The effects of processing on the amino acid profile of Oze (*Bosqueia angolensis*) seed flour

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Abstract

Oze (*Bosqueia angolensis*) is found in tropical rain forest and grows in thick humid forest of undisturbed land. It belongs to the family Moracea. Wholesome oze (*Bosqueia angolensis*) seeds were given different treatments, which included blanching, cooking, roasting and malting. The samples obtained from these treatments were analyzed for anti-nutritional properties, functional properties and proximate composition. It is observed that out of the common twenty-two amino acids present in food, seventeen were present in oze seed with glutamic acid (416.9 mg/g N), leucine (339.3mg/g N), aspartic acid (318.8 mg/g N), arginine (253.8 mg/g N) and valine (202.5 mg/g N) being predominant. Also the eight essential amino acids needed in the daily diet were all present in oze seeds in the levels 146.9 mg/g N (lysine); 113.8 mg/g N (histidine); 165.6 mg/g N (threonine); 202.5 mg/g N (valine); 48.9 mg/g N (methionine); 165.0 mg/g N (isoleucine); 336.3 mg/g N (leucine) and 163.8 mg/g N (phenylalanine). There were reductions in the levels of these amino acids as a result of the processing treatments. For instance, lysine was reduced by 25.2% and histidine 36.7% after 60 min boiling. However, there were also some increases in some of the amino acids in the roasted samples. For instance, lysine was increased by 12.6% and Alanine by 25.4%. Two sulfur containing amino acids (methionine and cystine) were present in oze seeds. The results suggest that Oze seed contains high quality proteins. [Life Science Journal. 2008; 5(4): 69 – 74] (ISSN: 1097 – 8135).

Keywords: amino acids; roasting; malting

1 Introduction

Oze (*Bosqueia angolensis*) referred to, as the "hospitality tree" in the cultural Igbo Community is a member of the botanical family, Moracea. It is a tropical rain forest tree and grows in the thick, humid forest of undisturbed land (Keay, 1989). The tree grows up to 30 – 40 meters high as it competes with other hard wood for sunlight (Irvine, 1961). Its green glossy leaves resemble those of 'Ogbono' (*Irvingia gabonensis*); but it is readily distinguished by the remarkably abundant latex flow observed immediately at a slash of its node. This plant called Oze in the Igbo speaking states of South Eastern zone of Nigeria, and called "koko eran" in the Yoruba speaking states of South Western states of Nigeria.

In most developing tropical countries the food situation is worsening owing to increasing population; shortage of fertile land, high prices of available staples and restrictions on the importation of food (Sadik, 1991; Weaver, 1994). This has resulted in a high incidence of hunger and malnutrition, a situation in which children and women, especially pregnant and lactating women, are most vulnerable (Coulter *et al*, 1988; Pelletier, 1994). Predictions of future rates of population increase and food production emphasize the seriousness of this problem (FAO, 1990). There seems to be no immediate single solution to the problem of food sufficiency, thus interdisciplinary approach is necessary (Avery, 1991). All information on new sources of food will be of value in dealing with the food problem as suggested by Masek (1966).

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While every measure is being taken to boost food production by conventional agriculture, a lot of interest is currently being focused on the possibilities of exploiting the vast numbers of less familiar food plant resources existing in the wild (RAO, 1994). Many such plants have been identified, but the lack of data on their chemical composition has limited the prospects for their broad utilization (Vijayakumari *et al*, 1994; Viano *et al*, 1995). Most reports on some less-known and unconventional crops indicate that they could be good sources of nutrients and many have the potentials of broadening the present narrow food base for human (Van Etten *et al*, 1967; Okigbo, 1977; Aletor and Aladetimi, 1989; Janick and Simon, 1990).

The aroma of roasted Oze seed is reminiscence (resembles) that of its family member, African breadfruit; but its usual traditional dehulling process is more laborious (drudgery) than that of African breadfruit seeds. This factor has limited the traditional processing of Oze to mere hot-ash roasting and a limited consequent utilization as snacking kernels, just as roasted cashew nuts. Thus Oze though aromatically and morphologically more like African breadfruit is utilized mainly as indigenous snacking nuts just like cashew nuts.

Usually, the consumption of hot ash roasted Oze seed results in high gasing phenomenon, suggesting the presence of some anti-nutritional factors. Also the gas has a smell reminiscence of hydrogen sulphide suggesting the presence of sulfur containing amino acids which are among the essential amino acids needed in our daily diet.

Application of different processing methods to Oze seed will give some information, which may increase the utilization of Oze seeds and enhance its potential in food formulations. It is envisaged that a more preferred process for the elimination or reduction of any detected anti-nutritional factor may be found for the production of safer Oze product. The knowledge of the quality and quantities of its amino acids will aid its utilization as food ingredient. A good result will increase the awareness of the plant and may promote it as a new cash crop. This will certainly encourage its cultivation thereby saving it from being endangered.

The objective of this study therefore is to: Investigate the effects of pretreatment on the amino acid profile of Oze seeds.

2 Materials and Methods

2.1 Materials collection and preparation

The Oze seeds with intact pulp were obtained from abandoned shrine spots at Ubomiri in Mbaitoli Local Government Area and Umuchima in Ideato South L.G.A both in Imo State. The pulp was washed off with water by rubbing with the hands. The seeds were then dried in the oven at 50 ºC – 55 ºC for 24 hours. The cleaned dry seeds were then given different treatments, which included blanching, cooking, roasting and malting after which they were dehulled. Blanching was carried out for 4, 6 and 8 minutes respectively; while cooking was carried out at boiling temperatures for 20, 40 and 60 minutes respectively. Roasting was carried out in the oven at a temperature of 150 ºC for 45 minutes. Malting was done by steeping the Oze seeds for 24 hours in water at a ratio of 1 : 2 (seed to water), then germinating the seeds at room temperature for 3 weeks before drying. All samples were dehulled and then milled using the manual grinder (Corona model), sieved to obtain fine powder, which were packaged in airtight plastic containers until needed for analysis.

2.2 Determination of amino acid profile

The amino acid profile in the Oze sample was determined. The Oze sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and then loaded into the Technicon Sequential Multisample Amino Acid Analyser (TSM).

2.2.1 Defatting of samples. A known weight of the dried samples was weighed into extraction thimble and the fat was extracted with chloroform/methanol mixture in ratio of 2 : 1 using soxhlet extraction apparatus as described by AOAC (1980). The extraction lasted for 15 hours.

2.2.2 Hydrolysis of the samples. Between 30 mg – 50 mg of the defatted Oze samples were weighed into glass ampoule. 7 ml 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 during hydrolysis). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 ºC ± 5 ºC for 22 hours to effect hydrolysis. The ampoule was allowed to cool before it was broken open at the tip and the content was filtered to remove the humins.

The filtrate was then evaporated to dryness at 40 ºC under vacuum in a rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in deep freezer.

2.2.3 Loading of the hydrolysate into the TSM analyzer. The amount loaded was between 5 to 10 microlitre. This was dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate

and analyse free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 75 min. The amino acids present in the samples were identified by matching their peak retaintion time in the chromatogram with those the peaks of standard mixture of amino acids with norleucine as internal standard.

2.3 Methods of calculating amino acid values from the chromatogram peaks

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

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

NE = (area of norleucine peak)/(area of each amino acid)

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

$S_{std.} = NE_{std.} \times$ Mol. weight $\times \mu MAA_{std.}$

Finally the amount of each amino acid present in the samples was calculated in grams per 100 g protein using the following formular:

Concentration (g/100 of protein) = NH × NH/2 × $S_{std.}$ × C

where: C = [Dilution × 16]/[Sample wt (g) × N% × 10 × vol. Loaded/NH × W (nleu)]

where NH = net height, W = width, nleu = norleucine

2.4 Statistical analysis

One-way analysis of variance was used to determine significant differences in the variables: crude protein and amino acids.

2.5 Measuring chemical quality using chemical scoring

The simplest way to evaluate a food protein's quality is to study the amino acid composition of the protein itself in the laboratory. The amino acid composition of a test protein can be compared with the composition of egg protein and a chemical score can be derived to express the theoretical value of the test protein. A test protein with a limiting amino acid that is present in only 70% of the amount found in egg for example, would receive a chemical score of 70. This was calculated according to the method of Eleanor *et al* (1996).

3 Results and Analysis

3.1 Amino-acid profile of Oze seed samples

Seventeen amino-acids were identified in Oze seeds namely: lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine (Table 1). Since asparagine and glutamine do hydrolyse to their acids (aspartic acid and glutamic acid respectively) under acidic or basic medium, they might have been present in the seed protein but converted to their acids (Lehninger, 1975). The relatively higher levels of aspartic acid (447 mg/g nitrogen) and glutamic acid (536 mg/g nitrogen) with the absence of asparagine and glutamine in this study for the raw seeds could be attributed to these conversions. These two amino acids and leucine (414 mg/g nitrogen) were the predominant amino-acids found in raw Oze seeds. The amino-acid tryptophan, was either not present in Oze seed protein or not in detectable level. The observed values for the other amino-acids ranged from 103 mg/g nitrogen (for protein) to 264 mg/g N (for arginine) with the exception of methionine, which was, least in value (56 mg/g N).

Lysine was the only amino-acid whose value was significantly ($P < 0.05$) higher in the malted samples than in the raw samples. Specifically, malting increased lysine from a raw seed value of 159 mg/g N to 219 mg/g N. Malting had no significant ($P > 0.05$) effect on the levels of phenylalanine, methionine, alanine, glycine, arginine and histidine of Oze seeds as no significant differences ($P > 0.05$) were observed between their values in raw and malted seeds. The malting operation significantly ($P < 0.05$) decreased the levels of aspartic acid, threonine, serine, glytamic acid, proline, cystine, valine, isoleucine, leucine and tyrosine in the treated seeds (Table 1). The reduction effect was observed more in some of the amino acids than in others. For instance proline suffered a reduction of 35.9% (103 – 66 mg/g N), cystine a reduction of 35.1% (111 – 72 mg/g N), aspartic acid a reduction of 28.4% (447 – 320 mg /g N), while glutamic acid had a reduction of 17.4% (336 – 443 mg/g N) and isoleucine had a reduction of 11.0% (200 – 78 mg/g N) (Table 1).

Roasting had no significant ($P > 0.05$) effect on the levels of agrinine, aspartic acid, threnine, glutamic acid and leucine in Oze seeds. For instance, while raw Oze seeds had 447 mg/g N and 414 mg/g N for aspartic acid and leucine respectively, roasted seeds had 436 mg/g N and 406 mg/g N for the two amino acids respectively (Table 1). There were significant differences ($P < 0.05$) between the levels of lysine (182 mg/g N); glycine (199 mg/g N) and alanine (189 mg/g N) in roasted Oze

Table 1. Mean values of amino acids in Oze seed flour samples

Protein	Raw	4 min	6 min	8 min	20 min	40 min	60 min	Roasted	Malted	Hulls	LSD (±)
Lysine	159^{c}	156^{c}	151^{c}	147^{e}	144^{c}	126^{ab}	119^{ab}	182^{d}	219^{e}	109^{a}	0.58
Histdine	128^{b}	126^{b}	114^{b}	113^{b}	112^{b}	105^{bc}	81^{a}	91^{a}	122^{b}	126^{b}	0.25
Arginine	264^{bc}	259^{bc}	254^{bc}	248^{bc}	242^{bc}	228^{b}	216^{b}	308^{c}	255^{bc}	313^{c}	1.49
Aspartic acid	447^{c}	437^{c}	423^{c}	409^{c}	378^{b}	321^{b}	319^{b}	436^{c}	320^{b}	174^{a}	1.42
Threonine	193^{d}	178^{cd}	175^{cd}	174^{cd}	166^{c}	162^{c}	116^{c}	168^{cd}	156^{c}	78^{a}	0.57
Serine	141^{b}	109^{ab}	108^{ab}	106^{ab}	103^{ab}	99^{a}	93^{a}	97^{a}	103^{ab}	138^{ab}	1.27
Glutamic acid	536^{c}	532^{c}	498^{bc}	479^{bc}	441^{b}	421^{b}	417^{b}	496^{bc}	443^{b}	259^{a}	0.32
Praline	103^{d}	89^{c}	82^{c}	69^{b}	62^{b}	48^{a}	41^{a}	69^{b}	66^{b}	41^{a}	0.32
Glycine	163^{b}	163^{b}	161^{b}	161^{b}	161^{b}	158^{b}	155^{b}	199^{c}	164^{b}	80^{a}	0.50
Alanine	141^{c}	136^{ab}	131^{ab}	127^{b}	126^{b}	126^{b}	118^{b}	189^{d}	144^{c}	51^{a}	0.57
Cystine	111^{e}	94^{d}	69^{c}	66^{c}	64^{c}	60^{c}	60^{c}	51^{b}	72^{c}	30^{a}	0.51
Valine	261^{d}	249^{cd}	242^{cd}	216^{c}	204^{c}	203^{c}	199^{bc}	166^{b}	221^{c}	81^{a}	0.87
Methionine	56^{bc}	54^{bc}	53^{bc}	50^{bc}	47^{b}	47^{b}	46^{b}	37^{a}	50^{bc}	22^{a}	0.17
Isoleucine	200^{f}	188^{e}	173^{e}	165^{d}	164^{d}	159^{c}	156^{c}	126^{b}	178^{e}	74^{a}	0.60
Leucine	414^{c}	407^{c}	340^{b}	338^{b}	336^{b}	336^{b}	331^{b}	406^{c}	352^{b}	209^{a}	2.03
Tyrosine	169^{c}	148^{d}	134^{c}	132^{c}	127^{b}	121^{b}	121^{b}	121^{b}	138^{c}	63^{a}	0.46
Phenylalanine	174^{d}	164^{c}	164^{c}	158^{c}	158^{c}	151^{b}	148^{b}	150^{b}	170^{d}	126^{a}	0.20
Tryptophasn	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0

seeds and their levels (159 mg, 163 mg and 141 mg/g nitrogen respectively) in raw seeds, with the exception of arginine where there was no significant difference ($P > 0.05$) between the level in the roasted and the raw sample. Specifically, the percentage increases in these same acids were 12.63% (159 – 182 mg/g N) for lysines, 14.29% (264 to 308 mg/g N) for arginine, 18.01% (163 – 199 mg/g N) for glycine and 25.40% (141 – 189 mg/g N) for alanine respectively (Table 1). However, thirteen of the amino-acids suffered reductions by the roasting operation, for instance, histidine had a reduction of 28.9%, serine 31.2%, proline 33.0%, cystine 54.1% valine 36.4%, methionine 33.9%, isoleucine 37.0% and tyrosine 28.4% (Table 2).

Boiling affected the amino-acid levels in moist-heat treated Oze seeds. Decreases in levels of the amino-acids were observed starting from the 4-minute blanched seeds to the 60-minute cooked samples. Though the boiling effected gradual decreases in the amino-acid levels at all stages of treatment, for most of them the levels at all stages of boiling were not significantly ($P > 0.05$) different (Table 1). The effect of boiling on the amino-acid of Oze seeds was more noticeable when the degree of the reductions for the individual amino acids was considered (Table 2). At 6 minutes boiling (blanching) serine suffered a reduction of 23.4%, proline, 20.4%, cystine 37.8% and tyrosine 20.7%. This observation indicated that these amino acids were more susceptible to hot-water leaching. When the seeds were boiled to "doneness" (ready-to-eat product) for 40 minutes, nine amino acids suffered reductions above 20% with proline (53.4%). At 60-minute cooking thirteen amino acids had reductions above 20% with proline still leading with 60.2% followed by cystine (45.95%). It could be stated at this point that proline was the most susceptible amino acid to hot water leaching followed by cystine. The observed data suggested that the Oze seed should not be cooked more than 40 minutes to prevent much leaching effect on the amino acid.

3.2 The essential amino acids of Oze seeds samples and the chemical score of its protein

All the eight essential amino acids lysine, methionine, threonine, histidine, valine, leucine, isoleucine and phenylalanine were found in Oze seeds. Using the amino acids contents in African bread fruit seed as reported by Nwokolo (1987) in comparism to their contents in Oze seed, it was observed that Oze seed had 1.74 times the level of lysine; 2.68 times of histidine, 2.85 times of threonine, 2.63 times of valine; 2.13 times of methionine, 2.54 times of isoleucine, 3.76 times of leucine and 2.15 times of phenylalanine as observed in African bread fruit (Table 3). This result suggested that Oze seed products are nutritionally high quality foods.

Table 2. Mean percentage reduction of amino acids in blanched, cooked, malted and roasted Oze seeds

Amino acid	Blanched (6 min)	Cooked (40 min)	Cooked (60 min)	Malted	Roasted
Lysine	5.03[b]	20.8[a]	25.2[a]	-	-
Histidine	10.9[d]	17.97[c]	36.7[a]	28.9[cd]	4.91[d]
Arginine	3.8[ac]	13.6[ab]	18.2[b]	-	3.41[ac]
Aspartic acid	5.4[b]	28.2[a]	28.6[a]	2.46[b]	28.4[a]
Threonine	4.3[a]	16.1[ab]	39.9[c]	12.95[ab]	19.2[b]
Serine	23.4[a]	29.8[a]	34.0[a]	31.2[a]	26.95[a]
Glutamic acid	7.1[b]	21.5[c]	22.2[c]	7.46[b]	71.4[a]
Proline	20.4[c]	53.4[b]	60.2[b]	33.0[ac]	35.9[a]
Glycine	1.2[a]	3.1[a]	4.9[a]	-	-
Alanine	7.1[ab]	10.6[a]	16.3[a]	-	-
Cystine	37.8[bc]	45.95[ab]	45.95[ab]	54.1[a]	35.1[b]
Valine	7.3[b]	22.2[a]	23.8[ad]	36.4[d]	15.3[ab]
Methionine	5.4[ab]	16.1[ac]	21.4[ced]	33.9[d]	10.7[abc]
Isoleucine	13.5[a]	20.5[a]	22.0[a]	37.0[d]	11.0[a]
Leucine	17.9[a]	18.8[a]	20.0[a]	1.9[b]	14.98[a]
Tyrosine	20.7[a]	28.4[a]	28.4[a]	28.4[a]	18.3[a]
Phylalanine	5.8[a]	13.5[a]	14.9[a]	13.8[a]	2.3[a]

Means down the column with the same suppressants are not significantly different at $P > 0.05$.

Table 3. Comparism of amino acid profile of African breadfruit and Oze

Amino acid	African breadfruit (g/100 g)	Oze seed (g/100 g)
*Lysine	0.79 – 1.35	2.35
*Histidine	0.39 – 0.68	1.82
Arginine	0.69 – 1.23	4.06
Aspartic acid	1.25 – 174	5.10
*Threonine	0.71 – 0.93	2.65
Serine	0.85 – 1.20	1.72
Glutamic acid	1.57 – 2.39	6.97
Proline	0.52 – 0.84	1.10
Glycine	0.92 – 1.27	2.60
Alanine	0.58 – 0.72	2.18
Cystine	0.08 – 0.12	0.96
*Valine	0.85 – 1.23	3.24
*Methionine	1.6	0.75
*Isoleucine	0.7 – 1.04	2.64
*Lencine	0.87 – 1.43	5.38
Tyrosine	0.62 – 1.06	2.11
*Phenylalanine	0.72 – 1.22	2.62

*Essential amino acids: Source Nwokolo (1987) for breadfruit. The comparison being made with Africa breadfruit was gotten from literature and we had to use the same units by converting our results to g/100 g.

Also the non-essential amino-acids present are higher in Oze seeds than the amounts found in African breadfruit. Like other amino acids these acids suffered reductions in value by the different treatments (Table 2) with threonine and histidine showing up to 39.9% and 36.7% reductions respectively by boiling and isoleucine, valine, methionine, and histidine showing up to 37.0%, 36.4%, 33.9% and 28.9% reductions respectively by roasting.

The chemical scores (a rating of the quality of a test protein by comparing its amino acid pattern with that of egg protein) of Oze protein at different treatments in this study is shown in Table 4. The relevant chemical score in this study was that obtained at the treatment stage where the seeds were ready-to-eat (40 minutes to 60 minutes cooking and roasting for 45 minutes). For cooked ready-to-eat seeds the limiting amino acid was lysine, which was only 29% and 26% of the amount found in egg. This was calculated using the method described by Eleanor *et al* (1996).

Thus with these treatments as the methods of preparation, the chemical scores for Oze protein were 29 and 26 respectively. Closely following lysine as a limiting amino acid was methionine which was only 30% of the amount found in eggs.

When roasting (45 minutes) was the method for preparation, the limiting amino acid was methionine which was only 25% of the amount found in egg protein. Thus with this treatment the chemical score for Oze protein was only 25. Under this condition, isoleucine was the next limiting amion acid.

The levels of methionine (37 mg/g N) and cystine (51 mg/g N) in the roasted (the method presently used in the preparation of seed for consumption) seeds might be responsible for the hydrogen sulphide – like smell perceived from gases evolved some hours after consumption as informed by rural consumers.

African breadfruit is the most common known tropical seed used locally used that resembles Oze both in seed nature habitat and nutritional composition. Hence it was used for comparison of their amino acid profile.

4 Conclusion

The results obtained from the project have shown that Bosqueia angolensis popularly known as Oze in Igbo speaking community yields flour which contain appreciable quantities of major nutrients like proteins

Table 4. Mean chemical scores for the essential amino acid of Oz' seed samples

Samples	Lysine	Cystine	Tyrosine	Threonine	Valine	Histidine	Isoleucine	Leucine
Raw	36[a]	46[a]	57[a]	65[a]	64[a]	86[a]	59[a]	77[a]
Blanched (4 min)	36[a]	41[b]	53[ab]	61[ab]	61[ab]	84[a]	55[ab]	75[a]
Blanched (6 min)	34[a]	33[c]	51[b]	60[bc]	59[b]	78[b]	51[bc]	63[b]
Blanched (8 min)	33[ab]	33[c]	51[b]	59[bcd]	53[cd]	78[b]	49[cde]	62[b]
Cooked (20 min)	33[ab]	33[c]	49[bc]	56[cde]	50[cd]	77[bc]	48[cde]	62[b]
Cooked (40 min)	29[b]	30[c]	49[bc]	55[de]	49[c]	73[c]	47[cd]	62[b]
Cooked (60 min)	26[b]	30[c]	46[c]	39[f]	49[c]	56[e]	46[d]	61[b]
Roasted	41[c]	25[d]	47[c]	57[bcde]	41[e]	63[d]	37[f]	75[a]
Malted	49[d]	34[c]	53[ab]	53[e]	54[d]	84a	52[bc]	65[b]

Means down the column with the same superscripts are not significantly different at $P > 0.05$.

and carbohydrates. The amino acid analysis revealed that Oze seed flour contains good quantity of histidine, which is essential for infants. So use of Oze seed flour should be encouraged for use in infant food formulation only when other analyses on biological value (BV) have been completed. Chemical score of the amino acids also revealed that lysine is the first limiting amino acid. To this effect maximum benefit from this product would be obtained by direct lysine supplementation or blending with lysine rich vegetable protein ingredients such as those of soybeans.

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