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Partial chemical composition of bambara pea [*Vigna subterranea* (L.) *Verde*]

Samuel R. Minka [a], Maud Bruneteau [b,*]

[a] *Ecole Nationale Supérieure de l'Agriculture et de l'Industrie, BP 455, Ngaoundéré, Cameroon*

[b] *Laboratoire d'Ecologie Microbienne du Sol, UMR-CNRS 5557, Université Claude Bernard, Lyon 1, Bâtiment 741, 43 Bd du 11 novembre 1918, 69622, Villeurbanne Cedex, France*

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Abstract

A partial chemical analysis of bambara peas [*Vigna subterranea* (L.) *Verde*], which are widely consumed in West and Central Africa, was undertaken. A flour sample of bambara pea was extracted with hot ethanol. The yield of crude lipids present in the ethanol-soluble material was 3.7% of the dry weight of sample flour. The water-soluble components present in the delipidated flour represent 10% of the dry weight of sample flour. The fatty acids, the sugars and the amino acids were analyzed. Linoleic, palmitic and linolenic acids were the most predominant fatty acids with average values of 44, 30 and 21%, respectively. Stearic acid was present in small quantities. Sugar analysis showed that 30% of the neutral sugars were present and identified essentially as glucose and galactose. The total essential amino acids amounted to an average of 32.7%. Lysine was the most predominant essential amino acid with average value of 10.3%.

Keywords: Polysaccharides; Proteins; Fatty acids; Bambara pea; *Vigna subterranea* (L.) *verde*

1. Introduction

Bambara peas [*Vigna subterranea* (L) *Verde*] are cultivated widely in West and Central Africa. It has been reported that most locally available legumes consumed in Africa are a very important source of nutritients, especially proteins, and an excellent source of complex carbohydrates (El-Mahdy, 1974; Minka, Mbofung, Gandon & Bruneteau, 1998; Moose & Baudet, 1983; Oshodi, Ipinmoroti, Adeyeye & Hall, 1995; Oshodi, Olaofe & Hall, 1993). Bambara peas are used to prepare many popular dishes in a variety ways. However, little information has been published about the nutritive values of this locally available vegetable. Thus, it was important to know the nature and the level of the major components of bambara pea, notably those of potential nutritional value for humans. In the present paper, we describe a first partial chemical composition of this legume.

2. Materials and methods

2.1. Preparation of flour sample

Bambara peas [*Vigna subterranea* (L.) *Verde*] were dried and stored for 24 months in a dry room. 120 g of kernels were soaked in distilled water and manually decorticated and then dried in a hot air draught oven at 50°C for 24 h. Then, the dried and cleaned kernels were ground to a fine powder (<250 μm) using a I.K.A. Universalmühe M 20 grinder.

2.2. Extraction of lipids and water-soluble components

Lipids (Residue 3, Fig. 1) and water-soluble components (Supernatant S_2, Fig. 1) were extracted from a flour sample (60 g) as previously described (Minka et al., 1998).

2.3. Isolation of polysaccharides and proteins

The supernatant S_2 was applied to a DEAE-cellulose column (Whatman, 2.5×30 cm) which had been equilibrated with 10 mM potassium phosphate buffer, pH = 7.

* Corresponding author.

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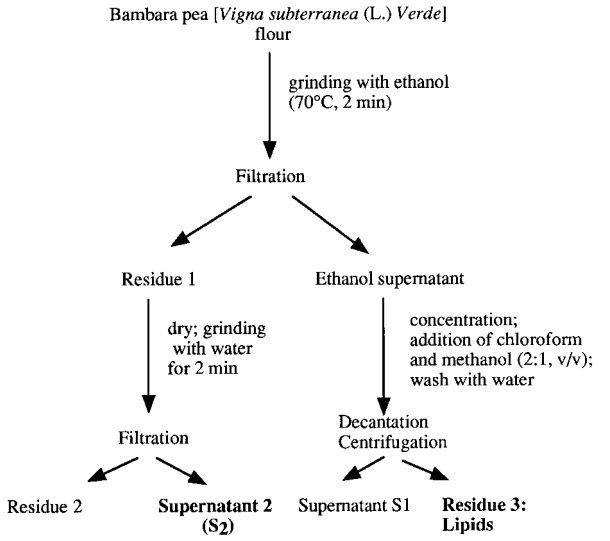

Fig. 1. Isolation of water-soluble components (Supernatant 2) and lipids (Residue 3) from flour sample obtained from raw bambara peas [*Vigna subterranea* (L) *Verde*].

Flow-rate was 18 ml/h. Neutral polysaccharides were eluted with the same buffer. The bound proteinaceous components were eluted from the column by a linear gradient of 0 to 1 M NaCl prepared in the same buffer. Bound and unbound (on DEAE-cellulose) fractions were dialyzed and subjected to chromatography on a column of Sephadex G-25 (Pharmacia LKB) eluted with water.

2.4. Analytical methods

Neutral sugar determination was performed, after hydrolysis in 0.1 M HCl (100°C, 48 h), with anthrone reagent (Shields & Burnett, 1960). Quantitative analysis of neutral sugars was carried out, after conversion of monosaccharides into alditol acetates according to the method of Sawardeker, Sloneker and Jeanes (1965) by gas chromatography (GC).

Proteins were determined by the Folin phenol reagent (Lowry, Rosebrough, Farr & Randall, 1951). Amino acid composition was carried out after hydrolysis in HCl 6 M, TFA (2:1, v/v) and 5% thioglycolic acid for 24 h at 100°C. Amino acids were quantified by post-column derivatization with ninhydrin on a Beckman 6300 amino acid analyzer.

Fatty acids were liberated by acid hydrolysis (4 M HCl, 100°C, 48 h). Free fatty acids were isolated by extraction of the hydrolysates with chloroform. After methylation with diazomethane, the fatty acids were analyzed by GC.

2.5. Polyacrylamide gel electrophoresis

Components of the proteinaceous fractions were resolved by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS–PAGE) on 15% polyacrylamide gels at pH = 8.3 according to Laemmli (1970). Gels were stained with 0.1% Coomassie Brilliant Blue.

2.6. Thin-layer chromatography

Lipids were analyzed by high-performance thin-layer chromatography (HPTLC) on silica gel 60 plates (Merck, Darmstadt, Germany) that were developed with the solvent system described by Heape, Juguelin, Boiron and Cassagne (1985). The detection of lipids was carried out by spraying the plates with various reagents: 0.2% (w/v) vanillin in sulfuric acid/water (1:9, v/v), Dittmer and Lester reagent as modified by Vaskovsky and Kostetsky (1968), Draggendorf reagent as modified by Wagner, Morhammer and Wolf (1961). Identifications were made by comparison with authentic standards.

2.7. Methylation analysis

A sample (1–5 mg) of polysaccharide dissolved in dimethyl sulfoxide (1 ml) was methylated according to the method of Hakomori (1964). The permethylated polysaccharide was hydrolyzed by heating in 85% formic acid (2 ml) at 100°C for 5 h and, after removal of the acid, in 1 M trifluoroacetic acid (2 ml) under the same conditions (Yamada, Kawaguchi, Ohmori, Takeshita, Taneya and Miyazaki, 1984). The methylated sugars were analyzed as alditol acetates by capillary GC.

2.8. General methods of gas chromatography and mass spectrometry

Gas chromatography was carried out on an Intersmat apparatus (Model 120 FL; Intersmat, Lyon, France) fitted with a capillary SP 2380 column (0.25 mm×20 m, 210°C) for sugar derivatives or with a capillary SP 2100 column (0.25 mm×25 m, 140 to 260°C) for fatty acid methylesters. Combined gas chromatography/mass spectrometry (GC/MS) was performed on a VG MM 305 apparatus (temperature 200°C, ionisation potential 70 eV and current intensity 200 μA), which was connected to a gas chromatograph equipped with a FFA1 capillary column (0.32 mm×50 m, 60 to 240°C) for fatty acid methylesters and with a BP1 capillary column (0.25 mm×60 m, 120 to 160°C, rate 5°C/min and 160 to 280°C, rate 2°C/min) for methylated sugars.

3. Results and discussion

The extraction of flour sample with hot ethanol, according to the procedure shown in Fig. 1, gave a crude lipid fraction (Residue 3, Fig. 1) obtained with a

yield of 3.7% of the dry weight of sample flour. The yield of water-soluble components (Supernatant S_2, Fig. 1) present in the delipidated flour extracted with distilled water was 10% of the dry weight of sample flour.

HPTLC analysis in the solvent system of Heape et al. (1985) for crude lipid fraction R_3 showed the presence of fatty acids ($R_F = 7.4$), glycolipids ($R_F = 6.3$), phosphatidylcholine ($R_F = 0.6$), phosphatidylserine ($R_F = 1.6$), phosphatidylethanolamine ($R_F = 2.7$), phosphatidic acid ($R_F = 2.8$).

Fatty acids were analyzed, after methylation, by gas chromatography/mass spectrometry (Table 1). The saturated acids are palmitic (16:0) and stearic (18:0), while polyunsaturated components are linoleic (18:2) and linolenic (18:3). Linoleic acid (18:2) is the most concentrated fatty acid (44%), palmitic acid (16:2) is second with an average of about 30% and linolenic acid (18:3) is in the third position with a value of about 21%. Stearic acid (18:0) is found at a low level of about 5%. This fatty acid composition is similar to that of lipids isolated from pigeon pea, chickpea, garden pea, cowpea and black-eyed pea (Lee & Mattick, 1961; Oshodi et al., 1993).

The fraction S_2 contained 12.8% of water-soluble proteins and 30.5% of neutral sugars. It was fractionated by an anion-exchange chromatography column. The material was eluted in two peaks S_2I and S_2II. The analysis of the first fraction S_2I, eluted with 10 mM potassium phosphate buffer showed that carbohydrates were the unique components. The S_2I fraction contained glucose and galactose in a molar ratio of 1:1. The fraction S_2II, obtained with a linear gradient of NaCl in the same buffer, contained only proteins. The S_2I fraction contained a mixture of polysaccharides S_2Ia and S_2Ib separated by column chromatography on Sephadex G-25. S_2Ia contained only glucose while S_2Ib contained glucose and galactose in a molar ratio of 1:1.4. Glycosyl linkage analysis of S_2Ib was established by methylation and hydrolysis. Methylated sugar derivatives were analyzed as alditol acetates by GC–MS. They were identified as 2,3,4,6-tetra- and 2,3,4-tri-*O*-methylhexitol acetates. These results indicate that S_2Ib contained 1→6 linkages. These findings seem in good agreement with the possible presence in S_2Ib of the oligosaccharides raffinose, stachyose and verbascose, found in the legumineuses and which are well know to cause flatulence.

Table 1
Fatty acid composition of crude lipid fraction (Residue 3) (results are expressed as a percentage of the total fatty acids present)

Fatty acid	
Palmitic (16:0)	29.7
Stearic (18:0)	5.7
Linoleic (18:2)	43.8
Linolenic (18:3)	20.8

The proteinaceous compounds recovered in the S_2II bound fraction on the DEAE-cellulose column were analyzed by SDS–PAGE (Fig. 2). Two major bands stained with Brilliant Coomassie Blue were observed with apparent molecular masses of 10 and 30 kDa as compared to protein standards. A broad band was also noted near 14 kDa. These results agree with those reported by Cheftel, Cuq and Lorient (1985) for water-soluble proteins present in the legumineuses.

The amino acid composition of the S_2II fraction given in Table 2 is similar to those found earlier for legume proteins (Khalil & Mansour, 1995; Ziena, 1989; Minka et al., 1998). Lysine formed 10.3% of the total essential amino acids and was the major essential amino acid.

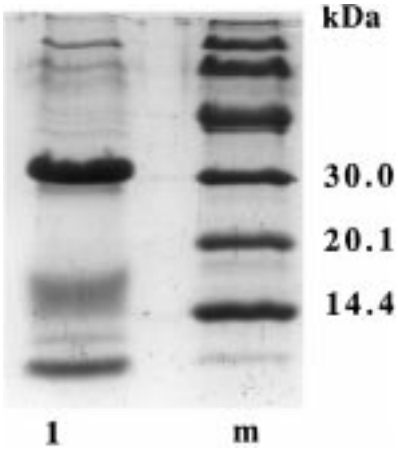

Fig. 2. 15% SDS–PAGE of S_2II fraction (lane 1, 50 μg of S_2II). The gel was Coomassie Brilliant Blue stained. m, Molecular mass markers.

Table 2
Amino acid composition of fraction S_2II

Amino acid	Residues (%)
Isoleucine	2.55
Leucine	4.38
Lysine	10.32
Cystine	n.e.[a]
Methionine	0.73
Tyrosine	1.62
Phenylalanine	2.40
Threonine	6.10
Tryptophan	n.e.
Valine	2.61
Histidine	2.71
Total essential amino acids	32.72
Arginine	3.86
Aspartic acid	12.30
Glutamic acid	18.72
Serine	5.84
Proline	6.15
Glycine	9.12
Alanine	9.85
Total non-essential amino acids	66.10

[a] n.e., not evaluated.

This result seems a characteristic of legume proteins (Moose & Baudet, 1983). Bambara pea is also a good source of leucine. In addition, this legume contains a reasonable amount of phenylalanine, histidine and valine. These results agree with those obtained by Oshodi et al. (1993) for pigeon pea.

Present results of a partial analysis of bambara pea show that the composition of this pea seems similar to those found earlier for other legumes. Thus, this locally available vegetable could have a possible role in overcoming protein malnutrition in Cameroon.

References

Cheftel, J. C., Cuq, J. L., & Lorient, D. (1985). *Protéines alimentaires: Classification des protéines de grains de céréales; Composition en amino-acides* (pp. 206–209). Technique et documentation. Paris: Lavoisier.

El-Mahdy, A. R. (1974). *Evaluation of* (Vicia faba) *bean as a source of protein and the influence of processing thereon*. Ph.D. thesis, University of Alexandria, Alexandria, Egypt.

Hakomori, J. (1964). Rapid permethylation of glycolipids and polysaccharides, catalyzed by methylsulfonyl carbanion in dimethyl sulfoxide. *Journal of Biochemistry, 55*, 205–208.

Heape, A. M., Juguelin, H., Boiron, F., & Cassagne, C. (1985). Improved one-dimensional thin-layer chromatographic technique for polar lipids. *Journal of Chromatography, 322*, 391–395.

Khalil, A. H., & Mansour, E. H. (1995). The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. *Food Chemistry, 54*, 177–182.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of plant defenses against microbiol attack. *Cell, 56*, 215–224.

Lee, F. A., & Mattick, L. R. (1961). Fatty acids of the lipids of vegetables I. Peas (*Pisum sativum*). *Journal of Food Science, 26*, 273–275.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Minka, S. R., Mbofung, C. M. F., Gandon, C., & Bruneteau, M. (1998). The effect of cooking with kanwa alkaline salt on the chemical composition of black beans (*Phaseolus vulgaris*). *Food Chemistry, 64*, 145–148.

Moose, J., & Baudet, J. (1983). Crude protein content and amino acid composition of seeds: variability and correlations. *Qualitas Plantarum. Plant Foods for Human Nutrition, 32*, 225–245.

Oshodi, A. A., Olaofe, O., & Hall, G. M. (1993). Amino acid, fatty acid and mineral composition of pigeon pea (*Cajanus cajan*). *International Journal of Food Sciences and Nutrition, 43*, 187–191.

Oshodi, A. A., Ipinmoroti, K. O., Adeyeye, E. I., & Hall, G. M. (1995). Amino and fatty acids composition of African yam bean (*Sphenostylis stenocarpa*) flour. *Food Chemistry, 53*, 1–6.

Sawardeker, J. J., Sloneker, J. H., & Jeanes, A. R. (1965). Quantitative determination of monosaccharides as their alditol acetates by gas–liquid chromatography. *Analytical Biochemistry, 12*, 1602–1604.

Shields, R., & Burnett, W. (1960). Determination of protein-bound carbohydrate in serum by a modified anthron method. *Analytical Chemistry, 32*, 885–886.

Vaskovsky, V. E., & Kostetsky, E. Y. (1968). Modified spray for the detection of phospholipids on thin-layer chromatography. *Journal of Lipid Research, 9*, 936.

Wagner, H., Morhammer, L., & Wolf, P. (1961). Dünnschicht chromatographic von phosphatiden and glycolipiden. *Biochemische Zeitschrift, 334*, 175–184.

Yamada, H., Kawaguchi, N., Ohmori, T., Takeshita, Y., Taneya, S. I., & Miyazaki, T. (1984). Structure and antitumor activity of an alkali-soluble polysaccharide from *Cordyceps Ophioglossoides*. *Carbohydrate Research, 125*, 107–115.

Ziena, H. M. S. (1989). *Hard-to-cook phenomenon in relation to physical, chemical and biological properties of faba beans* (Vicia faba *L.*). Ph.D. thesis, University of Alexandria, Alexandria, Egypt.