PRODUCTION AND CHARACTERIZATION OF ESSENTIAL AMINO ACIDS AND STARCH OF ROASTED AFRICAN BREADFRUIT SEEDS AS SOURCE OF PROTEIN AND ENERGY FOR INFANTS AND CHILDREN

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ABSTRACT: *Aim: 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 requirement. Breadfruit (v.Decne)seed is widely consumed legumes in tropics as subtropical regions of the world. In Nigeria it is staple and is an important source of dietary nutrients for adult, infants and children. The study aimed to identify the effect of roasting variable combinations of roasting temperature, time at 500g feed quantity that would yield the optimum eight essential amino acids incorporated into diet of infants and growing children towards the alleviation of malnutrition among children. Amino acid content and availability is important. Method: 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: 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 had significant ($p < 0.05$) effect on amino-acids. Lysine, leucine, methionine and phenyalanine showed high heat lability, with values significantly ($p<0.05$)different from amino-acid values of control. The lowest values of essential amino-acids evaluated indicating the detrimental effect of high temperature processes, 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 breadfruit seeds was observed at process variable combination of 140^0C and 40min of roasting condition. Conclusion: Results implied that appropriate combination these process variables condition will ensure adequate supply of essential amino acids in infant and children diet. it is also a useful template for addressing the nutritional challenges of sustainable development (SDG) goals and targets for developing nations. The flour of breadfruit (v.Decne)seeds is a good dietary 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.

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 Nigeria the insurgency in Northern Nigeria has reduced the availability of animal protein and increased its price beyond the reach of low income earners. Due to scarcity of animal protein children are faced with limited supply or essential amino acids needed for healthy

ISSN 2056-5798(Print), ISSN 2056-5801(online)

growth and wellbeing. To ameliorate the low intake of essential amino acids due to scarcity of animal protein from Northern Nigeria protein rich seeds of leguminous plants are added as substitute for animal protein in the food fed to infants and growing children (Ugwu and Ekwu 1996). Eight essential amino-acids needed by infants and children are Histidine, leucine, isoleucene, lysine, methionine, threonine, phenyalanine and valine (Francis 1986). Report in literature point to the influence of heating temperature and time on the nutritive value2 of proteins (Makinde, *et al* 1985, Nwosu *et al*, 2008).

Heating of protein foods leads to decomposition of amino acids especially valine, leucine, isoleucine, methonine. Histidine etc. [Makinde *et al* 1985] 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 [Anyalogba *et al* 2015]. Similar report by Makinde *et al* [1985) indicated 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 in the choice of mothers Soybean flour has a objectionable beany taste to infants. The flour of breadfruit seed is produced from roasted, milled and sieved seeds, and then mixed into diets or in oral rehydration solutions fed to infants and children for protein, energy and other nutrients. Breadfruits seed is an important source of essential amino acids [Okaka and Okaka 2005] When properly processed the flour of breadfruit seeds can furnish the Recommended daily allowances of essential amino acids for human beings. [Makinde *et al* 1985, Anyalogba 2015]

However the nutritional importance of breadfruit seed flour is challenged by inappropriate roast processing by illiterate mothers. Growth test on young rats showed a positive correlation between decreased nutritive value and temperature time effects on diets. In order to effectively use breadfruit seed flour as alternative to animal protein good processing is essential. To achieve this objective mothers need to be taught the appropriate processing and roasting condition needed to produce flour of excellent amino-acid properties. The dearth of information in literature on the effect of roasting on breadfruit seeds flour and quality of essential amino acids needed by children underscores the importance of this study. The result of this study is an indispensable aid to the realization of the Sustainable Development goals including the nutritional aspects of maternal and child health of developing nation, through the use of affordable indigenous and yet nutritious legumes.

MATERIALS AND METHODS

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 (28/30^{0}C).

Five groups of 500g each of the cleansed seeds were roasted in Fisher scientific oven for 30mins, 35min, 40min, 45min and 50min, dehulled using locally designed dehuller and evaluated for yield, ease of dehulling and condition of dehulled endosperm. 40mins was identified to be the best for roasting time, dehulling, yield and condition of the dehulled endosperm.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Treatment of sample

Group of 500g each of cleansed seeds of breadfruits were roasted experimentally in 8 factorial by 6 replications [Nwabueze *et al* 2007] at 120^{0}C, 140^{0}C, 160^{0}C, 180^{0}C and 200^{0}C for 40mins in the Fisher scientific oven. [655], 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) were not roasted, but shade dried at 28$^+$-2^{0}C for 24 hours dehulled, milled and sieved into flour with 2.0mm sieve.

Determination of amino-acid profile

The amino-acid profile of raw and roasted breadfruit flour were determined using the method described by Spackman et al., [1958]. 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

Determination of starch

Two point five (2.5)g of sample was mixed with 50ml distilled water. After allowed to stand for one hour, 20ml hydrochloric acid and 150ml distilled water were added to the starch solution. The mixture was refluxed in a 250ml round bottom flask for 2 hours. The mixture after reflux was neutralized with NaOH (5n) and made up to 250ml with distilled water.

One (1)ml of test sample solution mixed with 5ml anthrone reagent was heated in a water bath for 20 minutes then the absorbance read at 620nm (standard spectrophotometer). Mass of glucose is obtained from the concentration chat.

Mass of starch (g/100g)= mass of glucose x 0.9.

Analysis of Results

Data of the study were analysed and presented as tables for further descriptive analysis. For statistical evaluation, data of study 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 required by infants and children as described by the

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

ISSN 2056-5798(Print), ISSN 2056-5801(online)

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

3.0 RESULTS AND DISCUSSION

3.1 Characterization of Amino Acid Profile of Samples

The essential amino acid composition of the processed breadfruit seed flour is shown in Table 1. All essential amino acids in the control are 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 reduced as the roasting temperature was extended form 120^{0}C to 200^{0}C for 40mins with the highest effect (71.89%) on methionine and least (54.89%) on valine. 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. It has been inferred that roasting temperature has depleting effect on essential amino acids [Mauron 1982] Similar report by Adeyeye [2010] showed that roasting of groundnuts resulted in Maillard reaction losses in Lysine (15.9-27.6%) Histidine (4.23-16.5% threonine (40.1-60.6%) Methionine (38.0-63.4%) isoleucine (13.3-31.8%). Comparatively the amino acids of African bread fruit seeds is more heat susceptible than groundnuts. The reported maximum loss of essential amino acids occurred at 200^{0}C. Stepwise increase in roasting temperature by 20^{0}C resulted in losses in essential amino acid contents of the processed flour (Table1)

Table 1: ***Essential amino acid content of Processed Breadfruit seed flour***

	Essential Amino acids (g/16gN								
Treatment (^{0}C at 40min Roasting time)	**Histidine**	**Isoleucine**	**Leucine**	**Lysine**	**Methionine**	**Phenyalanine**	**Threonine**	**Valine**	**Starch (g/100g)**
Temperature (^{0}C)									
120	2.29	3.11	4.16	3.87	0.81	3.90	2.81	2.90	55.20
140	2.12	2.64	5.40	3.91	0.73	3.76	2.64	2.89	52.90
160	1.92	2.33	3.55	3.20	0.65	3.27	2.71	2.70	53
180	1.56	2.04	3.60	3.10	0.58	3.36	2.16	2.94	53.1
200	1.00	1.30	2.79	2.16	0.43	2.64	1.14	2.03	49.75
Control	3.16	3.60	6.80	7.10	1.53	6.10	3.22	4.50	56.90

The relationship and effects of roasting condition on the evaluated 8 essential amino-acids for infants and children is described in equations 1-8.

Histidine = -16.8033 + 0.1538^{RT}- 0.0005^{RT2} ....R^2 = 95.5%...(2)

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Isoleuline = 26.8837 – 1013^{RT} – 0.6994^{RM} –0.0021^{FR} +$0.0053R1^2$+0.0018^{RTRM} – R^2 $^=$83.05%..(3)

Leucime = 62.3574 – 0.2441^{RT} – 1.5157^{RM} – $0.022Y^{FQ}$ + $0.0188RT^{RM}$....... R^2 =7.15%...........(4)

Lysine = 5.65876 + 0.04183^{RT} – 0.11370^{RM} – 0.00307^{FQ} – 0.00229^{RTRM}....R^2 =90%................(5)

Methionine = 2.33251- 0.00534 ^{RT} – 0.04203^{RM} – 0.0003^{RTRM}..........R^2 = 93.37%...................(6)

Phenyalanine = 19.3587 – 0.0919^{RT} – 0.2623^{RM} – 0.0085^{FQ} + 0.0029^{RTRM} – R^2 = 78.19%.......(7)

Threonine = 6.41759 – 0.06402 ^{RT} – 0.39543^{RM} – R^2=84.88% ....(8)

Valine = 24.9529 – 0.0489^{RT} – 0.7546^{RM} + 0.0085^{RTRM} – R^2 = 59.07%.....................................(9)

The variation in essential amino acids (Table 2) and percent losses are described on Table 3. Results show that different essential amino acids have various degrees of susceptibility to heat as shown in percent loss on the Table 3.

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

The effects of roasting temperature and tine are highly significant for histidine, lysine and methionine

Implications of Roasting on the Eight-Essential Amino-Acids for Infants and Children

Histidine

Histidine content of processed breadfruit seed flour ranged from 1.0 to 2.29g/16gN. Percentage loss in histidine ranged from 26.90% to 68.35%. At the mid temperature of 160^0C the loss in histidine was 39.24%. The significant effect of Roasting temperature on histidine was observed at 200^0C. Reductive effect of temperature was linear to mid temperature (160^0C) then squared at higher temperature, reaching the peak loss of 68.35%. Less than 50% of histidine was lost at 160^0C. Histidine a component of haemoglobin is needed by infants and growing children for growth, tissue maintenance, formation of antibodies and good reproductive development [Francis 1986, Enwere 1986].

Low histidine in infant diets places extra demand on leucine, tryptophan and valine [passmore 1981] leading to nitrogen imbalance and adverse growth and metabolism. Processed breadfruit seed flour can furnish the RDA (20-30mg/kg) requirement for histidine [FAO/WHO 1991]. However it is important to note that roasting of African breadfruit seeds used for flour must not exceed 160^0C. Processing above 160^0C would result to severe loss and pressure on leucine,

ISSN 2056-5798(Print), ISSN 2056-5801(online)

tryptophan and valine whose values are threshold in breadfruit (v.Decne)seed flour. [Makinde *et al* 1985].

Table 2: ***Loss variation of Essential Amino Acids of processed Breadfruit seed flour at 20^{0}C Temperature Intervals***

	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}
Phenyalanine	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

Table 3: ***Percent loss of Essential Amino Acid at Low –Mid- Highest Points Temperatures***

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

Isoleucine and leucine

Isoleucine and leucine make up one third of the human muscle [Renie 2015] 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 1.30g-3.11g/16gN. Percentage loss was between 13.61% and 63.88% with a 35.27% loss of mid temperature (160^{0}C). Loss of isoleucine was similar to histidine at midpoint temperature. The Reductive effect of roasting on isoleucine was significant ($p< 0.05$) at 200^{0}C. Unlike isoleucine which showed heat stability at lower to mid temperature, leucine exhibited rapid losses at lower

ISSN 2056-5798(Print), ISSN 2056-5801(online)

(120^0C) to mid (160^0C) temperatures. From an initial value of 6.80g/16gN (control) leucine losses were 2.20g/16gN (120^0C) 1.40g/16gN (140^0C) 3.24g/16gN (160^0C) 3.20g/16gN (180^0C) and 4.01/16gN (200^0C). The loss of leucine in the processed flour ranged from 32.35 (120^0C) to 47.06% at midtemperature(160^0C) to 58.97% at 200^0C. The loss of leucine of that value is nutritionally important. Though leucine showed heat stability at 1400-160C, effect of roasting temperature was linear and significant. Roasted breadfruit seed flour is adequate in meeting RDA of isoleucine (28-83mg/kg) for infants and children [FAO/WHO 1991]. Results showed that roasted breadfruit seed flour used to supplement diet of infants and growing children is deficient in leucine. The deficiency in leucine is compensated by isoleucine content.

The complementary role of isoluecine and leucine in diet reduces the adverse consequences of their deficiency. Mental and physical disorders in children are common consequences of such deficiency. Roasting breadfruit seeds used for diet of infants and children is detrimental to its isoleucine and leucine contents. Breadfruit seed flour is an ideal source of leucine and isoleucine for infants and growing children. Though the deficiency in leucine can be compensated by isoleucine [FAO/WHO 1991]. 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 requirements of disease conditions during insurgency as observed in northern Nigeria

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) losses of lysine were between 45. 49% (120^0C), 53.52 (160^0C) and 69.58% (200^0C). Roasting temperature effect on lysine was significant and linear. Loss of lysine is important for infants as it is needed for absorption of calcium and formation of collagen [Balch and Balch 2000]. Lysine deficiency in infant diet could lead to poor bone formation, poor skin integrity, poor immunity and certain childhood diseases [Balch and Balch 2000].

Though the lysine is within RDA (22-99mg/kg) requirement, processing breadfruit seeds into flour for infant and children diets at above 140^0C has adverse effect on availability of lysine, [Borton 1978]. In their diet. The abundance of lysine in legumes makes legume-cereal complements an ideal nitrogen balance method in diets. But in regions without adequate supply of legumes lysine deficiency threatens the its importance in bone formation and healthy skin

Methaionine

Methionine values in processed breadfruit seed flour ranged from 0.81 – 1.53g/16gN. Methionine showed heat stability at 120^0C-140^0C, followed by a rapid loss (57.52%) at 160^0C. Comparatively methionine is more stable to roasting temperature than lysine (0.81 – 1.53g/160gN (200^0C) 3.19 -7.10/16Gn (120^0C).Compared with other essential amino-acids, methionine suffered the highest (57.52%) loss at mid point (160^0C) temperature. The effect of roasting temperature on methionie is linear and significant ($p< 0.05$) at 200^0C. 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 [Iwe and Ngoddy 2001]. 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 [Passmore and Eastwood 1981]. Deficiency in methionine or lysine result to inter methionine-lysine pressure and nitrogen imbalance in the amino-acid

ISSN 2056-5798(Print), ISSN 2056-5801(online)

pool of infants especially when the diet is deficient in threoonine. 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 140^0C inorder to preserve methionine in the diet of infants and growing children

Phenyalanine

Phenylanine is less stable than methionine during roasting of breadfruit seeds into flour. From initial value of 6.10g/16gN (control) phenyalanine losses ranged from 34.43% to 56.72%. Loss of phenyalanine through roasting temperature is comparable with leucine. The rapid depletion observed at low temperature (120^0C) emphasized the importance of low temperature processing for phenyalanine retention. Roasted breadfruit seed flour can furnish the RDA (22-141mg/kg) of phenyalanine for infants and children [FAO/WHO 1991]. As an important constituent of thyroid hormones, phenyalanine is needed by infants and children for learning, vitality and alertness (Mindel 2003). Complementation of breadfruit seed flour with soybean flour has been reported to be an important means against phenyalanine deficiency in infant and children diets. [Enwere 1998].

Threonine

Threonine exhibited two regimes of losses. First, linear loss (12, 72 to 15.84%) in value from 120^0C to 160^0C, second, sharp loss (to 60.81%) from 180^0C to 200^0C. As shown in sample the loss of threonine in the processed flour ranged from 12.73% to 60.81%. At 200^0C the effect of temperature on threonine was significant. Threonine is responsible for bone formation, good muscle, good skill and sugar metabolism in children [Francis 1986]. The content of threonine in roasted (120 to 180^0C) breadfruit seed flour is adequate to furnish the RDA (28-68mg/kg) requirement of threonine for infants and children [FAO/WHO 1991]. However any deficiency can be ameliorated by high lysine content of the flour.

Valine

Valine is an essential amino-acid needed by infants and children for good metabolism energy generation, mental alertness and calm disposition (Lehningers *et al* 2000). Valine in the processed flour ranged from 2.90g/16gN to 2.03g/16gN. Valine loss was between 35.55% and 54.89%. The effect of roasting temperature on valine was directly proportional to temperature up to 160^0C. Breadfruit seed flour meets the RDA (98mg/kg) requirement of valine for infants and children [FAO/WHO 1991].

Effect of Roasting variables on *Starch*

Independent process variables i.e. temperature, time and feed quantity (Table 1) showed significant (P = 0.05) relationship with starch content as describe in equation 10

Starch $= 74.1121\text{-}2.1713^{RM} - 0.012^{RT2} + 0.050^{RTRM} - 0.040^{RTFQ}$ …… $R^2 = 89.70\%$........... (10)

Increasing roasting temperature and time had significant effect on starch content in proportion to the mass of breadfruit.

The relationship between amino acids and starch contents due to roasting to be an indirect measure of extent of maillard reaction between amino acids and sugars. The energy value is describe in figure 1. Kilo joules contribution by starch to gross energy ranged from 187.85 to 2081.04 Kilo joules

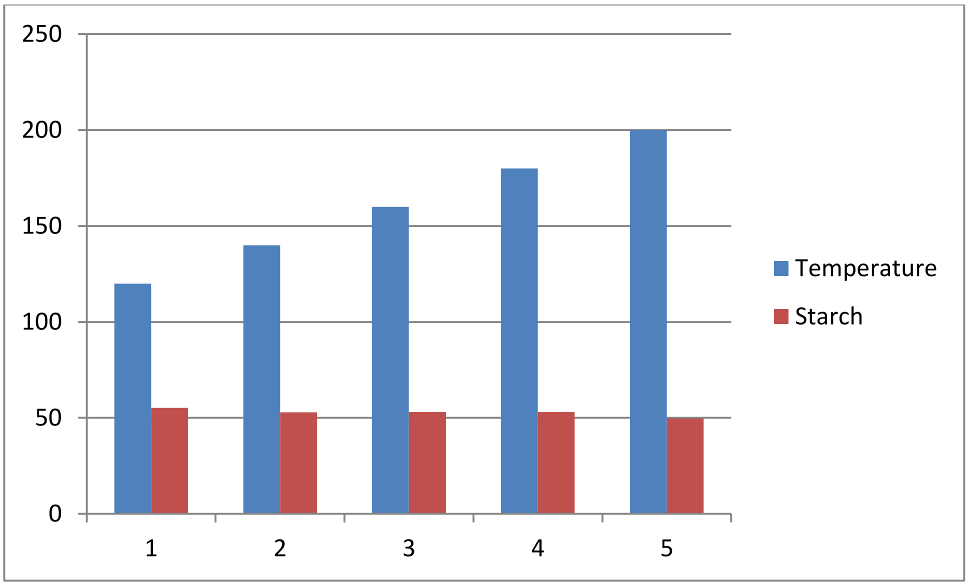

Figure 1: Effect of independent process variables on starch content

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. Except for isolencine and threonine, roasting temperature above 120^0C showed different effects on the concentration of amino acids of roasted breadfruit seeds. Breadfruit seed flour is an nutritious substitute for animal protein for the supply of essential amino acids needed by infants and children. The flour also provides a reasonable supply of energy. Proper harnessing of lesser known legumes of developing nations is an important means of addressing childhood malnutrition during periods of food insecurity when regular animal protein supply is limited due to economic and agricultural dislocations.

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