	-24.13*
Tyrosine	18.45	-11.82*	13.58
Cysteine	1.49	-10.73*	15.29

**these amino acids showed increase after processing (boiling).*

The comparison of the amino acid profile of the raw and processed Moringa oleifera seed and difference (D_1) in g/100g protein for each amino acid present in the samples is shown in Table 1. Table 2 shows the comparison of amino acid profile of raw and processed (boiled) Moringa oleifera leaf. The comparison of the amino acid profile of the raw and processed Moringa oleifera is shown in Table 3. Table 4 highlights the percentage reduction of amino acid in processed (boiled) Moringa oleifera leaf, root and seed.

DISCUSSION

The amino acid profiles of Moringa oleifera seed, leaf and root shown in Table 1,2 and 3 respectively showed that a total of 17 amino acids were present in each of the analyzed samples while 3 amino acids- Asparagine, Glutamine and Tryptophan were absent. This trend is similar to that reported by Anhwange et al [13] but slightly different from that reported by Bridgemohan and Knights [14]. They reported the presence of 12 amino acids in Moringa oleifera seeds after subjecting these seeds to varying treatments and analyzed the amino acid profile using the Technicon Sequential Multi-Sample Amino acid Analyzer. The difference may be attributed to differences in the pretreatment and method of amino acid analysis.

A cursory glance across Table 4 leads to the observation that most of the amino acids in the Moringa oleifera seed, leaf and root protein were reduced after processing by boiling. The amino acids with the highest percentage reduction were Tyrosine (18.45), Alanine and Aspartate (10.20) and Lysine (21.82) in the Moringa oleifera seed, leaf and root respectively. It may therefore be suggested that processing reduced the availability of amino acids in the Moringa oleifera samples. This is because vitamins, amino acids and other nutrients are prone to destruction during food processing due to sensitivity to pH, oxygen, light, heat and the combination of these factors [15].

Results in Table 4 also showed that few amino acids increased after processing. In the Moringa oleifera seed, the amino acids- Methionine (18.56%), Phenylalanine (0.88%) and Histidine (7.46%) increased. In the leaf protein, it was Proline (0.75%), Tyrosine (11.82%) and Cysteine (10.73%) while in the root, Histidine (9.94%) and Arginine (24.13%) were the two amino acids that increased. These results compare favorably to that obtained by Nwosu et al [16] for the Oze (Bosquein angolensis) seed flour.

The decision that processing (by boiling) has a significant effect on the availability of amino acids in Moringa oleifera seed, leaf and root proteins was reached by statistically analyzing data from Table1 (Raw seed vs. Processed seed), Table 2 (Raw leaf vs. Processed leaf) and Table 3 (Raw root vs. Processed root) using the Student t-test at 5% level of significance. In all 3 cases, the calculated values of the test statistic (3.49, 3.29 and 2.98) respectively lied in the critical region i.e. they exceeded the critical value (1.75). Hence, the null hypothesis was rejected in each case and the alternative hypothesis which stated that processing had significant effect on the amino acid profiles of Moringa oleifera seed, leaf and root was accepted.

CONCLUSION

This results of these study revealed that the amino acid profiles of the Moringa oleifera seed, leaf and root were affected when processed by boiling i.e. there was an observed decrease in the overall (total) amino acid content in each case. A further comprehensive study of effect of various types of processing other than boiling and various durations is recommended to better understand and optimize the utilization of these morphological parts of Moringa oleifera if they are to be incorporated into commercial feeds to boost their amino acid contents.

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