



Effect of Processing on Total Amino Acid Profile of Maize and Cowpea Grains

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Abstract: During the processing of cereal grains, a substantial proportion of the nutrients are lost, most especially the proteins in the form of the smallest moiety called amino acids. The objective of this study is to investigate the proportional loss due to processing, in order to throw more light in external fortification of the processed grains as may be necessary for the future.

This investigation carried out the amino acid profile of drum dried; freeze dried and spray dried products of processed maize and cowpea grains in order to establish the after effects of these processing methods on the amino acid profile of the residual products. The processed methods investigated have been reported to be ideal for the preservation of processed grains in the powdered forms and amino acids availability in the products will determine the final protein nutritional value of the processed food products.

Keywords: Cereal grains processing, Cereal proteins, Sprouting, Drying.

1. Introduction

1.1 Modern Processing

1.1.1 Modern Dry Milling of Cereal Grains

A good amount of work has been carried out on the dry milling of cereals by Ladipo (1977), Olatunji *et al.*, (1980), and Kent (1980). The aim of modern processing of cereals for food uses is to separate the grain into three fractions, which are:

- i. Hulls and Bran: Which constitute the outer layer of the grain. Although rich in vitamins and minerals, these are largely indigestible by man. They are removed from the grain, partly because their removal improves the functional properties as well as the colour of the resulting flour.
- ii. Germ: This fraction is high in oil. It is enzymatically active and is responsible for the development of rancidity if not removed.
- iii. Endosperm: Contains a large proportion of the starch and proteins of the grain and are the desired fractions for milling into food flour.

Kent (1980) classified the series of operations in dry maize milling as follows:

1. Cleaning
2. Conditioning classifying
3. Degerminating
4. Drying and cooling
5. Grading and aspirating
6. Grinding on fluted rolls
7. Sifting and classifying
8. Purifying and aspirating
9. Drying
10. Packaging

1.2 Effect of Processing on the Nutritive Value of Maize

1.2.1 Effect of Traditional Processing

Akinrele (1966) discovered that the fermentations which occurred as a result of steeping and the souring of maize during the traditional processing method did not significantly affect the protein content. However, the biological value, the net protein utilization and the protein efficiency ratio of the final product "ogi" were found to be inferior to those of

whole maize meal. This he attributed to losses in milling and sieving during processing. The traditional processing method was found to remove a considerable portion of the hulls, aleurone layer and germ. In his report, there was a slight indication that the ogi fermentation *per se* brought about a slight enrichment in the thiamine and niacin content of maize, but this did not make up for the loss of vitamins which took place milling and sieving.

Oke (1965) found Nigerian maize to be peculiarly high in ash content, with special reference to calcium and iron, when compared with American maize. However, he found the nutritive advantage arising from this to be lost in the processing of ogi.

Although the discovery of the use of the opaque-2 mutant gene in changing the amino acid composition of maize protein raised considerable hope for the maize-eating population, studies by Fetuga *et al.*, (1974) showed ogi from high-lysine maize to be inferior to that from normal maize. They found that the biological value, net protein utilization and net protein retention were lower with opaque-2 maize flour than with normal maize flour. The rats lost more weight in 10 days when fed processed opaque-2 maize than when fed processed normal maize.

During the traditional processing of maize into “tortilla” (“tortilla” is a Mexican maize dish prepared from maize by steeping it in about one percent lime solution, wet milling and cooking into a flat cake), Cravioto *et al.*, (1945) found that there was a 40 percent loss in the carotene of the yellow corn; losses of thiamine were relatively small, there was no significant loss of riboflavin. As to mineral content, the calcium content increased by 2000 percent, the phosphorus by 15 percent and the iron by 37 percent. The high calcium content of tortilla was attributed to the treatment of the corn with limewater.

Similar studies carried out by Massieu *et al.*, (1949) on tortilla preparation revealed the following amino acid losses;

Amino Acid	Amino Acid Losses %
Tryptophan	30
Threonine	26
Histidine	26
Arginine	16

1.2.2 Effect of Modern Processing

The modern processing of maize into flour involves total separation of the hulls and bran as well as the germ from the endosperm. The endosperm, which contains the major proportion of the starch and proteins of the grain, is the desired fraction for milling into food flour.

The removal of the bran is accompanied by the loss of a high proportion of the minerals, since the bran layer contains a higher proportion of minerals than the endosperm, while removal of the hull or chaff is

associated with removal of the cellulose (Perten, 1976 and Desikachar, 1976).

Work carried out by Perten (1976), Desikachar (1976) and recently by Olatunji *et al.*, (1980) has shown reduction in oil, protein, crude fibre and dash content of processed maize grains. However, pearling was reported to improve the appearance of the grains and the traditional dishes prepared by using the flours and grits were found to improve eye and taste appeal. Goussault and Adrian (1976) showed that the reduction of the indigestible carbohydrate material during the pearling enhanced higher digestibility of the final flow product.

1.2.3 Effect of Heat Treatment on Cereals

Mitchell *et al.*, (1949) carried out a series of metabolic studies with young rats on the effect of dry and moist heat on the nutritive value of the protein contained in maize. They found dry heating (toasting of the “cut” grain at 176.7⁰C – 204.4⁰C for 3 to 4 minutes to increase the digestibility of the proteins by about 8 percent and caused no change in their biological value. In the process of “flaking”, however, in which the cut grain, before being toasted, was subjected to steam pressure for 20 to 45 seconds, they revealed a diminished digestibility of the protein by about 15 percent and the biological value by about 6 percent.

Bender (1971) in his experiments concluded that no damage was noticed when cereals are cooked in water, and that the damage accompanying baking is limited to the outer and consequently more severely heated portions, and that the only processes which cause really severe damage are the puffing-exploding process used in the preparation of certain breakfast cereals, and the baking of biscuits.

These effects of heat had been taken into consideration in the choice of different drying operations used in this study –drum drying which imparts direct heat on the food product, freeze drying which is expected to impart less heat on the food product during drying, and spray drying which comes in between the two earlier mentioned drying operations in terms of imparting of heat on drying.

1.2.4 Improving Nutritional Quality of Maize Diets

Work by Sure (1948) has shown that, when milled white cornmeal is replaced by an equivalent amount of dried brewer’s yeast or primarily grown cultured yeast, there resulted large gains of body weight and a marked increase in protein efficiency ratio. As evident from gain in weight per gram of protein intake. This probably resulted from the contribution by the yeast of the amino acids lysine and tryptophan, which are limited in maize.

Akinrele (1966) and Akinrele *et al.*, (1971) found that when full-fat soya flour, which has been heated to destroy the anti-tryptic factor was added to “ogi” at the 30 percent level, the protein efficiency ratio

of the mixture was 2.21, about three times the value for “ogi” (0.77).

Jones and Divine (1944) found that the protein efficiency ratio of full-fat soya bean meal was equal to that of casein. Assuming this to be so, the protein efficiency ratio of the experimental soya-ogi diet should theoretically be 1.49 according to Akinrele (1967). He, therefore, stated that his observed value of 2.21 clearly confirms the observation of Chick (1951), that soya protein had a special supplemental value to cereals, probably because of its high lysine content.

Work by Elias and Bressani (1971) had shown that, for the improvement of the protein quality of tortilla flour (a maize diet in Central America), there were three possible ways:

- i. Supplementation with synthetic amino acids;
- ii. Supplementation with the use of protein concentrates;
- iii. Supplementation with protein concentrates plus amino acids.

2. Materials and Methods

2.1 Total Amino Acid Determination

2.1.1 Grain Samples

The maize (*Zea mays*) used in the present study was a white variety obtained from the National Cereals Research Institute, Badeggi, Nigeria, designated FARZ 27. The cowpea seeds (*Vigna unguiculata*) were of the same source as the maize, but designated FARV 34; all the samples were cleaned by passing the grains over screens to remove broken grains and debris.

2.1.2 Maize and Cowpea Flour Processing

i. Corn flour Preparation using a Drum Dryer

The processing of corn into corn flour included the preliminary stages of cleaning, steeping and wet milling using a liquidizer and sieving as described by Akinrele (1971). The corn mash (70% moisture) was dehydrated on an atmospheric double drum dryer (Brooks Motors Ltd., Huddersfield Model B.SS 168) with 15cm diameter x 20cm in length. The drum speeds were varied between 2-4 rpm with a clearance between the drums of approximately 0.04cm. The drums were heated internally by steam at a pressure of 45 psi (45lb/in²). The corn mash was applied using a peristaltic pumping machine to prevent accumulation and excessive gelatinization, and also to give a regular throughput. Samples of the dried corn flakes were crumbled into smaller bits. The flaked corn was later milled into flour using a liquidizer and later sieved through a No. 500 British Standard Sieve (a 1mm screen) to give a flour of uniform particle size.

ii. Cowpea Flour Preparation Using a Drum Dryer

The cowpea was cooked using a steam pressure (121⁰C; 15 psi, for 20 minutes in a pressure cooker after preliminary cleaning, soaking for 18 hours and manually dehulled, as Described by Onayemi and Potter (1976). The cooking was to inactivate the anti-nutritional substances such as trypsin inhibitors, which reduce biological utilisation. The cooking also inactivates the lipid enzymes, which are associated with the development of off-flavours during prolonged storage (Rackis, 1972).

The cooked mash was blended using a Waring blender to give slurry of uniform particle size. While the blending proceeded, the puree was diluted to a solid content of 30 percent to facilitate handling on the drum dryer. The slurry was also drum dried as in the case of corn, using the same drum drier.

The cowpea flakes were later milled into flour using a Waring blender and sieved through a No. 500 British Standard Sieve. The flour was packed into airtight plastic containers and kept in a deep freezer at a temperature of -16⁰C.

iii. Corn Flour Preparation Using a Spray Dryer

The processing of corn using a spray dryer included similar preliminary stage of cleaning, steeping, milling and sieving as described using a drum dryer. The slurry obtained was reconstituted to 25 percent total solids. The inlet temperature was 110⁰C while a temperature of 90⁰C was maintained at the outlet. The fine powder collected was packed into airtight plastic boxes and kept in a deep freezer at a temperature of -16⁰C.

iv. Cowpea Flour Preparation Using a Spray Dryer

The cowpea used in this study was subjected to similar preliminary unit operations as described for the drum dryer, before spray drying operations.

v. Corn Flour Preparation using a Freeze Dryer

Processing corn flour using a freeze dryer involved then initial stage of cleaning, soaking, milling and sieving. The freeze dryer used was a Stokes laboratory freeze dryer (F.J. Stokes Corporation, Philadelphia 20, PA, U.S.A.). The product was frozen in a deep freeze at a temperature of -16⁰C before being introduced to the condenser plate at a temperature of -20⁰C. The shelf temperature was 45⁰C and the sample was dried for 24 hours. The moisture of the slurry before the commencement of the freeze drying operation was brought to 45 percent using cheesecloth to strain off some of the liquid. The freeze-dried products were finally crushed into fine powder using a Waring blender and later sieved through a No. 500 British Standard Sieve. The fine powder resulting was packed in an airtight plastic container and kept in a deep freeze at -16⁰C.

vi. Cowpea Flour Preparation using a Freeze Dryer

The cowpea used was subjected to the same preliminary unit operations as described above, before the final freeze-drying operation.

2.2 Sample Preparation (Hydrolysis)

A sample of the test proteins containing 6-8mg nitrogen (20mg in the case of cowpea grain) were weighed into screw capped 25ml tubes. 6ml hydrochloric acid was added, depending on the weight of the sample (HCl 500 x weighs of sample), 10ml for example for 20mg cowpea sample. Nitrogen gas was gently bubbled through each tube for 20 minutes (timed by stop clock to prevent oxidation of the samples). The samples were later incubated for 24 hours at a temperature of $110 + 1^{\circ}\text{C}$. At the end of the incubation, each hydrolysate was cooled, transferred with distilled water (~ 30ml) to a 250ml round-bottomed flask, and the contents of the flask were evaporated to dryness under vacuum at a temperature a little below 60°C . 25ml of citric acid buffer pH 2.2 was added to each residue, the whole thoroughly shaken and filtered into a Universal bottle through a No. 1 Whatman filter paper. The samples were frozen.

2.3 Sample Analysis

Amino acid composition in the hydrolysates was determined by automatic ion exchange column chromatography, based on the method of Spackman *et*

al., (1958), using a JEOL amino acid analyser 9 type JLC-5AH (Japan Electron Optics Co. Ltd., Tokyo). The chromatography was carried out on two columns, both containing cation exchange resin (JEOL resin, type LC-R-1) of particle size 11-14 μ . The columns, which had dimensions of 0.8 x 15cm and 0.8 x 70cm, were for the analysis of basic amino acids and ammonia and neutral and acidic amino acids, respectively. The basic amino acids were eluted with 0.35M sodium citrate buffer of pH 5.28 at a flow rate of 1.22ml/min. Under these conditions, lysine, histidine, arginine and ammonia were separated in about 2 hours. The concentration of each amino acid was determined after reaction with ninhydrin by reference to the internal standard of 2-amino = 3-guanidinopropionic acid. The neutral and acidic amino acids were eluted initially with 0.2M sodium citrate buffer pH 3.16 at 0.83ml/min. After 130 min. the elution was continued using 0.2m citrate buffer of pH 4.25 also at 0.83ml/min. Under these conditions, the neutral and acidic amino acids were well separated in about 5 hours.

The levels of amino acids were calculated with reference to the response of a standard mixture of amino acids run on the same programme. Peak areas were digitised with an online JEOL model DK integrator and visualised on a JEOL model 1-2 printer. The analysis of each sample was completed in about 7 hours.

Table 1. Effect of Processing on Total Amino Acid Profile of maize expressed as g/16g N and analysed with a JEOL Amino Acid Analyser.

Amino Acid	PROCESSING OPERATION					STANDARD ERROR OF DIFFERENCE (SED)		VARIANCE RATIO (F)		
	Raw Maize Flour	Drum-Dried Maize Flour	Sprouted Drum-Dried Maize Flour	Freeze Dried Maize Flour	Sprouted Freeze Dried Maize Flour	Sprouting and Drying	Drying x Sprouting	Sprouting	Drying	Drying x Sprouting
Lysine	3.17	2.23	2.10	2.29	2.02	0.04	0.05	32.8	0.1	3.6
Histidine	3.69	3.89	3.73	3.15	2.46	0.18	0.26	5.4	31.7	2.2
Ammonia	4.29	3.41	3.52	4.78	5.49	0.04	0.06	87.1	1428.2	44.8
Arginine	3.56	4.48	4.39	5.08	4.66	0.08	0.11	10.8	23.6	7.9
Aspartic Acid	5.61	6.96	4.54	7.22	5.67	0.11	0.16	320.3	39.4	15.0
Threonine	3.19	3.41	2.77	3.22	3.97	0.24	0.34	0.1	4.5	8.6
Serine	4.77	5.49	5.53	6.35	3.80	0.03	0.04	1951.6	234.1	2092.6
Glutamic Acid	19.74	15.56	18.18	16.87	17.73	0.04	0.05	2363.3	145.6	598.1
Proline	7.41	7.59	8.58	7.53	8.33	0.19	0.20	22.7	0.7	0.3
Glycine	3.59	3.39	3.57	3.44	3.73	0.20	0.29	1.4	0.3	0.7
Alanine	8.40	6.87	6.82	7.70	7.96	0.10	0.14	1.2	101.0	2.5
Cystine	1.62	1.74	1.82	1.73	1.94	0.07	0.11	2.1	5.7	3.1
Valine	3.81	4.38	4.28	4.16	3.93	0.03	0.04	37.6	111.1	5.6
Methionine	1.75	1.78	1.64	1.68	1.78	0.08	0.11	14.9	27.2	0.6
Isoleucine	3.33	3.87	3.92	3.15	2.58	0.06	0.08	14.9	27.2	32.4
Leucine	13.44	12.66	12.88	14.50	15.71	0.11	0.15	44.1	470.5	20.0
Tyrosine	4.92	6.59	5.33	4.35	4.44	0.09	0.12	46.3	328.5	62.2
Phenylalanine	5.29	7.96	8.21	5.27	6.35	0.11	0.16	34.4	394.5	13.1

F values for 1 and 4 degrees of freedom

5%	1%	0.1%
5.71	21.2	74.4

*Mean of two replicates (results for raw maize flour only included for comparison).

Table 2. Effect of Processing on Total Amino Acid Profile of cowpea expressed as g/16g N and Analysed with a JEOL Amino Acid Analyser.

Amino Acid	PROCESSING OPERATION					STANDARD ERROR OF DIFFERENCE (SED)		VARIANCE RATIO (F)		
	Raw Maize Flour	Drum-Dried Maize Flour	Sprouted Drum-Dried Maize Flour	Freeze Dried Maize Flour	Sprouted Freeze Dried Maize Flour	Sprouting and Drying	Drying x Sprouting	Sprouting	Drying	Drying x Sprouting
Lysine	7.45	7.37	6.68	6.52	6.89	0.08	0.12	3.4	14.6	36.9
Histidine	4.08	3.29	3.62	3.42	3.56	0.13	0.18	3.3	0.6	0.6
Ammonia	3.23	4.58	3.60	3.79	3.79	0.13	0.18	7.4	1.4	7.2
Arginine	8.21	8.28	8.73	8.15	8.11	0.11	0.16	3.2	10.9	4.7
Aspartic Acid	11.57	9.58	10.15	10.17	10.20	0.08	0.12	28.0	5.2	24.5
Threonine	5.31	4.57	4.13	4.71	4.54	0.13	0.18	5.5	4.7	1.2
Serine	5.49	4.79	5.81	6.40	6.23	0.04	0.05	138.6	804.8	278.7
Glutamic Acid	18.52	20.48	15.76	15.80	15.80	0.14	0.20	280.0	270.4	280.6
Proline	3.35	4.52	4.58	5.05	4.81	0.28	0.39	0.1	1.9	0.3
Glycine	3.38	3.69	5.68	5.89	5.10	0.24	0.34	6.5	11.7	34.1
Alanine	3.83	5.08	5.25	5.95	4.99	0.25	0.35	2.6	1.5	5.3
Cystine	0.65	0.67	0.74	0.69	0.93	0.06	0.08	13.1	6.8	3.4
Valine	3.67	1.62	4.36	4.14	4.44	0.17	0.24	82.8	61.0	53.1
Methionine	1.23	1.41	1.33	1.54	1.33	0.06	0.09	6.1	1.0	1.0
Isoleucine	3.49	4.11	4.55	4.31	4.04	0.30	0.42	0.8	0.3	1.4
Leucine	8.41	6.43	4.39	4.13	4.86	0.15	0.21	19.4	37.9	85.9
Tyrosine	3.34	3.76	3.36	3.12	3.65	0.09	0.13	0.6	3.5	24.4
Phenylalanine	5.14	6.73	6.76	6.42	6.68	0.14	0.20	1.1	2.2	0.7

F values for 1 and 4 degrees of freedom

5%	1%	0.1%
5.71	21.2	74.4

*Mean of two replicates (results for raw cowpea flour only included for comparison).

3. Results and Conclusion

3.1 Results

Results in Tables 1 and 2 show the effect of processing on the total amino acid profile of maize (*Zea mays*) and cowpea (*Vigna unguiculata*) expressed as g/16g N and analysed with a JEOL amino acid analyser, respectively.

Table 1 shows no significant changes ($P \geq 0.05$) in the two drying operations; drum drying and freeze-drying of maize of maize grains, as far as lysine, proline, threonine and glycine amino acids content are concerned. On the other hand, significant changes ($P \geq 0.01$) were recorded for the rest, except cystine, which was found to be significant only at the 5 percent level of significance.

Table 2 shows that there were no important variation between the arginine content of the raw cowpea and the processed grains.

The important differences in amino acid contents as a result of processing are a general increase in serine, proline, glycine, alanine, cystine, methionine, isoleucine and phenylalanine. Among the processed samples the differences in the amino acid profile between the sprouted and unsprouted samples were in general not significant ($P > 0.05$).

3.2 Conclusion

Although there were no remarkable differences between the freeze-dried samples of maize and the drum-dried ones (Table 1), the sprouted drum-dried maize samples showed significant increases in phenylalanine, valine, isoleucine, cystine, praline, tyrosine and arginine. On the other hand, lysine, the most important amino acid as far as nutritive value of cereals is concerned, was notably reduced by the drum-drying and freeze-drying operations.

Contrary to expectations, it, therefore, appears that the high temperature for a short time in drum-drying caused less destruction of lysine than lower temperature for a long time applied in freeze-drying.

The total loss to the amino acids can be attributed to losses due to soaking and wet sieving of maize materials prior to the final drying. In the present study, therefore, the two-adopted methods did not improve the total amino acid profile to any appreciable extent.

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