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Production and evaluation of optimum values of some dietary amino acids of roasted African breadfruit seeds as an alternate source of protein.

Umezuruike Azubike. Chris[a] and Titus. Ugochukwu. Nwabueze[a],

Department of Food Science and Technology, College of Applied Food Sciences And Tourism. Michael Okpara University Of Agriculture, Umudike, Nigeria

Email: realmira4ac@gmail.com

ABSTRACT

This study aimed to identify the effect of roasting variable combinations of roasting temperature, time at 500g feed quantity that would yield the optimum of some essential dietary amino acids from roasted African breadfruit seeds. African breadfruit seeds were experimental roasting using predetermined variable values of temperature (120, 140, 160, 180, 200 centigrade) at 40 min and Feed mass of 500 g. The essential amino acids content of African bread fruit seed flour of different treatment condition were determined using Technicon sequential multi-sample acid analysis. Results showed that both raw and processed flour contained the same types of amino-acids. Analysis of of results showed that Roasting temperature had significant ($p < 0.05$) effect on amino acids of samples.Lysine, leucine, methionine and phenylalanine showed high heat sensitivity with values significantly ($p<0.05$)different from amino-acid values of control. The lowest values of essential amino-acids evaluated were recorded at 200^0C, Leucine and valine profiles of the processed flour were similar. Optimum contents of the eight Essential amino-acids of processed flour of African breadfruit seeds was observed at process variable combination of 140^0C and 40min for roasting temperature and time respectively.Using these process variables condition will ensure optimum harnessing of essential amino acids from African breadfruit seeds. Results of this study point to African breadfruit seed flour is a good dietary supplement for alleviating hunger and malnutrition among a deprived population.

Keywords: African breadfruits, roasting, optimum essential amino acids**.**

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1. INTRODUCTION

Protein is an important source of amin acids needed by human for proper development and metabolism. Essential amino acids such as histidine, leucine, isoleucine, lysine, methionine, threonine, phenylalanine and valine must be supplied in diets as humans are unable to synthesize them appropriately to meet our metabolic requirements (Renie 2015). Animal protein constitutes the major source of amino acids in human diets. For some reasons such as insurgency and population displacements animal protein may become scarce resulting in limited supply of amino acids in diets. To ameliorate the low access to animal protein, protein rich seeds of leguminous plants are added as substitute for animal protein in the food for supply of essential amino acids (Ugwu and Ekwu, 1996). However, processing method determines the quality and bioavailability of amino acids from the unconventional sources. Studies have shown that process temperature and time as important determinants of nutritive and biological values of protein (Makinde, *et al* 1985, Nwosu *et al,* 2008). Decomposition of amino acids especially valine, leucine, isoleucine, methonsin when protein products are heated (Makinde *et al* 1985) than the decomposition due to decarboxylation, deamination, or maillard reaction reduces biological values of Protein diets (Anyalogba *et al* 2015).

Information in literature points to African breadfruit (*Treculia africana*) seeds as an important alternative source of nutrients for human. When properly processed the flour of African breadfruit seeds can furnish the Recommended daily allowances for essential amino acids for human beings. (Makinde *et al* 1985, Anyalogba 2015)
The primary challenge facing the exploitation of African breadfruits is inappropriate roast processing techniques. For optimum harnessing of amino acids of African breadfruit seeds a process template is important to guide processors. The absence of such information in literature motivated this study.
It was envisaged that the results of this study would fill the observed information gap for roast processing and utilization of African breadfruit seeds as alternate source of amino acids in human diets as nations strive to achieve the Sustainable Development Goal of food security.

2. MATERIALS AND METHODS

2.1 Sample Collection and Pretreatment of Samples

African breadfruit (*Treculia africana) d*ry seeds were purchased from Ubani market, Umuahia Nigeria. The seeds were screened for contaminants such as stones, soils, etc., and stored in plastic bowls. The screened seeds were washed and air dried under shade at ambient temperature.

Five sets of 500g each of the cleansed seeds were roasted in oven (Fisher scientific) for 30, 35,40, 45and 50 min, and dehulled using locally designed dehuller and evaluated for yield, ease of dehulling and condition of dehulled endosperm. Forty(min) was identified to be the best roasting time, dehulling, yield and condition of the dehulled endosperm. Forty minute of roasting time was selected as center point of the factorial experimental design.

2.2 Treatment of sample

Group of 500g each of cleansed seeds of African breadfruits were roasted experimentally in 8 factorials by 6 replications (Nwabueze *et al* 2007) at 120 °C, 140 °C, 160° C ,180 °Cnd 200°C for 30mins, 35mins, 40mins, and 45mins in oven (Fishers scientific company USA). The roasted seeds were cooled, dehulled, using locally designed dehuller, milled using a hand mill (Corona Model, Landers/CIA SA) and sieved into flour using 2.0mm sieves, labelled and used for essential amino-acid assay. The control (raw seeds) was un-roasted asted flour of African breadfruit seeds.

2.3 Determination of amino-acid profile

The amino-acid profile of raw and roasted African breadfruit flour were determined using the method described by Spackman et al., (1958) The samples were dried to constant weight in oven, defatted, hydrolyzed, then evaporated using rotary evaporator. The evaporated samples were loaded into amino acid analyzer (Technicon sequential multi-sample TSM). Thirty milligram of each sample was mixed with 7ml of NHCl in a glass ampoule. Oxygen was expelled from the mixture by passing nitrogen into the ampoule. The glass ampoule was heat sealed using a Bunsen burner flame and placed in an oven (105^0C) for 22 hours. After heating the glass ampoules was allowed to cool, the tip opened and the content filtered. The filtrate was evaporated to dryness using rotary evaporator at 40^0C.
The residue was dissolved in 5ml acetate buffer (pH.2.0) and stored in refrigerator using plastic bottle. Five to ten (5-10) microliters of each sample was placed in amino-acid cartridges and loaded into the amino-acid analyzer. The TSM analyzer separates and analyzes amino acids into acidic, basic and neutral amino acids. The data generated from the Technicon

Sequential Multi-sample analyzer were quantitatively determined against the standard Technicon auto analyzer chart (No 011-648-0; Technicon Instruments, Tarrytown New York, USA

2.4 Data Analysis

Data of the study were analysed and presented as tables for further descriptive analysis. For statistical evaluation, data was analyzed using Minitab statistical software version 15 of Minitab Inc. Pen. USA. Regression analysis was used to evaluate the effect of roasting condition on the eight essential amino acids as described by equation 1.

$$Y = \beta o + \sum \beta i\ x\ i + \sum \beta ii\ x_{12} + \sum \beta ji\ xixje\ldots.\ (1)$$

Where Y = response, βo = intercept xiji = independent variables, e = error

3. RESULTS AND DISCUSSION

3.1 Amino Acid Profile of Samples

The essential amino acid composition of the processed African breadfruit seed flour is shown in Table 1. The essential amino acids are histidine, Isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine. The values of all the amino acids analysed progressively reduced as the roasting temperature was increased from 120 °C to 200 °C for 40mins (Table 2). The reported values of the essential amino acids are attributable to roasting conditions (different roasting temperature and time). The essential amino acid contents differ significantly in line with applied temperature. Our results are in consonance with the reported temperature depleting effect on essential amino acids (Mauron 1982; Adeyeye2010). The relationship and effects of roasting conditions on the analysed 8 essential amino acids indicated various degrees of susceptibility to heat as shown on the Table 3. Effect of roasting variables on amino acid content were 95.5%, 83.05% ,71.50% ,90%,93.37%, 78.19%, 84.88%, and 59.07% for histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine respectively.

Table 1. ***Essential amino acid content of Processed African Breadfruit seed.***
Essential Amino acids (g/16gN)

Treatment (^{0}C at 40min Roasting time)	Histid ine	Isoleu cine	Leucin e	Lysine	Methio nine	Phen ylalan	Threo nine	Valine
Temperature (^{0}C)								
120	2.29	3.11	4.16	3.87	0.81	3.90	2.81	2.90
140	2.12	2.64	5.40	3.91	0.73	3.76	2.64	2.89
160	1.92	2.33	3.55	3.20	0.65	3.27	2.71	2.70
180	1.56	2.04	3.60	3.10	0.58	3.36	2.16	2.94
120	1.00	1.30	2.79	2.16	0.43	2.64	1.14	2.03
Control	3.16	3.60	6.80	7.10	1.53	6.10	3.22	4.50

The variations in concentration of the essential amino acids in the test samples have been reported to reflect degree of maillard reaction between amino-acids and sugars, deamination (Nkatamiya *et al* 2007).

Table 2. Average variation of Amino Acid content of processed African Breadfruit seed flour samples.

	Content (g/16g N)					
Amino Acids	**Control**	**120^{0}C**	**140^{0}C**	**160^{0}C**	**180^{0}C**	**200^{0}C**
Histidine	3.16	0.85^{ce}	1.04^{ed}	1.24^{cd}	1.60^{ae}	2.16^{ad}
Isoleucine	3.60	0.49^{ef}	0.96^{df}	1.27^{cd}	1.56^{ad}	2.30^{ad}
Leucine	6.80	2.20^{cd}	1.40^{de}	3.24^{bb}	3.20^{bb}	4.01^{ab}
Lysine	7.10	3.23^{ad}	3.19^{ad}	3.80^{bb}	4.00^{ab}	4.94^{aa}
Methionine	1.53	1.53^{cf}	0.80^{df}	0.81^{ee}	0.95^{cf}	1.10^{bf}
Phenylalanine	6.10	2.10^{cd}	2.34^{bc}	0.83^{ee}	2.74^{bc}	3.46^{ac}
Threonine	3.22	0.41^{ef}	0.48^{ee}	0.57^{df}	0.96^{bf}	1.96^{af}
Valine	4.50	1.60^{dd}	1.61^{cd}	1.80^{cb}	1.56^{ee}	2.47^{ad}

Subscripts depict significant variations

3.2 Implications of Roasting on Individual Amino-Acids.

3.2.1 Histidine

Percentage loss in histidine showed a higher than average loss. At the mid temperature of 160^{0}C the loss in histidine increased to 39.24%. The significant effect of roasting on histidine was observed at 200^{0}C. Reductive effect of temperature was linear to mid temperature (160^{0}C) then reached the peak loss at 200 °C.

Processed African breadfruit seed flour can furnish the requirement for histidine (FAO/WHO 1991) However it is important to note that roasting of African breadfruit seeds used for flour must not exceed 160^{0}C. Above 160^{0}C processing would result to severe loss and pressure on already limiting values of leucine, tryptophan and valine in African breadfruit seed flour. (Makinde *et al* 1985)

Table 3. Amino acid Losses Across Experimental Temperatures at 40 min.

	Percent (%) loss at Temperature (^{0}C)		
Amino acids	**Low (120^{0}C)**	**Midpoint (160^{0}C)**	**Highest (200^{0}C)**
Histidine	**26.90**	**39.24**	**68.35**
Isoleucine	**13.61**	**35.27**	**63.88**
Leucine	**32.35**	**47.06**	**58.97**
Lysine	**45.49**	**53.52**	**69.58**
Methionine	**0%**	**57.52**	**71.89**

Phenylalanine	**34.43**	**13.60**	**56.72**
Threonine	**12.73**	**15.84**	**60.81**
Valine	**35.55**	**40.00**	**54.89**

3.2.2 Isoleucine and leucine

Percentage loss in isoleucine was between 13.61% and 63.88%. Loss of isoleucine was similar to histidine at midpoint temperature. The Reductive effect of roasting on isoleucine was significant ($p< 0.05$) at 200^0C. Isoleucine showed heat stability at lower to mid temperature, against leucine that exhibited rapid losses at lower (120^0C) to mid (160^0C) temperatures. The loss of leucine in the processed flour is reflective of both temperature and native values of native seeds. Though leucine showed heat stability at 140°C to 160°C, effect of roasting temperature was linear and significant. Results showed that roasted African bread fruit is deficient in leucine. The deficiency in leucine could be compensated by isoleucine content in food products.

The complementary role of isoluecine and leucine in diet reduces the adverse consequences of their deficiency. Though the deficiency in leucine can be compensated by isoleucine, it is nutritionally important that the eight essential amino-acids should be adequate for metabolic requirements of humans(FAO/WHO,1991).

3.2.3 Lysine

Lysine showed higher heat liability than other essential amino-acids of the processed flour.

Processing African breadfruit seeds above 140^0C had adverse effect on lysine. The abundance of lysine in legumes is a plus for achievement of nitrogen balance in diets with limited sulphur containing amino acids.

3.2.4 Methionine

Methionine showed heat stability at 120^0C-140^0C, followed by a rapid loss to 57.52%. Methionine is more stable to roasting temperature than lysine. Methionine had the highest loss at mid- point (160^0C) temperature. The effect of roasting temperature on methionine was observed to both linear and significant ($p< 0.05$) at extreme temperature. Methionine can be synthesized from cysteine but maintains a synergy relationship with lysine for proper metabolism in human (Passmore and Eastwood 1981). The synthesis of methionine from cysteine is critical for tryptophan. Since cysteine and tryptophan are limiting in African breadfruits, it is important that processing temperature must not exceed 140^0C in order to preserve methionine content.

3.2.5 Phenylalanine

Phenylalanine was observed to be less stable than methionine during roasting of African breadfruit seeds. Loss of phenylalanine through roasting temperature is comparable with leucine. The rapid depletion observed at low temperature (120^0C) emphasized the importance of low temperature processing for phenylalanine retention. Supplementation with soybean flour has been shown as an important means ameliorating phenylalanine deficiency in diets. (Enwere 1998).

3.2.6 Threonine

Threonine exhibited both linear loss and quadratic lossess in roasted African breadfruit seeds. The effect of temperature and time on threonine was significant. The content of threonine in African breadfruit seed flour roasted at 120-160 °C is adequate to furnish the required dietary allowances of threonine for humans (FAO/WHO 1991). However, any deficiency in threonine can be ameliorated by high lysine content.

3.2.7 Valine

Valine losses ranged between 35.55% and 54.89%. The effect of roasting temperature on valine was observed to be linear up to 160^{0}C. Results of this study show that roasted African breadfruit seed flour is not deficient in valine

4. CONCLUSION

The effects of roasting on the eight essential amino acids of African breadfruit seed were determined by comparing the values of amino acids in roasted samples and control. Effect of roasting temperature and time were significant for histidine, methionine, isoleucine, leucine and threonine. Roasting time and mass of breadfruit seeds had more effect on lysine content of samples. All variables impacted negatively on phenylalanine content of processed flour.

This study has shown that essential amino acids needs for good health and well being can be optimally harnessed from roasted African breadfruit seeds through appropriate roasting condition. Thus African breadfruit seed flour is an excellent substitute for animal protein for the supply of essential amino acids. We infer that proper harnessing of lesser known legumes of developing nations is highly desirable as a means of addressing hunger and malnutrition during periods of food insecurity when regular animal protein supply is limited due to insurgency or internal displacement of population.

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