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Process Variables Combination of Roasted African Breadfruit Seed Flour and the Essential Amino Acids Needs of Infants and Children

Umezuruike Azubuike C.*
Department of Food Science and Technology, College of Applied Food Sciences and Tourism. Michael Okpara University of Agriculture, Umudike, Nigeria

T. Ugochukwu Nwabueze
Department of Food Science and Technology, College of Applied Food Sciences and Tourism. Michael Okpara University of Agriculture, Umudike, Nigeria

Abstract

Infants and growing children require eight Essential amino acids for healthy growth and wellbeing. The amino acids must be at optimum values in order to satisfy their metabolic requirements. Breadfruit (v.Decne) seed is a widely consumed legume in tropics and subtropical regions of the world. In Nigeria it is staple and an important source of dietary nutrients for adult, infants and children. The study aimed to identify the roasting conditions of temperature and time at 500g seed quantity that would yield the optimum contents of eight essential amino acids in flour incorporated into the diets of infants and growing children for the alleviation of malnutrition among children. Experimental roasting used the factorial design. The essential amino acids content of 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 amino-acids essential for infants and growing children. Analysis of amino acids showed retention of the eight essential amino acid for infant and children. Roasting temperature and roasting time had significant ($p < 0.05$) reductive effect on evaluated amino-acids. Summation of the evaluated amino acids showed average ranges of 22.56 to25.61 g/16gN,17.14 to 23.86g/16gN and 18.56 to 19.90 g/16gN respectively for flour samples of seeds roasted at 140°C,160°C,180°C at 35,40,45 mins. The low values of some essential amino-acids evaluated indicated the detrimental effect of higher temperature processing on African breadfruit seeds. Processing African breadfruit seeds into flour at 140 and 160°C would yield four of atleast 76.0% of the RDA for essential amino acids for humans. Results identified 140°C-40min-500g as the best process variables combination for adequate supply of essential amino acids in infant and children diets. Roasting severely depleted the content of tryptophan of the evaluated flour samples. The flour of breadfruit (v.Decne) seeds is a good source of amino acids supplement for infants and growing children. Which should be introduced to mothers in developing nations.

Keywords: Breadfruits; Roasting condition; Essential amino acids; Infant and children diets.

1. Introduction

Malnutrition of infants and children is a serious challenge to the realization of Sustainable Development Goals (SDG) goals for material and child health in developing nations. Low income, illiteracy insurgency, food, insecurity, infection and diseases are some of the factors responsible for global nutritional challenges.

In a reduced availability of animal protein due to disruption in supplies with increase in prices beyond the reach of low income earners children are faced with limited supply or essential amino acids needed for healthy growth and wellbeing. To ameliorate the low intake of essential amino acids due to scarcity of animal protein, some protein rich seeds of leguminous plants are added as substitute for animal protein in the food fed to infants and growing children [1]. Eight essential amino-acids needed by infants and children are Histidine, leucine, isoleucene, lysine, methionine, threonine, phenyalanine and valine [2]. Reports in literature point to the influence of heating temperature and time on the nutritive value of proteins [3, 4].

Heating of protein foods leads to decomposition of amino acids especially valine, leucine, isoleucine, methonine. Histidine etc. [3] The decomposition due to decarboxylation and deamination, produce harmful products and reduces biological values of protein.Which could be responsible for decreased growth of animals fed with more severely heated protein diets [5]. Makinde, *et al.* [3] reported similiar loss of biological value of proteins due to excessive cooking.

The growing preference for roasted breadfruit (v. Decne) seed flour over soybean flour for complementation of infant and children foods is evident by the preferential choice of mothers for African breadfruit seed flour .Soybean flour has a objectionable beany taste to infants. The flour of breadfruit seed is produced from roasted seeds is usually mixed as supplements into diets fed to infants and children for the supply of protein, energy and other nutrients. Breadfruits seed is an important source of essential amino acids [6] When properly processed the flour of breadfruit seeds can furnish the Recommended daily allowances of essential amino acids for human beings. [3, 5].

However the nutritional importance of breadfruit seed flour is challenged by inappropriate roast processing of African breadfruit seeds by illiterate mothers. Growth test on young rats showed a positive correlation between

*Corresponding Author: realmira4ac@gmail.com.

decreased nutritive values and growth rates of diets. In order to effectively use breadfruit seed flour as alternative to animal protein good processing is essential. To achieve this objective, mothers should be taught the appropriate processing and roasting condition needed to produce flour of excellent amino-acids. The optimization model developed by Nwabueze [7] for heat treatment of African breadfruits is too professional and technologically sophisticated for domestic applications. There is the need for a simple and practicable template for heat processing of African breadfruits. Moreso information in literature point to experimental safe ranges for amino acids. which further suggests the necessity for appropriate roasting of African breadfruit seeds as source of amino acids for infants and growing children. The dearth of information in literature on the effect of roasting on the essential amino acids of African breadfruit seeds flour and the nutritional importance of these essential amino acids for infants and children underscore the importance of this study. The result of this study is intended to aid the realization of the nutritional aspects of Sustainable Development goals in relation to maternal and child health of developing nation, through the exploitation of affordable indigenous and yet nutritious legumes.

2. Materials and Methods

2.1. Sample Collection and Pretreatment of Samples

Breadfruit (v.Decne) dry 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 (28 ±2^{o}C)

Pretreatment roasting,dehulling and evaluation of 500g mass of cleaned seeds using an Electric oven and locally designed dehuller ,identified the ideal roasting points to be 140°C and 40 mins for roasting temperature,time, dehulling yield and condition of the dehulled endosperm.

2.2. Treatment of Sample

The Study design employed 3 factors by three levels experimental layout. The optimization model of Umezuruike and Nwabueze [8] and parametric values in literature for roasting of African breadfruit seeds were employed for allocation of values to roasting temperature and time at a mass of 500g. For ease of use by local processors, three standout points used were 140°C, 160°C, 180°C and 35mins, 40mins and 45mins for roasting temperature and time respectively. The cleansed breadfruits seeds were divided into groups (500g) and roasted experimentally at 140^{0}C, 160C and 180^{0}C for 35 mins 40mins and 45mins in oven (Fisher scientific oven 655, Fishers scientific company USA). The roasted seeds were cooled, dehulled using locally designed dehuller, milled with a hand mill (Corona Model, Landers/CIA SA) and sieved into flour using 2.0mm sieves, The samples were appropriately labelled and used for essential amino-acid assay. The control (raw seeds) were not roasted, but shade dried at 28$^+$-2^{0}C for 48 hours, dehulled, milled and sieved into flour with 2.0mm sieve.

2.3. Determination of Amino-Acid Profile

The amino-acid profile of raw and roasted breadfruit flour were determined using the method described by [9]. The samples were dried to constant weights 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^{0}C) 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^{0}C.

The residue was dissolved in 5ml acetate buffer (pH.2.0) and stored in refrigerator using plastic bottle. 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. Interpretation of Results

For statistical evaluation, data of study was analyzed using Minitab statistical software version 15 of Minitab Inc. Pen. USA .Analysis of variance ANOVA and characterization of variables at 95% confidence limit were used to describe level of differences among the test samples at mid and extreme roasting temperatures and roasting time.

3. Results and Discussion

3.1. Amino Acid Content of Samples

The essential amino acid compositions of the processed breadfruit seed flour samples are presented on Tables 1-3. All essential amino acids in the control were present in the processed flour. These essential amino acids are histidine, Isoleucine, leucine, lysine, methionine, phenyalanine, threonine and valine. The values of all the amino acids analysed progressively decreased as the roasting temperature was extended from 140^{0}C to 180^{0}C for the duration of 35 to 45 mins.The reported content of the essential amino acids are attributable to roasting condition (different roasting temperature and time). The essential amino acid contents differ significantly in line with applied temperature and duration as consistent with report in litreature on the depleting effect of roasting on essential amino acids [10] Similar report by Adeyeye [11] showed that roasting of groundnuts resulted in losses in contents for lysine

(15.9-27.6%) Histidine (4.23-16.5%) threonine (40.1-60.6%) Methionin (38.0-63.4%), isoleucine (13.3-31.8%). Comparatively the amino acids of African bread fruit seeds are more heat susceptible than groundnuts .The difference in heat liability of the amino acids could be due to differences in the physical dimensions of groundnuts and African breadfruits and heat transfer rates. As observed by Nwabueze [12] the physical dimensions of African breadfruit seeds is an important factor of heat processing. .The maximum loss of essential amino acids occurred at 180^{0}C.Delamination,Millard reactions and other biochemical reactions have been reported to be responsible for changes in the content of amino acids [13] and these biochemical reactions could account for the observed variations in content of test samples.

Results of the amino acid contents of flour samples produced with seeds roasted at 140°C for 35,40,45 mins are shown on Table 1.

Table-1. Amino Acid Content (g/16gN) of Flour of Seeds Roasted at 140^{o}C

Amino Acids	35min	40min	45min	Standard Deviation	Standard Error of Mean
Histidine	2.20	2.23	2.20	0.5831	0.3366
Isoleucine	3.01	2.90	2.89	0.5578	0.3219
Leucine	6.00	5.70	5.01	0.4670	0.2696
Lysine	4.27	4.18	3.11	0.6137	0.3543
Methionine	0.75	0.73	0.70	0.0416	0.0240
Phenylalanine	4.05	3.99	3.80	0.1887	0.1090
Threonine	2.73	2.70	2.75	0.2203	0.1271
Value	2.41	3.18	2.1	0.2157	0.1245
Covariance	0.993	0.978	0.972		

At 35min amino acids contents did not differ significantly except for methionine that reduced (50.77%) significantly. At 35 and40min roasting time, the difference in content of amino acids was only 0.74%.Similarly only slight difference in values were observed for 40 min roasting time. Compared with control the different roasting time resulted in total amino acid contents of 70.50%,(35min),71.12%(45min)and 62.64%(45min).

Results of the amino acid contents of flour samples produced with seeds roasted at 160°C for 35,40,45mins are presented on Table2.

Table-2. Amino Acid Content (g/16gN) of Flour of Seeds Roasted at 160^{o}C

Amino Acids	35min	40min	45min	Standard Deviation	Standard Error of Mean
Histidine	2.30	2.20	1.19	0.3056	0.1766
Isoleucine	3.11**	2.28	2.05	0.6658	0.0384
Leucine	4.53**	3.99	3.60	0.5076	0.2930
Lysine	3.64	3.49	2.51**	0.6453	0.3725
Methionine	0.69	0.63	0.61	0.0251	0.0145
Phenylalanine	3.47	3.35	3.10	0.1305	0.0753
Threonine	2.71	2.31	2.35	0.2516	0.0145
Valine	2.41	2.01	2.07	0.5560	0.3211
Covariance	0.990	0.985	0.982		

Contents of Lysine, methionine and phenylalanine were significantly reduced in flour samples. Observed decreases across roasting time ranged from 7.10-2.5g for lysine, 1.5 to 0.61g for methionine and 6.10 to 3.10g for phenylalanine. Summations of essential amino acids contents showed that flour samples produced at 140°C and 160°C differed by 8.38%. Compared with control, flour samples produced with seeds roasted at 160°C were 66.26%,56.29% and 48.46% for 35,40,45mins respectively.

The process variables combination of 160°C-40min-500g marked the start-point of significant ($p<0.05$) decreases in the amino acid contents of roasted African breadfruits.

Roasting at 180°C for 35,40,45 mins resulted to significant decreases in amino acids contents (Table3).

Table-3. Amino Acid Content (g/16gN) of Flour of Seeds Roasted at 160^{o}C

Amino Acids	35min	40min	45min	Standard Deviation	Standard Error of Mean
Histidine	1.80	1.50	1.10	0.3511	0.2028
Isoleucine	1.70	2.38	2.30	0.3716	0.2145
Leucine	4.03	3.39	3.46	0.3510	0.2027
Lysine	3.30	2.91	2.88	0.2343	0.1353
Methionine	0.60	0.57	0.55	0.0251	0.0145
Phenylalanine	3.20	3.39	3.27	0.0960	0.0555
Threonine	2.21	2.01	2.10	0.1001	0.0578
Valine	3.05	2.90	2.90	0.0866	0.5000
Covariance	0.941	0.971	0.939		

Histidine, isoleucine, lysine, methionine and phenylalanine showed high heat liability to high temperature roasting. Summations of amino acid contents showed that flour samples produced with seeds roasted at 180°C for 35,40,45mins were 55.26%,52.62%and 51.54% respectively compared with control.

3.2. Implications of Roasting Variable Combinations on Aminoacids

3.2.1. Histidine

Histidine content of processed breadfruit seed flour ranged from 1.9 to 1.29g/16gN. Percentage loss in histidine ranged from 26.90% to 65.19%. The effect of Roasting temperature and time on histidine was significant ($p<0.05$). Reductive effect of temperature was linear to mid temperature (160^{0}C) then squared at higher temperature, reaching the peak loss of 65.19% at 180°C. Less than 50% of histidine was lost at 160^{0}C.

The relationship between roasting variables combination with histidine was described as

Histidine =-16.8033+0.1538RT--0.005RT^2------------R^2=95.56%-------(1)

3.2.2. Isoleucine and Leucine

Isoleucine and leucine make up one third of the human muscle [14] which underscores their importance in the nutrition of infants and growing children. As part of haemoglobin, isoleucine and leucine are needed for hormonal synthesis and energy generation in cells and tissues. The isoleucine content of the processed flour ranged from 2.050g-3.11g/16gN. Percentage loss was between 13.61% and 63.88% with a average loss of 35.27% at mid temperature (160^{0}C) roasting conditions. Loss of isoleucine was comparable with histidine.The effect of roasting on isoleucine contents of the flour samples was significant ($p< 0.05$). Unlike isoleucine which showed heat stability at lower to mid temperature, leucine exhibited rapid losses at lower (140^{0}C) to mid (160^{0}C) temperatures. From an initial value of 6.80g/16gN (control) leucine loss of leucine in the processed flour ranged from 32.35 to 47.06% at mid temperature (160^{0}C) to 58.97% at 180°C. The loss of leucine of that value is not nutritionally important. Though leucine showed heat stability at 140-160^{0}C, effect of roasting temperature was significant.

The effects of roasting variables combinations on isoleucine and leucine were described as

Isoleucine=26.8837- 0.6990RM^2+0.0010RTRM------R^2=83.05%--------(2)

Leucine. =62.3514-1.5151RT+0.0185RT----------------R^2=70.51%------(3)

3.2.3. Lysine

Lysine showed higher temperature sensitivity compared with other essential amino-acid s of the processed flour. From an initial lysine content of 7.10g/16gN (control)the pattern of) losses of lysine were reflective of temperarure - time relationship. Roasting temperature effect on lysine was significant and described by

Lysine =5.65976-0.007RT-----------------------------R^2=90.08% (4)

3.2.4. Methionine

Methionine values in processed breadfruit seed flour ranged from 0.55 to 0.75g/16gN. Methionine showed a rapid significant ($p<0.05$) loss (57.52%) in content. Comparatively methionine is more stable to roasting temperature than lysine.Compared with other essential amino-acids, methionine suffered the highest (57.52%) loss at mid point (160^{0}C) temperature. Roasting time was a significant term and its effect on methionine under the experimental conditions was significant. The effect of roasting time on methionine could be explained by the equation

Methionine =2.3325 + 0.0052RM^2------------------------R^2=(93.39)%---- (5)

3.2.5. Phenylalanine

Phenylalanine is less stable than methionine during roasting of breadfruit seeds for flour. From initial value of 6.10g/16gN (control) phenylalanine losses ranged from 34.43% to 56.72%. Loss of phenylalanine through roasting temperature is comparable with leucine. The rapid depletion observed at temperature (140^{0}C) emphasized the importance of low temperature processing for phenylalanine The equation describing the effect of roasting time on contents of phenylalanine can be written without the non significant terms as

Phenylalanine=19.3587 -00919RT--------------R^2=78.91% ----------- (6)

3.2.6. Threonine

Threonine exhibited heat stability up to 180°C process variables conditions. Losses of threonine in the processed flour ranged from 25.37 % to 30.81%. Temperature was a significant term on threonine retention in roasted African breadfruit seed flour. The significant relationship could be explained using the equation

Threonine=6.4176 +0.0055RM^2-------------R^2=84.88 % ------------ (7)

3.2.7. Valine

Valine is an essential amino-acid needed by infants and children for good metabolism energy generation, mental alertness and calm disposition [15]. Losses in the Valine contents of the roasted seeds flour peaked at

54.89%.(180°C). The squared effect of roasting temperature c on valine was directly proportional to temperature up to 160^{0}C.The tolerable values and RDAs for essential amino acids tfor infants andchildren are shown on Table 4'

Table-4. Amino Acid Content (g/16gN) of Flour of Seeds Roasted at 160^{o}C

Amino Acids	Control	Tolerable g/day	FAO/WHO (1982) g/day	FAO/WHO (1991) g/day
Histidine	3.16	-	-	2.50
Isoleucine	3.60	4.5	-	4.80
Leucine	6.80	5.6	4.0	4.90
Lysine	7.10	14	7.0	4.30
Methionine	1.53	5.8	5.5	2.30
Phenylalanine	6.10	-	3.5	2.2-4.1
Threonine	3.22	6.2	4.0	2.90
Valine	4.50	-	5.0	4.60

Histidine a component of haemoglobin is needed by infants and growing children for growth, tissue maintenance, formation of antibodies and good reproductive development [2, 16].

Low histidine in infant diets places extra demand on leucine, tryptophan and valine [17] leading to nitrogen imbalance and adverse rates of growth and metabolism. Processed breadfruit seed flour can furnish the RDA (20-30mg/kg) requirement for histidine [18]. However it is important to note that roasting of African breadfruit seeds used for flour must not exceed 160^{0}C at 30-40 mins. Processing above 160^{0}C would result to severe loss and pressure on leucine, tryptophan and valine ,whose values are already at threshold in breadfruit (v.Decne)seed flour. [3].

Roasted breadfruit seed flour is adequate in meeting RDA of isoleucine (28-83mg/kg) for infants and children [18]. Results show that roasted breadfruit seed flour used to supplement diet of infants and growing children is high in leucine.

The complementary role of isoluecine and leucine in diet reduces the adverse consequences of their deficiency [19] Mental and physical disorders in children are common consequences of such deficiency. Roasting of breadfruit seeds used for diet of infants and children is detrimental to its isoleucine and leucine contents. Roasted Breadfruit seed flour is a major source of leucine and isoleucine for infants and growing children [18]. Leucine content is a major risk factor for major metabolic disorders in infants and children, Excess leucine was observed to impede the migration of other amino acids to brain and protein synthesis in the muscles, leading to retardation in mental and physical growth. It is nutritionally important that the eight essential amino-acids required by infants and growing children should be adequate with overages as to compensate for metabolic self regulating mechanisms.

Loss of lysine is of nutritional importance for infants as it is needed for absorption of calcium and formation of collagen. Lysine deficiency in infant diet could lead to poor bone formation, poor skin integrity, poor immunity and certain childhood diseases [20].

Though the lysine contents are within RDA (22-99mg/kg) requirement, processing breadfruit seeds into flour for infant and children diets at above 140^{0}C had adverse effect on the yield of lysine.The abundance of lysine in legumes makes legume-cereal complementation an ideal nitrogen balance method in diets. But in regions without adequate supply of legumes lysine deficiency is an important concern over limitations against healthy bone formation and healthy skin in human.

Methionine is needed for thiamine synthesis and important for infant and children. Its deficiency will result to hyperactivity and poor skin, nail and hair formation in children.Methionine and lysine maintain a synergy in human body. it is essential for absorption of calcium, collagen formation, nitrogen balance, fat metabolism, anti-oxidation and proper development of the immune system [17]. Deficiency in methionine or lysine result to inter methionine-lysine pressure and nitrogen imbalance in the amino-acid pool of infants especially when the diet is deficient in threonine. The RDA (22-49mg/kg) of methionine for infants and children is marginally met as observed in test flours. Though, methionine can be synthesized from cysteine, a critical draw on cysteine leads to excessive draw on tryptophan. As both cysteine and tryptophan are limiting in breadfruits, it is important that processing temperature must not exceed 140^{0}C for 40 mins inorder to preserve methionine in the diet of infants and growing children

Roasted breadfruit seed flour can furnish the RDA (22-141mg/kg) of phenylalanine for infants and children [18]. As an important constituent of thyroid hormones, phenylalanine is needed by infants and children for learning, vitality and alertness [21]. The phenylalanine content of African breadfruit seed flour suggested the complementation of breadfruit seed flour with soybean flour [16] is not necessary in infant and children diets.

Threonine is responsible for bone formation, good muscle, good skill and sugar metabolism in children [2]. The content of threonine in roasted (120 to 180^{0}C) breadfruit seed flour can furnish the RDA (2.90g/day) requirement of threonine for infants and children [18]. However any deficiency can be ameliorated by high lysine content of the flour

Breadfruit seed flour meets the RDA (98mg/kg) requirement of valine for infants and children [18].

4. Conclusion

The effects of roasting on the eight essential amino acids of breadfruit (v. Decne) seed were determined by comparing the values of amino acids in roasted samples and control.

This study has shown that essential amino acids needs of infants and children for good health and well being can be optimally harnessed from breadfruit seed flour through appropriate roasting condition. Results of the study implied the promise of roasted Breadfruit seed flour as an important source for the supply of essential amino acids needs of infants and children. Proper harnessing of lesser known legumes is an important means addressing infant and childhood Protein -Energy malnutrition among children of economic disadvantaged parents and during periods of limited regular animal protein supply due to dislocations of agricultural activities.

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