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The effect of cooking with *kanwa* alkaline salt on the chemical composition of black beans (*Phaseolus vulgaris*)

Samuel R. Minka [a], Carl M.F. Mbofung [a], Corinne Gandon [b], 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

The effect of cooking with *kanwa* alkaline solution on the chemical composition of black beans (*Phaseolus vulgaris*) was analyzed. Crude lipids and water-soluble fractions were extracted from three flour samples obtained from raw black beans and black beans cooked in *kanwa* solution or distilled water. Heat processing resulted in significant reduction in crude lipids, proteins and neutral sugars. Neither heat treatment induced changes in the levels of palmitic, stearic, oleic or linoleic acids, present in the crude lipid fraction. There was a significant change in both the total amino acids and the total essential amino acids. Heat treatment in alkaline solution decreased the levels of isoleucine, lysine, tyrosine, phenylalanine, threonine and valine. There was also a decrease in the galactose content after this heat processing.

Keywords: Cooking; Kanwa; Black beans; *Phaseolus vulgaris*

1. Introduction

Black beans (*Phaseolus vulgaris*) are among the most common legumes consumed in Africa. Like most legumes, they are very important sources of nutrients, especially proteins, and an excellent source of complex carbohydrates. The storage of beans under adverse high temperature and high humidity renders them susceptible to a hardening phenomenon generally known as the hard-to-cook defect requiring long cooking times and much energy (Vindiola, Seib, & Hoseney, 1986). However, the long cooking times reduces the nutritive value of legumes (El-Mahdy, 1974; Kon & Sanshuck, 1981; Youssef, Hamza, Abdel-Aal, Shekib, & El-Banna, 1986; Ziena, 1989; Khalil & Mansour, 1995).

It has been reported that the use of salts to cook beans reduces the cooking times (Uzogara, Morton, & Daniel, 1988) but little information is available regarding the effect of these salts on the composition of the cooked black beans. In west and central Africa, a common natural alkaline rock salt known as *kanwa* or *trona* (Na_2CO_3, $NaHCO_3$, xH_2O), is very often used as a tenderizer in the cooking of beans (Makanjuola & Beetlestone, 1975).

The present study was carried out to determine the effect of cooking with this salt on the chemical composition of black beans (*Phaseolus vulgaris*).

2. Materials and methods

2.1. Preparation of flour samples

Black beans of the *Phaseolus vulgaris* family which had been stored under tropical conditions for 30 months and had developed the hard-to-cook defect were used for the experiment. A known quantity of the beans was cleaned of debris and sand, briefly washed and rinsed in distilled water and then soaked for 16 h in distilled water. At the end of the soaking period the sample was divided into two portions A and B. Sample A was cooked for 90 min in 0.1% *kanwa* or *Trona* solution while sample B was cooked for the same period in distilled water. At the end of cooking, the beans were removed from the cooking solution and dried in a hot air draught oven at 90°C for 24 h and then ground to a fine powder (<250 μm) using a Moulinex grinder with steel blades. A third sample, sample C, consisted of raw beans which were washed clean and dried to constant weight and then converted into flour.

* Corresponding author.

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2.2. Extraction of lipids and water-soluble components

The flour samples A, B and C (60 g) were homogenized in 80 ml of hot ethanol (70°C) for 2 min with an Ultra-Turrax homogenizer. After filtration, the ethanol-soluble material was treated in 60 ml of chloroform and 30 ml of methanol followed by 75 ml of distilled water. The mixture was shaken and left until it separated into two phases. Lipids (Residue 3, Fig. 1) were obtained in the low layer. Delipidated flour (Residue 1, Fig. 1) was treated with distilled water and homogenized for 2 min with an Ultra-Turrax macerator. The supernatant S2 (Fig. 1), obtained after centrifugation, contained the water-soluble components.

2.3. 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 analysis was carried out after hydrolysis in 6M HCl, 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.

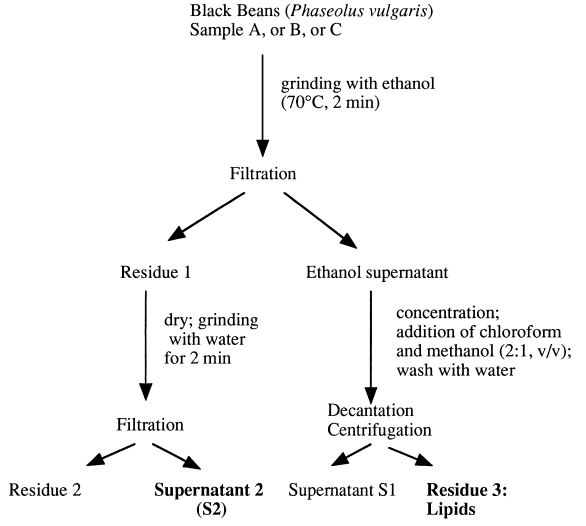

Fig. 1. Isolation of water-soluble components (Supernatant 2) and lipids (Residue 3) from black beans (*Phaseolus vulgaris*): sample A, flour sample obtained from heat-treated black beans in *kanwa* solution; or B, flour sample obtained from heat-treated black beans in distilled water; or C, flour sample obtained from raw black beans.

2.4. 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), Dragendorff reagent as modified by Wagner, Morhammer, and Wolf (1961). Identifications were made by comparison with authentic standards.

2.5. 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

Table 1
Chemical composition of flour samples A, B and C

Component	Sample[a]		
	A	B	C
Crude lipids[b]	0.7	0.8	2.2
Water-soluble components[b]	7	6.9	10.0

[a] A, flour sample obtained from heat-treated black beans in *kanwa* solution; B, flour sample obtained from heat-treated black beans in distilled water; C, flour sample obtained from raw black beans.

[b] Crude lipids: Residue 3, Fig. 1; water-soluble components: Supernatant S2, Fig. 1. The yield values are expressed as a percentage of dry weight of sample flour.

Table 2
Chemical composition of fraction S2 isolated from flour samples A, B and C

Component	Sample[a]		
	A	B	C
Proteins[b]	10.7	14.9	27.7
Neutral sugars[b]	15.8	29.8	33.3

[a] A, flour sample obtained from heat-treated black beans in *kanwa* solution; B, flour sample obtained from heat-treated black beans in distilled water; C, flour sample obtained from raw black beans.

[b] The values are expressed as a percentage of dry weight of the fraction S2.

Table 3
Fatty acid composition of crude lipid fraction (residue 3) from flour samples A, B and C (results are expressed as a percentage of the total fatty acids present)

Fatty acid	Sample[a]		
	A	B	C
Palmitic (16:0)	20.3	19.9	20.1
Stearic (18:0)	5.4	4.8	6.2
Oleic (18:1)	57.6	58.0	56.6
Linoleic (18:2)	16.7	17.3	17.1

[a] A, flour sample obtained from heat-treated black beans in *kanwa* solution; B, flour sample obtained from heat-treated black beans in distilled water; C, flour sample obtained from raw black beans.

Table 4
Amino acid composition of fraction S2 isolated from flour samples A, B and C

Amino acid	Sample[a]		
	Residues (%)		
	A	B	C
Isoleucine	1.53	2.03	2.76
Leucine	7.56	6.65	8.06
Lysine	5.22	10.4	7.37
Cystine	n.e.	n.e.	n.e.
Methionine	0.21	0.23	0.31
Tyrosine	0.40	0.61	1.12
Phenylalanine	1.79	1.89	5.53
Threonine	3.65	4.51	5.15
Tryptophan	n.e.	n.e.	n.e.
Valine	2.92	4.72	4.78
Histidine	2.04	2.27	1.89
Total essential amino acids	25.11	33.1	36.66
Arginine	6.06	3.20	2.89
Aspartic acid	16.0	15.8	15.9
Glutamic acid	26.8	20.8	16.0
Serine	5.00	5.74	6.67
Proline	4.34	4.15	4.45
Glycine	6.46	7.31	7.64
Alanine	8.03	9.62	9.31
Total non-essential amino acids	72.8	66.6	62.8

n.e., not evaluated.
[a] A, flour sample obtained from heat-treated black beans in *kanwa* solution; B, flour sample obtained from heat-treated black beans in distilled water; C, flour sample obtained from raw black beans.

connected to a gas chromatograph equipped with a FFA1 capillary column (0.32 mm×50 m, 60 to 240°C).

3. Results and discussion

The extraction of flour samples with hot ethanol, according to the procedure shown in Fig. 1, gave a crude lipid fraction. The water-soluble components present in the delipidated flour were extracted with distilled water. The yields of crude lipids (Residue 3, Fig. 1) and water-soluble components (Supernatant 2, Fig. 1) are given in Table 1. Significant differences were observed between raw and heat-treated black beans. Both heat treatments caused the same decrease in components of black bean flours.

Table 5
Neutral sugar composition of fraction S2 isolated from flour samples A, B and C (results are expressed in molar ratio)

Sugar	Sample[a]		
	A	B	C
Galactose	0.25	0.50	0.50
Glucose	1.00	1.00	1.00

[a] A, flour sample obtained from heat-treated black beans in *kanwa* solution; B, flour sample obtained from heat-treated black beans in distilled water; C, flour sample obtained from raw black beans.

An analysis of proteins and neutral sugars present in fraction S2 is shown in Table 2. Both heat-treatments decreased the contents of proteins and of neutral sugars. In addition, cooking in alkaline solution was more effective than heat-treatment in water in the reductions of these compounds.

HPTLC analysis in the solvent system of Heape et al. (1985) for total lipids showed the presence of fatty acids (R_F=7.4), glycolipids (R_F=5.4), phosphatidylcholine (R_F=0.5) and phosphatidylethanolamine (R_F=0.49) as major compounds in the three flour samples. Phosphatidylserine (R_F=1.7) and phosphatidic acid (R_F=2.7) were present in small amounts.

Fatty acids were analyzed after methylation by gas chromatography/mass spectrometry. Table 3 shows that the fatty acid composition is the same in raw and heat-treated black beans. The saturated acids are palmitic (16:0) and stearic (18:0), while polyunsaturated components are oleic (18:1) and linoleic acids (18:2). Oleic acid (18:1) is the most concentrated fatty acid (56–58%), palmitic acid (16:0) is second with an average of about 20% and linoleic acid (18:2) is in the third position with a value of about 17%. Stearic acid (18:0) is found at a low level in the three flour samples. Caprilic (8:0), lauric (12:0) and myristic acids (14:0) are absent. These data show that neither heat treatment affected the fatty acid composition of black beans. This fatty acid composition is similar to that of lipid isolated from various legumes (Lee & Mattick, 1961; Privett, Dougherty, Erdahl, & Stolyhwo, 1973; Mahadevappa & Raina, 1978; Paul & Southgate, 1985; Oshodi, Olaofe, & Hall, 1993). Recently, Oshodi, Ipinmoroti, Adeyeye, and Hall (1995) reported a high percentage of linoleic (18:2) and palmitic acids (16:0) in African yam bean.

The amino acid compositions of raw and heat-treated black beans given in Table 4 show a wide range of variation in the essential amino acids. These results agree well with those reported by Salunkhe, Kadam, and Chavan (1985). This amino acid composition is similar to those found earlier for legume proteins (Ziena, 1989;

Oshodi et al., 1995; Khalil & Mansour, 1995). As seen from the results in Table 4, both heat-treatments caused a reduction in the total essential amino acids and an increase in the total non-essential amino acids. These results agree with those obtained by Ziena (1989) for faba beans but they differ from those reported by Khalil and Mansour (1995). Leucine and lysine were the major essential amino acids in raw and heat-treated black beans. This is in line with what was observed earlier for legume proteins (Moose & Baudet, 1983; Leung & Salunkhe, 1985; Khalil & Mansour, 1995; Oshodi et al., 1995). The cooking conditions utilized in this study showed that black beans cooked in distilled water had higher content of lysine, histidine, arginine, glutamic acid and alanine than raw black beans. Also cooking in *kanwa* solution caused an increase of the level of the same amino acids and of aspartic acid. In addition, as Youssef et al. (1986) found in their study of faba bean, heat-treatments decrease some essential amino acids, especially, isoleucine, tyrosine, phenylalanine, threonine and valine.

The neutral sugars were identified, by gas chromatography, as galactose and glucose (Table 5). These hexoses compose the oligosaccharides raffinose, stachyose and verbascose, which are well known to cause flatulence. These results show that the three flour samples contain the same sugar residues but that their glucose to galactose ratios were different in black beans cooked in the alkaline solution. Galactose was decreased by this heat-treatment. These findings are in good agreement with those reported by Mansour and El-Adawy (1994) and Khalil and Mansour (1995).

From these results, it was evident that cooking modified the yield of crude lipids, proteins and sugars extracted from the black bean flour according to the procedure adopted in this study. In addition, an effect of cooking in alkaline solution for a short time was especially observed in the contents of proteins and neutral sugars. Effectively, cooking of the beans in *kanwa* solution caused significant decrease of the level of essential amino acids such as lysine, isoleucine, tyrosine, phenylalanine, threonine and valine and the content of galactose which composed the flatulence factors. However, this heat treatment did not seem to induce significant changes in the composition of lipid and water-soluble fractions since we observed the same distribution of fatty acids in the crude lipid fraction and of amino acids and neutral sugars in the water-soluble fractions from the three flour samples.

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