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EFFECT OF PARTICLE SIZE DISTRIBUTIONS ON THE AMINO ACID COMPOSITION AND *IN-VITRO* PROTEIN DIGESTIBILITY OF TWO VARIETIES OF BEAN

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Abstract
The study examined the alterations in the proximate composition, *in-vitro* protein digestibility and amino acid composition of two varieties of bean with respect to particle size distributions. Brown and white beans were processed and milled to obtain flour samples of fine particles. The flour samples were subjected to particle size analyses using Endecott sieve with pore sizes of 300, 315, 500 and 600 μm. The bean flours with different particle sizes were evaluated for proximate composition, protein digestibility and amino acid composition. The results showed that the highest yields obtained for the brown and white flours were 40 % (315 micron) and 35 % (600-micron) respectively. The ash, protein and fibre contents of the samples varied between 4.51 to 4.61 % and 3.72 to 3.78 %; 24.74 to 24.87% and 22.76 to 22.95 %; 3.24 to 5.23 % and 2.11 to 5.15 % for brown and white bean varieties respectively. The highest values for the protein digestibility were obtained in 315μ for both the brown and white bean flours. The results also showed that the amino acid composition of the samples varied with respect to the particle size and the variety of bean flour. The study concluded that particle size of flour should be considered as a quality factor of flours to enhance derivation of nutritional benefits of flours.
Keywords: Food powders; improved functional properties; legumes; flavor improvement

Introduction

Leguminous crops such as soybean, cowpea, peanuts, Bambara nuts etc. play significant roles in the dietary pattern of sub-Sahara African countries of the world, due to the high protein contents and low cost. The crops are usually used as replacement for animal proteins among the low resource population. Cowpea is a widely consumed leguminous crops around the globe. The Food and Agricultural Organization reported that the world production of cowpea has been on the increase annually at about 3% rate since the year 2000 (FAO, 2019). Nigeria contributes a total of 40.2 % to the annual global production of cowpea (FAO, 2021), with cultivation mainly concentrated in the Northern part of the country, due to favorable climatic condition that supports its growth. Cowpea*, (Vigna unguiculata L. Walp)* exist in different species (Brink & Belay, 2006). In Nigerian markets, the seed of cowpea is available in different colour, such as black, blackeye, brown eye, red, pinkeye, cream etc. The nutritional composition of cowpea may be influenced by climatic conditions, colour of the seeds, species varieties and agronomic practices (Afrifah *et al.*, 2021). Cowpea contain high amounts of proteins (23-37 %) with appreciable level of essential amino acids, except methionine and cysteine (FAO and USDA, 2021). Cowpeas also contain other nutrients, such as fibre and mineral elements.

The utilization of cowpea, in wet or dried form, as bean pudding, bean cake or as nutrient enhancer in food involves milling and reduction in particle sizes. Variations in the particle sizes of flours or food powders have been reported to influence the quality characteristics of the resulting products. It has been revealed that quality attributes of food powders, such as protein composition, starch properties, stretchability, and foldability are affected by the particle sizes (Ma, *et al.*, 2019; Guerra-Olivera *et al.*, 2022; Sinaki *et al.*, 2023). In some food products, fine particle sizes result in high quality in terms of sensorial and nutritional values while in some cases, bigger particle sizes are required to produce high quality food products. For instance, Patwa *et al.* (2014) reported that small particle sizes of wheat flour enhanced its flowability and reduced interparticle cohesive forces. Ma *et al.* (2019) also showed that wheat flour with small particle sizes have increased starch damage, enhanced creep protein recovery, increase the rate of cooking loss and decreased the amount sulfhydryl content of gluten protein.

However, the literature appears to be full of information regarding the effect of particle size distribution on wheat flour, a cereal grain, with limited data on legumes, especially cowpea, which can be transformed into another category of food using particle sizes. Therefore, since cowpea is a protein rich crop, an understanding into the effects of the particle sizes distribution of the proteins involves an investigation of the particle sizes of the flours as it affects the protein digestibility and the amino acid content. This work was designed to provide an insight on how particle size distribution affects some protein content related parameters, such as amino acid composition and intro protein digestibility of two varieties of cowpeas in the Nigeria market.

Materials and Methods

Materials

The materials used in this work include white and brown beans which were obtained from popular market in king market, Ado-Ekiti, Ekiti State, Nigeria. All chemicals used in this work were purchased from accredited wholesalers in Nigeria.

Methods

The beans were sorted and cleansed to remove foreign materials, stones and unwanted grains that were present in the beans to prevent contamination. The beans were milled using the laboratory blender (Kenwood) set at speed 4 (max). The milling operation was done three times at sixty seconds each, to ensure that the finest particle sizes of the flour was obtained from the milling stage. The flours were weighed and packaged as starting material for the particle size analysis.

The milled flour samples obtained from the two different bean varieties were subjected to particle size analyses using the Endecott's sieve of various pore sizes. Four pore sizes were selected for this process (300, 315, 500 and 600 μ). The sieves were arranged using the top-bottom approach (the big size at the top and the small pore size at the bottom). Bean particle bigger than each pore size of the sieve are retained on the sieves. Bean particle smaller than the pore size of the sieves pass through the pores and enter the next sieve. The particles that passed through the pore sizes were regarded as the sizes of the flour particle. The process continues until the bean flour is used up. The percentage retention on each sieve was calculated

Percentage retained (yield)=

$$\frac{\text{amount retain on the sieve size}}{\text{Initial weight of bean flour}} \times 100 \qquad (I)$$

Determination of the proximate composition of the flours

The flour samples of different particle sizes were subjected to proximate composition (Moisture content, Ash content, Fibre content, Fat content, crude protein content and carbohydrate content) using the method of AOAC (2012).

Determination of the *In-vitro* protein digestibility

In-vitro protein digestibility of the samples was measured according to the method described by Chavan *et al.* (2001). Two hundred fifty milligrams of the sample were suspended in 15 mL of 0.1 M HCl containing 1.5 mg pepsin (> 250 units/mg), followed by gentle shaking for 1 h at room temperature. The resultant suspension was adjusted to pH 7.0 with 0.5 M NaOH and treated with 4.0 mg pancreatin (> 250 units/mg) in 7.5 mL of phosphate buffer (0.2 M, pH 8.0). The mixture was shaken for 2 h at room temperature. The mixture was then filtered using Whatman No 1 filter paper and the residue washed with distilled water, air-dried and used for protein determination using Lowry method (Markwell *et al.* 1978) as described earlier. Protein digestibility was obtained using the equation

$$Invitro\ protein\ digestibility(\%) = \left(\frac{I - F}{I}\right)x100$$

where, I = protein content of sample before digestion

F = protein content of sample after digestion

Determination of the amino acid composition

Amino acid composition was determined following the method described by Gbadamosi *et al.* (2012) using S433 Amino Acid Analyzer (SYKAM, Eresing, Germany). Samples were freeze-dried and then hydrolysed for 24 h at 110 °C with 6 M HCl. After hydrolysis, the samples were freeze stored in sodium citrate buffer at pH 2.2. When ready for analysis, a 50 µL of the hydrolysates was directly injected into the analyser. Tryptophan was determined separately by hydrolysis of the sample with sodium hydroxide. Cysteine and methionine were determined after performic acid oxidation prior to hydrolysis in 6 M HCl, and measured as cysteic acid and methionine sulphone respectively (Girgih*et al.*, 2011).

Statistical Analysis

Data were analysed using statistical package for social sciences (SPSS, V20). Means were separated using Duncan multiple range test and significance were chosen at $p<0.05$.

Results and Discussion

Yield of bean flours

The yield of the bean flours per pore size of each variety is shown in Figure 1. The result showed that 40 % of the brown bean flour passed through 315 microns size. However, the amount of white bean flour for the 500- and 600-micron sizes were not significantly ($p<0.05$) different from each other. The result showed that almost half of the brown bean flour had lower particle size but the white bean flour contained higher particle sizes 500-600 micron than the smaller ones. Partwa *et al.*, (2016) applied the principles of particle sizes to determine the food quality of two variety of wheat flour and reported that the quality of final product of food powders depend in a large extent the distribution of the particles. The differences in the yields of flours with respect to particles sizes and bean varieties agreed with the submission of Patwa *et al.* (2015) on the differences in the yield of flours as a function of different pore sizes of two different classes of wheat and attributed this to the level of cohesion between the type of flours used.

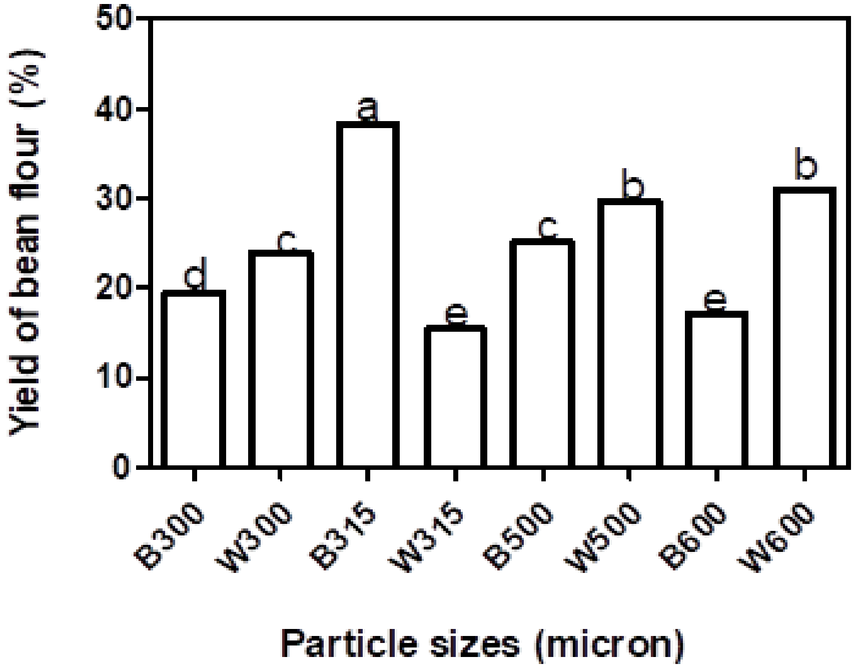

Figure 1: Yield of bean flour per pore size

Values are mean± s.d of triplicate determinations. Values with different superscript on the bars are different significantly ($p<0.05$) from one another
B300; Brown bean of 300-micron size, B315; Brown bean of 315 micron size, B500; Brown bean of 500 micron size, B600; Brown bean of 600 micron size, W300; White bean of 300 micron size, W315; white bean of 315 micron size, W500; White bean of 500 micron size, W600; white bean of 600 micron size, WWF: White whole bean flour; WBF: whole brown flour

Proximate composition

The proximate composition of the bean flour with respect to the pore sizes is shown in Table 1. The result showed no significant difference ($p>0.05$) in the moisture content of B500 and B600; B300 and B315. Also, there was also no significant difference ($p>0.05$) in the moisture content of the white bean flours with respect to particle sizes of the flours. The result showed moisture content of the bean flours, irrespective of the varieties did not have effect as per the particle size distributions.

The trend obtained for the fat content of the samples was almost similar with the fat content of the samples. In this case, there was no significant difference ($p>0.05$) in the fat content of the brown and white bean flours with respect to the differences in the particle size distributions. However, bean flours that passed through each pore size was significantly different ($p<0.05$) when compared with the whole bean flours for the white and the brown samples.

The ash content of the bean flours with respect to particle sizes varied between 4.51 to 4.61 % and 3.72 to 3.78 % for brown and white beans respectively. there was no significant difference ($p<0.05$) in the values obtained for the brown flour and white flour with respect to particle size distribution. This implies that irrespective of the particle sizes of the flours, the ash content was the same for both the white and brown flours. The result appeared different in the case of the fibre content of the bean flours separated into different particle sizes. The values obtained for the fibre content of the samples ranged between 3.24 to 5.23 % and 2.11 to 5.15 % for the brown and white bean variety respectively. The result showed that increase in the particle size of the bean flour increased the fibre content of the samples and the values were significantly ($p<0.05$) different from each other. The results also revealed that particle sizes of the brown bean flour had higher amounts of fibre when compared with the values obtained for the white flour.

The protein content of the samples assumed similar trend as the ash and fat content of the bean flour samples. The value ranged between 24.74 to 24.87% and 22.76 to 22.95 % for brown and white bean variety respectively. The values obtained for the different particle sizes were numerically comparable but statistically different ($p<0.05$) from the whole bean flours. The result obtained for the protein content of the samples only showed that protein content of the bean flour, irrespective of the particle sizes does not affect significantly the protein content but showed variations with respect to the bean varieties. The carbohydrate content of the samples varied significantly ($p<0.05$) from one another and ranged between 55.28 to 57.21 % and 58.10 to 61.45 % for the brown and white varieties. However, the results showed an inverse relationship between the particle sizes of the bean flour and the carbohydrate contents.

Table 1: Proximate composition of variety of bean flours of different particle sizes

Pore sizes	Moisture content (%)	Fat content (%)	Ash content (%)	Fibre content (%)	Protein content (%)	Carbohydrate content (%)
B300	9.45±0.43 ^{b}	0.64±0.10 ^{b}	4.62±0.55 ^{c}	3.24±0.42 ^{d}	24.84±1.04 ^{b}	57.21±2.03 ^{d}
B315	9.28±0.44 ^{b}	0.61±0.02 ^{b}	4.53±0.31 ^{c}	4.23±0.13 ^{c}	24.87±1.11 ^{b}	56.48±2.11 ^{e}
B500	9.79±0.83 ^{a}	0.65±0.12 ^{b}	4.51±0.21 ^{c}	5.08±0.05 ^{b}	24.74±1.04 ^{b}	55.33±0.94 ^{f}
B600	9.44±0.31 ^{a}	0.66±0.02 ^{b}	4.61±0.09 ^{c}	5.23±0.09 ^{a}	24.78±1.04 ^{b}	55.28±1.22 ^{g}
W300	9.41±0.66 ^{a}	0.52±0.09 ^{b}	3.75±0.05 ^{d}	2.11±0.01 ^{e}	22.76±1.06 ^{c}	61.45±0.44 ^{a}
W315	9.47±0.74 ^{a}	0.51±0.03 ^{b}	3.72±0.11 ^{d}	3.13±0.13 ^{d}	22.82±0.84 ^{c}	60.35±0.31 ^{b}
W500	9.41±0.08 ^{a}	0.53±0.01 ^{b}	3.78±0.11 ^{d}	4.10±0.16 ^{c}	22.88±0.44 ^{c}	59.30±0.44 ^{c}
W600	9.53±0.33 ^{a}	0.51±0.01 ^{b}	3.76±0.04 ^{d}	5.15±0.11 ^{a}	22.95±0.21 ^{c}	58.10±1.04 ^{c}
Brown whole	9.43±0.04 ^{a}	4.01±0.55 ^{a}	5.11±0.25 ^{b}	4.74±0.43 ^{f}	25.64±0.42 ^{a}	51.07±1.21 ^{f}
White whole	9.11±1.32 ^{b}	3.95±0.53 ^{a}	5.55±0.55 ^{a}	4.68±0.32	24.44±0.31 ^{b}	52.27±1.44 ^{e}

Values are mean± s.d of triplicate determinations. Values with different superscript down the column are different significantly ($p<0.05$) from one another

B300; Brown bean of 300-micron size, B315; Brown bean of 315-micron size, B500; Brown bean of 500-micron size, B600; Brown bean of 600-micron size, W300; White bean of 300-micron size, W315; white bean of 315-micron size, W500; White bean of 500-micron size, W600; white bean of 600-micron size, WWF: White whole bean flour; WBF: whole brown flour

In-vitro protein digestibility

Figure 2 shows the *in-vitro* protein digestibility (IVD) of the bean flour with respect to different particle sizes, which is an indication of the quality of protein in food samples (Nnamezie *et al.*, 2021). There was no significant difference (p>0.05) in the brown and white bean flours of 300-micron size. The result also showed significant difference in the quality of protein present in the brown and white bean flour of 315-micron size and the trend of result is the same for the 500-micron size flour. it was revealed from this result that the whole bean flour (not subjected to particle size distribution) had significantly (p<0.05) lower when compared with the flours subjected to particle size distribution. The result indicated that particle size of bean flour influenced the quality of protein in the bean flour and by extension may have implications in the quality of the product made from the flour.

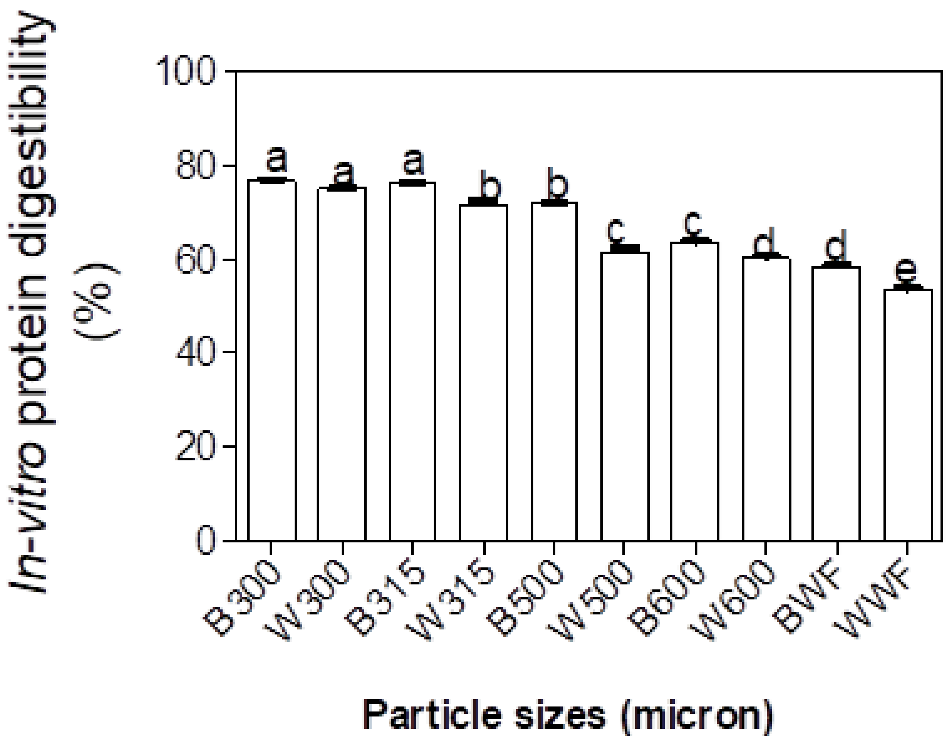

Figure 2: *Invitro*-protein digestibility of the samples

Values are mean± s.d of triplicate determinations. Values with different superscript on the bars are different significantly (p<0.05) from one another
B300; Brown bean of 300-micron size, B315; Brown bean of 315-micron size, B500; Brown bean of 500-micron size, B600; Brown bean of 600-micron size, W300; White bean of 300micron size, W315; white bean of 315micron size, W500; White bean of 500-micron size, W600; white bean of 600micron size, WWF: White whole bean flour; WBF: whole brown flour

Amino acid composition

The amino acid composition of the bean flour with respect to the particle sizes is shown in Table 2. The results showed that the individual amino acids for the brown bean flours with respect to particle sizes were higher when compared with the white bean flours. On the amino groupings, the hydrophobic amino acids (HAA) were higher in the white bean flours when compared with the brown bean flours, except for 600-micron sizes(B-600) where the brown flour was higher than white flour. The trend of result was also similar for the other groupings of the amino acids, such as the aromatic amino acid (HAA), Sulphur containing amino acids (SCAA), positively charged amino acid (PCAA), negatively charged amino acid and branched chains amino acids were higher in the brown bean flours when compared with the white bean flours. Also, each of the particle sized bean flours for the brown variety was higher when compared with the individual particle of sizes white bean flours. The results also showed that the amino acid composition of the flour samples was different with variations in the particle sizes, for both the white and brown bean variety but no definite trend could be traced. However, it is clear from the result that small particle sizes flours (300-315 microns) appeared to be more favored in terms of the quantities of these amino acids than the bigger sized bean flours. There is dearth of information in the literature regarding the effect of influence of particle size distribution on proximate, invitro and amino acid composition of foods and this has limited the comparison of the result in the present study with previous research. However, it is hoped that this piece of work would serve as a reference literature for further works on particle size distribution and food powders.

Table 3: Amino acid composition (%) of the samples

	Whole flour (white)	Whole flour (brown)	B300	B315	B500	B600	W300	W315	W500	W600
Aspartic/ asparagine	10.759	10.950	10.996	11.094	11.037	10.867	10.758	10.795	10.792	10.828
Threonine	4.415	4.504	4.557	4.585	4.597	4.613	4.425	4.446	4.418	4.436
Serine	6.601	5.909	5.895	5.757	5.713	5.712	6.549	6.561	6.637	6.435
Glutamic/ glutamine	24.262	24.545	24.167	24.163	24.154	24.165	24.299	24.214	24.393	24.360
Proline	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Glycine	4.291	4.260	4.294	4.290	4.301	4.321	4.282	4.299	4.305	4.289
Alanine	4.016	4.066	4.102	4.097	4.106	4.118	4.015	4.040	4.034	4.043
Cysteine	0.939	0.921	0.849	0.845	0.846	0.850	0.926	0.945	0.931	0.933
Valine	5.272	5.000	5.001	5.009	5.036	5.044	5.282	5.309	5.279	5.304
Methionine	0.819	0.802	0.799	0.803	0.787	0.791	0.819	0.807	0.810	0.803
Isoleucine	4.672	4.628	4.666	4.681	4.690	4.698	4.679	4.692	4.693	4.682
Leucine	7.501	8.140	8.162	8.178	8.212	8.237	7.518	7.523	7.521	7.528
Tyrosine	3.601	3.483	3.500	3.475	3.480	3.488	3.593	3.604	3.599	3.589
Phenylalanine	6.044	5.872	5.929	5.908	5.929	5.924	6.032	5.913	5.775	5.917
Histidine	2.662	2.653	2.680	2.681	2.673	2.685	2.650	2.667	2.642	2.674
Lysine	6.130	6.405	6.493	6.509	6.521	6.529	6.144	6.142	6.154	6.155
Arginine	6.901	6.736	6.778	6.799	6.804	6.829	6.923	6.945	6.913	6.932
Tryptophan	1.114	1.124	1.133	1.126	1.116	1.129	1.107	1.096	1.103	1.093
HAA	10.759	10.479	10.562	10.510	10.525	10.541	10.732	10.614	10.477	10.599
AAA	17.446	17.769	17.828	17.868	17.938	17.979	17.479	17.524	17.493	17.514

SCAA	31.107	31.190	31.358	31.348	31.453	31.510	31.119	31.082	30.901	31.063
PCAA	15.693	15.793	15.951	15.989	15.997	16.042	15.717	15.754	15.709	15.760
NCAA	35.021	35.496	35.163	35.257	35.191	35.032	35.057	35.009	35.185	35.188
BCAA	1.757	1.723	1.647	1.647	1.632	1.641	1.745	1.752	1.741	1.736

Conclusion

The study examined the impacts of particle size distribution of bean flours on the proximate composition, invitro protein digestibility and the amino acid composition. The study revealed that particle size distribution of bean flour had influence on the nutritional composition of the bean flours, in termns of the amino acid composition and the quality of protein. It also showed that lower particle sized flour had greater nutrient quality when compared with larger particle sized. This may be as a result of increased surface area. It is therefore advisable to ensure that the right particle size of flour is selected to ensure maximum nutrient exploitation of food powders.

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