FRACTIONATION AND FUNCTIONAL PROPERTIES OF CARBOHYDRATE FROM FIVE COMMON COWPEA CULTIVARS IN NIGERIA

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ABSTRACT
The proportions of starch component of the cowpea cultivars were determined. The proportions of amylose to amylopecin starch were 21.52% to 73.26% in Ife brown and 19.11% to 76.46% in Patasco beans. The highest content of amylopectin occurred in patasco beans. The functional properties of carbohydrate extracted from the five commonly consumed cowpeas in Nigeria were determined. Emulsion capacity, swelling index and foamability were highest in Ife-brown cultivar. Fat and water absorption capacities were also highest in this cultivar. The high foaming capacity (15g/g) and better foam stability (2.0g/g) of sokoto cultivar explains why this cultivar is often used in preparing *akara*. Ife-brown cultivar may qualify as a health food.

Key words: Cowpea, Cultivars, Carbohydrate, starch fractions, functional properties.

INTRODUCTION
Cowpea (*Vigna unguiculata* (L.) Walp) is a plant food, which belongs to the class of foods known as legumes or pulses, and is a member of *Papilionoideae* and *Leguminosaea* family. It originated in Africa and is the pulse crop grown in the largest quantity in the tropical Africa (Ihekoronye and Ngoddy, 1985, Ofoya and Akhidue, 2005). Cowpea is the most important food legume in Africa, Asia and parts of central and southern America. Globally, cowpea is cultivated in over 12.5 million ha with annual production exceeding 3.5 million tons (Ofuya and Akhidue, 2005). About 70% of the production is in West and Central Africa where it is the major source of protein in the daily diet of urban as well as rural masses. Nigeria is the largest producer of cowpea (IITA, 1999, Ofuya and Akhidue, 2005) producing 2.1million tones of the total world production of 3.5million tones. Cowpea, with about 24% protein and high mineral content complements starchy staples (cereals, roots and tubers), and is important to the livelihood of millions of poor people in less developed countries of the tropics. Cowpea is an important source of dietary protein in Nigeria where animal protein is scarce and expensive. In Nigeria, as well as other sub-Saharan countries, cowpeas are regarded as "poor man's" protein. Two out of every three Nigerian child feed and depend on legumes, including cowpeas, as sources of protein.

Cowpea is widely consumed as a source of protein, though the fact remains that it is also high in carbohydrate (CORP,1981, Ofuya and Akhidue, 2005). Legumes including cowpea contain up to 60% carbohydrate mainly in the form of starch granules- amylose and amylopectin (Ihekoronye and Ngoddy, 1985). The high content of carbohydrate in cowpea notwithstanding, cowpea meals is still recommended as a diabetic food, and for people who do not need much sugar in their system (Sathe, *et al.*, 1984, Miller, 2002). The reason for this recommendation is probably because of the presence of other nutrients in cowpea apart from carbohydrate. The bran of cowpea is rich in vitamins and minerals, which help in carbohydrate digestion, utilization and elimination from the body. From the report of FAO (1997) we discover that all carbohydrate are not the same, and do not affect blood sugar equally. The presence of amylose in legumes has been reported to influence starch solubility as explained by the crystal region of the starch granules (Chen, 2003). Variations in the proximate (Ofuya and Akhidue, 2005) and rheological properties of some cowpea cultivars have been reported (Ojimelukwe, *et al.*, 1999). It has been discovered recently that different cowpea cultivars affect human blood sugar differently after consumption (Onyeka, 2006). We are anticipating that the differences may be due to the differences in their starch content and characteristic as well as other factors such as processing methods and presence of phytochemicals, which have direct link to the health benefit of food (Anderson, 2004). This present work is designed to investigate the differences in carbohydrate composition and characteristics of the commonly consumed cowpeas in Nigeria.

MATERIALS AND METHODS

Sample collection

Five commonly consumed cultivars (*Sokoto, Patasco, Iron-beans, Isiocha and Ife-brown*) of dry cowpea seeds were identified and collected from National Cereal Research Institute, Amakama, Umuahia, Nigeria.

Experiments

Total Dietary Fibre Determination.

The total dietary fibre was determined according to the method of A.O.A.C (1995) with slight modifications. One gram of each sample (dry basis) was put into a beaker and 10mls of distilled water was added and the mixture stirred. The beaker with the sample was then put into a water bath containing boiling water and was gelatinized over a hot plate whilst stirring. When the sample had gelatinized, the pH of the gelatinized sample was adjusted to 6.0. Two drops of Termamyl was added and incubated at 100°C for 30min. This was followed by protease incubation and lastly by amyloglucosidase incubation at pH 4.5 and 60°C for 30mins. At the end of amyloglucosidase incubation, the sample was precipitated with four volumes of ethanol and acetone. The sample was then dried in a moisture removal oven (GallenKamp, London) at 100°C until constant weight was achieved. Total dietary fibre was calculated as the difference in weight.

Available Carbohydrate Determination

This was done using the Anthrone method described by James (1995). Fifty grams of each flour sample was measured using a sSartorious weighing balance and transferred into 250ml conical flask. Dilute hydrochloric acid solution was added in a ratio of 1.10 (w/v). The mixture was boiled on a hot plate with stirrer until it tested negative to simple iodine starch test. The hydrolysate was filtered through Whatman No 42 filter paper and the filtrate was used for the analysis. An aliquote of the hydrolysate (1ml) was put in a test tube. An equal volume of distilled water and the same volume of standard sugar solution (glucose) were put in separate test tubes to serve as reagent blank and standard, respectively. Six mls. of the Anthrone reagent was added to each of the tubes. The tubes were stoppered and boiled in a water bath for 10min. They were cooled (and filtered to remove the precipitate) and their absorbance measured in a spectrophotometer at 620nm wavelength with the reagent blank at zero. The carbohydrate content was estimated using the relationship below.

$$\%\,sugar = \frac{100}{w} \times \frac{au}{as} \times \frac{C}{10^3} \times \frac{vf}{va} \times D);$$

% CHO = 0.9 x % sugar. *where* **w** = wt of samples hydrolysed., **C** = concentration of standard sugar (mg/ml), **vf** = Total vol of hydrolysate, **va** = vol of hydrolysate analysed, **D** = Dilution factor where applicable., **au** = absorbance reading of sample **as** = absorbance reading of standard

Cowpea flour extraction

Wholesome (insect un-infested) seeds from each cultivar were carefully sorted. Each sample was milled into flour in a Kenwood portable mill and sieved through 1mm sieve. The flour samples were put into glass jar and sealed hermatically prior to starch isolation and other analysis

Isolation of Starch

The method described by Daladodalan *et al*, (1988) was employed. The starch was obtained by homogenizing the cowpea flour in distilled water. The homogenate was filtered through a two-fold muslin cloth and the filtrate was allowed to settle at room temperature for 6h. After the starch portion had settled and the supernatant decanted, the starch sediment was spread on a laboratory tray and dried in the oven at 50°C until it was dried to constant weight. The starch was used for analysis after cooling in a dessicator.

Fractionation of cowpea starch

The method described by Daladodalan *et al*, (1988) was employed. Ten grams of the starch from each sample was dispersed in 100mls of distilled water and stirred to suspend the particles in the water. Two mls. of 0.15N NaOH sol. was added and mixing continued until the solution became clear, but care was taken to stir gently to avoid damaging the gel structure of the amylopectin. The mixture was allowed to stand for 5min before 400mls of 5% NaCl solution was added to it and its pH adjusted to 7.0 using dil. HCl. After standing overnight (16h) the supernatant was separated from the settled gel by suction (siphoning). Then the fitrate was mixed with 1-butanol and stirred with a magnetic stirrer for 60min at room temperature. The amylose (having formed complex with the butanol) was precipitated and left to settle for 2h. The complex precipitate was recovered by centrifugation at 3000X9 for 15min.The butanol was blown off with a steam of air, and the amylose quantified by weight relative to the weight of the sample analysed. The amylopectin (contained in the gel) was recovered by centrifugation at 8000X9 for 20min. The supernatant was discarded and the residue was mixed with 1% NaCl solution. It was then centrifuged at 3000X9 for 15min. The gel was precipitated with excess ethanol and the amylopectin content calculated by weight relative to the weight of the sample analysed

Emulsion Capacity and Blue value index Determination

The method of Beuchat (1977) was used for the determination of the emulsion capacity while the method of Kawabata *et al*., (1984) was used for blue value index.

Swelling Index

Swelling index was determined according to the method of Ukpabi and Ndimele (1990) with slight modification. Three grams portion of each flour was transferred into a clean dry graduated (50ml) cylinders. The flour samples were gently leveled and the volumes noted. Thirty mls of tap water was added to each sample and the cylinder was swirled and allowed to stand for 60minutes. The swelling power of each four was calculated as volume of the sample after swelling relative to the original volume.

Foaming capacity and foam stability

Foaming capacity and foam stability were determined as described by Lawhen *et al*, (1972). One gram flour was mixed with 10ml-distilled water using a magnetic stirrer (PHYWE) at 10 Ruhrer speed at room temperature. The suspension was stirred again for 5min using the magnetic stirrer. The content along with the foam were poured into 25ml measuring cylinder and the foam volume read after 30sec. The volume of foam at 30sec was regarded as foam capacity and the volume of the foam over a time period of 30 – 120min as foam stability.

Oil and water Absorption Capacities

The method of Beuchat (1977) was followed with slight modification for the determination of water and oil absorption. Flour samples weighing 1g each were measured and mixed with 10ml turkey brand vegetable oil or water for 1minute by hand shaking. The samples were allowed to stand at room temperature for 30minutes and then centrifuged at 3000rpm (Hettich Universal) for 30minutes. The volume of the supernatant in a 10ml-graduated cylinder was noted. The mean of duplicate determinations was reported as g oil or water absorbed per g of sample

Statistical Analysis

The data obtained from the experiments were computed into means. Analysis of variance (ANOVA) was performed in order to detect any significant difference as a result of differences in cultivar. Fishers Least Significant Difference ($LSD_{0.05}$) procedure was used to separate the means where significant difference existed using the method described by Ihekoronye and Ngoddy (1985).

RESULTS AND DISCUSSION

Carbohydrate, and Dietary Fibre Content of Cowpea Cultivars

The starch content of all the cowpea examined ranged from 56.39 to 68.15% (Table 1). Iron-beans cultivar had the highest starch content of 68.15% followed by Isiocha (61.50%) and Patasco had the lowest value of 56.39%. These figures are in close range with those reported by Ihekoronye and Ngoddy, 1985 (61.01%); IITA, 1999 (67%), Ojimelukwe, *et al* 1999 (56-66%), Kungu *et al* 1999 (55.66-60.90%) and Ofuya and Akhidue, 2005 (56-68%). The cowpeas had net/available carbohydrate in the range of 28.35-32.40% (Table 1). The figures for the net carbohydrate content of the cowpeas were slightly lower than those reported by Naivikul and Appolonia, 1979 (39-42%); FAO 1997 (49%); Ofuya and Akhidue, 2005 (31-48%). There were significant (p=0.05) variations in the available carbohydrate content of the different cultivars (Table 1). Sokoto cultivar had the highest amount of available carbohydrate (32.40%) while Patasco had the lowest (28.35%), The net carbohydrate contents of Ife-brown, Isiocha, Iron-beans were about the same. A significant (p=0.05) difference in dietary fiber content of the cowpea cultivars was also observed (Table 1). Iron beans had the highest amount of dietary fibre (38.65%) while Patasco had the lowest (28.04%). The values for dietary fiber reported in this work (28.04-38.65%) are higher than those reported for many pulses by Ofuya and Akhidue, (2005) but tally with the report of FAO, (1997). The high dietary fiber content of the cowpeas indicate that cowpea could qualify as a health food since dietary fiber has been postulated to have some important physiological effect such as reducing the transit time in mammalian gut and reducing the incidence of diabetes (Montonen *et al,* 2003).

The amylose starch content of Isiocha, Sokoto and Ife brown were not significantly (P=0.05) different from each other (Table 1.). Their values ranged from 21.40% to 22.42%, but Patasco cultivar had the lowest amylose starch fraction of 19.11%. Lowest content (73.26%) of amylopectin was observed in Ife-brown while the highest content (76.46%) was found in Patasco. Incidentally Patasco, which had the highest amylopectin content among the cowpea cultivars, had the lowest starch content. The amylose content reported in this research is close to those reported by Naivikul and Dappolonia (1979) for mung beans (19.5%), faba beans (24.0%) and navy beans (22.1%). The low glycemic index of Ife-brown as reported by Onyeka, (2006) could be as a result of the low amylopectin content of this cultivar coupled with the low starch content, and probably the brown colour of the beans bran. According to FAO (1997) amylopectin fraction of starch causes a sharp rise in blood sugar more than the amylose fraction.

Table 1: Fractions (%) of the carbohydrate of commonly consumed cowpeas*

Cowpea	Amylose content	Amylopectin	Total DF	Starch content
Isiocha	21.41[a]	76.18[a]	35.00[b]	61.50[b]
Patasco	19.11[b]	76.46[a]	28.04[c]	56.39[c]
Iron-beans	21.40[a]	75.28[a]	38.65[a]	68.15[a]
Ife-Brown	21.52[a]	73.26[b]	28.69[c]	59.74[cb]
Sokoto	22.42[a]	74.84[ab]	30.00[cb]	62.40[b]
LSD $_{(p=0.05)}$	**2.43**	**2.21**	**3.44**	**5.02**

Values reported are averages of three determinations DF = Dietary fiber

The blue value index (BVI) of the cowpea cultivars was significantly (p=0.05) different from each other. Ife-brown had the highest blue value index of 51.25% while Iron-beans had the lowest value of 37.25% (Table 2). Since blue value index shows the extent of starch damage, this result implies that there are less starch damage in Ife-brown than the other cultivars. In terms of swelling index, Iron beans had the lowest value of 1.20 while Ife-brown had the highest value of 1.61. The test for swelling index in this work was done with cold water indicating that protein played the major role in the results observed. Since Ife-brown had the highest swelling index, this implies that this cultivar had higher soluble proteins compared to the rest of the cultivars. This result confirms the earlier result obtained on blue value index since decrease in swelling index is also attributed to starch damage (Ezema, 1989). The extent of swelling of legumes has been found to depend on temperature, availability of water, species of starch, and extent of starch damage, etc. (Ezema, 1989). Relating this result to that of BVI one observes that the high swelling index of Ife-brown could be caused by its low starch damage.

Table 2. Functional Characteristics of carbohydrate of commonly consumed cowpeas*

Cowpea	BVI	Swelling Index	Foaming Capacity (g/g)	Foaming Stability (g/g)	WAC (g/g)	FAC (g/g)	Emulsion Capacity (g/g)
Isiocha	49.5[a]	1.49	13.00[c]	1.00	1.30	1.40	2.0[ab]
Patasco	50.00[a]	1.29	11.50[c]	1.00	1.20	1.30	2.0[ab]
Iron-beans	37.50[c]	1.20	7.50[a]	1.00	1.73	1.50	1.4[b]
Ife-Brown	51.25[a]	1.61	17.00[a]	1.00	1.80	2.03	3.5[a]
Sokoto	37.51[c]	1.39	15.00[b]	2.00	1.10	1.60	2.4[ab]

Means in a column with the same superscript are not significantly (P>0.05) different.
BVI=Blue Value Index WAC= Water Absorption Capacity FAC Fat Absorption Capacity

In terms of foam capacity it was again discovered that Ife brown had the highest faomability value of 17g/g while Iron-beans had the lowest value of 7.0g/g. But in the case of foam stability, Sokoto was observed to exhibit the highest value of 2.0 while the rest of the samples had the same value of 1.0. Since foam formation and stability are functions of protein (Kinsella, 1976), it means that Sokoto cultivar has more intact flexible protein molecules, which help to stabilize the foam in solution. According to Kinsella (1976), foamability relates to the rate of decrease of the surface tension of the air-water interface caused by absorption of protein molecules. Foamability could also be caused by phytochemicals. Direct relationship between foamability and protein solubilities has been reported except for fish protein concentrate which lacked solubility but had good foamability (Okaka and Potter, 1979). Foaming capacity and foam stability are useful when preparing *akara* as the foam formed when cowpea paste is whipped holds the incorporated air and enables the bean balls to retain a firm and spongy shape after frying. The high foaming capacity (15g/g) and better foam stability (2.0g/g) of Sokoto cultivar observed in this work may suggest why this cultivar is often used in preparing *akara*.

The highest values for water absorption capacity (WAC) and fat absorption capacity (FAC) of 1.80g/g and 2.03g/g respectively (Table 2) were observed in Ife-brown cultivar. Sokoto cultivar had the lowest value (1.10) for WAC while Patasco had the lowest (1.30g/g) for FAC. The high WAC together with the high swelling index of the Ife brown cultivar indicates that the polar groups of the flour formed hydrogen bond with water or water soluble substances. That is, it has more water-soluble substances than other cultivars

(Iwuoha, 2004). Proteins can increase water-binding capacity if their swelling capacity is enhanced. Greater starch swelling ability might contribute to higher water binding of flour.

Again Ife-brown had the highest emulsion capacity of 3.5g/g while Iron beans had the lowest value of 1.4g/g. The values for the emulsion capacity of the other cultivars were not significantly different (P>0.05) from each other. The high protein content of this cultivar might have caused the higher value of emulsion capacity of Ife-brown. Proteins act as surface-active agents and lower the interfacial tension between immiscible liquids such as oil and water, thereby enabling a mixture of the two (McWatters and Holmes, 1979; McWatters and Brantlay, 1982).

CONCLUSION

The different cultivars of cowpea are likely to affect health differently when consumed. That is, the cultivars would have different physiological effects. Since the cultivars have varying contents of starch, amylose, amylopectins and also varying functional properties. It is therefore important to note the cultivar of cowpea consumed. Among the cultivars, Ife-brown seems to have the best composition and functional characteristics as to qualify as health food. The contributions of the brown pigment of Ife-brown cultivar need to be further investigated.

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