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Comparative Study of Nutritional Qualities of Defatted Cottonseed and Soybean Meals

J. M. Rouanet, O. Henry, B. Caporiccio & P. Besançon

Laboratoire de Nutrition, Centre de Génie et Technologie Alimentaire, Université de Montpellier II, France

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ABSTRACT

Experiments were conducted on rats to investigate the quality of glandless cottonseed flour compared to soybean flour prepared in the same way. In order to inactivate toxic residual factors, an additional heat treatment (steaming at 112°C for 15 min) was applied on both flour samples. Cottonseed flour contained 39% protein and about 1·7% ether extract. Dietary fibres ranged from 19·2 to 20·6%. Antinutritional compounds were absent, except phytic acid (4·4%) which was still present after steaming. Nutritional parameters, used as criteria in this work, i.e. protein efficiency ratio (PER), biological value (BV) and net protein utilization (NPU), demonstrated a good intrinsic nutritional quality for cottonseed protein (unless it had a low lysine content), when compared with casein and soybean. Moreover, enzymic and light microscopic studies, which were performed on the digestive tract of animals, showed no noxious effect.

INTRODUCTION

The protein content in cottonseed flour is relatively high compared with many other vegetable protein sources (Bressani, 1965); numerous studies have shown its potential for utilization in human diets (Bressani, 1965; Srikantia & Sahgal, 1968; Alford *et al.*, 1977; Thomas *et al.*, 1979), especially infant and children's food (Braham *et al.*, 1965; Graham *et al.*, 1969, 1970). These proteins are shown to be well balanced in their essential amino acid composition. Since soybeans are already widely used in human diets, it

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would be of particular interest to compare the nutritional value of defatted cottonseed and soybean meal which are simultaneously and identically treated. Gossypol is considered as a handicap for cottonseed utilization; thus, glandless cottonseed production (Lawhon *et al.*, 1970; Martinez *et al.*, 1970) is of promising value. This study will deal only with the glandless cottonseed variety. Nevertheless, other antinutritional and toxic compounds, which are ubiquitously encountered in vegetable seeds (legume and cereals), i.e. enzyme inhibitors (Liener & Kakade, 1969), lectins (Rouanet *et al.*, 1985) and phytic acid (Wozenski & Woodburn, 1975), and which may affect the protein quality, should be investigated in cottonseed products. Thus, we have tested the presence of some antinutritional factors, and potential toxicity in the digestive tract.

MATERIALS AND METHODS

Glandless cottonseeds (ISA BC4 variety) and soybean seeds came from a breeding managed by the Ivorian Company for Textiles Development (Bouaké, Ivory Coast); they were cultivated on an industrial scale. The standard industrial process for trituration was conducted as follows: after harvesting, seeds were dehulled and then kernels conditioned (11% moisture, 60°C); subsequently, flakes (flattening at 60°C for 15 min) were exhaustively defatted by hexane extraction (60°C) followed by desolventization (90°C). This technology led to representative materials. Defatted cottonseed flour (CF) and soybean flour (SF) were prepared from these oilcakes by the Grands Moulins de Paris. In order to inactivate residual antinutritional factors, an additional heat treatment was applied on a part of each flour by steaming at 112°C for 15 min in a Hydrolock sterilization simulator, then lyophilized; these flours were, respectively, denominated CFHT and SFHT.

Chemical techniques

Moisture, ash, nitrogen and lipid extract contents were determined on flours by AOAC methods (AOAC, 1980). Dietary fibres were assayed according to Van Soest (Van Soest & Wine, 1963, 1967). The amino acid determinations were performed after an acid hydrolysis using 6N HCl at 110°C for 24 h in an air-oven and under nitrogen at atmospheric pressure. After evaporation of HCl under reduced pressure at 40°C, the amino acids were determined using a DURUM amino acid analyser (Moore & Stein, 1954). Tryptophan content was measured after alkaline hydrolysis using 4N sodium hydroxide at 110°C

during 16 h and under nitrogen atmosphere (Hugli & Moore, 1972). Sulfur amino acid analyses (methionine and cysteine/cystine) were performed after performic acid oxidation according to Hirs (1956); methionine content was obtained as methionine sulfone and the pair cysteine/cystine as cysteic acid. On the basis of amino acid composition, the chemical score was calculated as the percentage of the most deficient amino acid in the protein (Mitchell & Block, 1946) using FAO/WHO pattern recommendation (FAO, 1973). The available lysine was estimated following Booth's technique (Booth, 1971). Trypsin and chymotrypsin inhibitors were quantified spectrophotometrically according to Rackis *et al.* (1974) using benzoyl-DL-arginine *p*-nitroanilide and glutamyl-L-phenylalanine, respectively, as substrates. Lectins, amylase inhibitor and phytic acid were, respectively, tested according to Liener (1965), Jaffé *et al.* (1973) and Davies and Reid (1979).

Biological techniques

The diets used for PER determination and nitrogen balance studies were isoenergetic and isonitrogenous (10% of protein). Ingredients and diet composition used are described in Table 1. For PER and *in vivo* digestibility, Sprague–Dawley rats, 25–30 days old, and housed in individual metabolic cages (6 rats/dietary group), were used. The temperature of the animal house was regulated at 25°C. Food and water intake were *ad libitum*. The experiment was carried out for 21 days, and food consumption and body weight were individually recorded twice a week. During the last week, the urine and feces were collected; the feces were lyophilized before nitrogen determination. At the end of the experiment, rats were killed after anesthesia. Duodenum and proximal jejunum were immediately removed. After flushing, the intestinal mucosae were scraped, homogenized at 4°C in 20 ml saline and stored at −80°C until assayed. Alkaline phosphatase (duodenum) and maltase (jejunum) were tested according to Bessey *et al.* (1946) and Dahlqvist (1968), respectively. Protein concentration was determined by the Lowry procedure (Lowry *et al.*, 1951). Immediately after killing and prior to scraping the mucosa, specimens were taken from identical parts of the digestive tract of each animal (end of the proximal jejunum), fixed in Bouin's solution, embedded in paraffin, cut perpendicular to the longitudinal axis and stained with haemalun-eosin. The lengths of the villi and adjacent crypts were measured by means of a light microscope (Carl Zeiss, Iena) equipped with a projective apparatus. Results were given as mean ± SEM. Data were compared by analysis of variance (ANOVA); the significances of comparisons were determined by a multiple range Student's *t*-test.

TABLE 1
Composition of Experimental Diets (dry basis)

Ingredients	*Dietary groups (g/100 g of diet)*				
	A	*B*	*C*	*D*	*E*
Casein (87% protein)	11·50				
CF		25·31			
CFHT			25·57		
SF				18·67	
SFHT					21·41
Corn starch	61·95	52·58	52·10	56·93	55·01
Glucose	4·92	3·73	3·72	4·06	3·93
Cellulose	4·92	3·73	3·72	4·06	3·93
Fat	7·08	5·97	5·95	6·50	6·29
Salt mix[a]	9·73	8·21	8·18	8·94	8·65
Vitamin mix[b]	0·89	0·75	0·75	0·81	0·78
Protein and energy level in each diet					
N × 6·25	10·00	10·00	10·00	10·00	10·00
kcal (calcd)[c]	393	394	394	393	394
kJ (calcd)	1 643	1 645	1 645	1 643	1 645

[a] Composition of the salt mix in g/kg of mix: calcium dihydrogen phosphate: 430·0; potassium chloride: 100·0; sodium chloride: 100·0; magnesium chloride: 50·0; ferric oxide: 30·0; ferric sulfate: 30·0; manganese sulfate: 2·45; zinc sulfate: 2·00; cupric sulfate: 0·50; cobalt sulfate: $4{\cdot}0 \times 10^{-3}$; potassium iodide: $8{\cdot}0 \times 10^{-3}$.

[b] Composition of the vitamin mix, expressed as units or g/kg of mix: retinyl acetate: 1 980 000 IU; cholecalciferol: 600 000 IU; DL-tocopheryl acetate: 17·0; menadione: 4·0; thiamin-HCl: 1·0; riboflavin: 1·5; calcium pantothenate: 7·0; pyridoxin-HCl: 1·0; inositol: 15·0; cyanocobalamin: $5{\cdot}0 \times 10^{-3}$; ascorbic acid: 80·0; nicotinic acid: 10·0; choline-HCl: 136·0; folic acid: 0·5; *p*-aminobenzoic acid: 5·0; D-biotin: $3{\cdot}0 \times 10^{-2}$.

[c] Calculated as: total carbohydrates × 4 kcal/g; fat × 9 kcal/g; protein 4 kcal/g.

RESULTS AND DISCUSSION

Chemical composition

Results of the proximate analysis of the flours are given in Table 2. The protein content in CF was 39·5% (N × 5·30) and 53·5% (N × 5·70) in SF. The fat content of these defatted flours was less than 1·7%. The fibre content was higher in cottonseed samples than in soybean ones because hulls were added to kernels during the extracting process in order to improve the oil extraction yield. The ash content ranged from 7·2–7·5%. It was verified that steaming did not significantly modify the chemical composition of the samples.

TABLE 2
Chemical Composition of the Flours (g/100 g of flour)

Sample	*Protein*[a]	*Fat*	*Fibre*[b]	*Ash*	*Moisture*
CF	39·50	1·62	20·62	7·23	4·18
SF	53·54	1·68	9·90	7·52	5·73

[a] Cotton: N × 5·30; soya: N × 5·70.
[b] As NDF.

TABLE 3
Amino Acid Composition (g/16 g of N)

Amino acids	*FAO/WHO pattern*[a]	*Casein*[b]	*CF*	*CFHT*	*SF*	*SFHT*
Ile	4·0	5·5	2·98	2·80	4·11	4·11
Leu	7·0	9·7	5·71	5·36	7·18	7·18
Lys	5·5	8·3	4·25	3·59	5·47	4·92
Met	3·5[d]	2·9	1·18	0·96	1·15	1·02
Cys		0·4	1·79	1·64	1·60	1·57
Phe	6·0[e]	5·3	4·73	4·46	4·71	5·03
Tyr		5·9	3·29	2·84	3·80	3·60
Thr	4·0	4·9	3·18	2·95	3·64	3·75
Try	1·0	1·7	1·18	N.D.	1·11	N.D.
Val	5·0	6·9	4·25	4·00	4·18	4·13
Arg		3·8	11·43	10·64	7·13	6·94
His		3·0	2·53	2·27	2·29	2·20
Ala		3·1	4·03	3·83	4·15	4·19
Asp		7·3	9·32	8·86	11·50	11·90
Glu		22·5	18·35	17·55	17·83	19·23
Gly		2·0	3·98	3·78	3·80	3·91
Pro		11·8	3·02	2·95	3·93	4·15
Ser		6·2	4·19	3·89	4·87	5·03
Total AA		111	89·39	81·87	92·45	92·86
Ratio E/T[c]		46·3	36·40	34·32	39·96	38·02
Available lysine (%)			83·00	78·00	85·00	79·00

[a] FAO (1973).
[b] Recalculated from data of FAO (1970).
[c] Essential amino acids/Total amino acids.
[d] As Met + Cys.
[e] As Phe + Tyr.

Amino acid composition

Amino acid content (Table 3) was similar to that previously reported by Martinez and Hopkins (1975) in CF as well as in SF samples. Chemical score was estimated by comparing amino acid composition of the protein with the reference pattern of amino acids (FAO, 1973) and is shown in Table 4. Limiting amino acids were in cottonseed samples: sulfur amino acids (first limiting amino acid), lysine and threonine, and in soybean samples: sulfur amino acids and valine. Although cottonseed samples scored higher than soybean, cottonseed provided only three amino acids (aromatic amino acids and tryptophan) in quantities in a relative excess regarding the pattern (Table 3), whereas in soybean flours all the amino acids, except total sulfur amino acids, were provided in larger amount. This is an important point since cottonseed or soybean proteins were promised to be mixed with other protein sources in order to get a complementarity effect. Nevertheless, amino acid imbalance was less pronounced in cottonseed samples. Steaming did not notably affect amino acid content except a slight loss in lysine. As shown in Table 3, the available lysine, expressed as per cent of total lysine, was slightly decreased after steaming of the flours; nevertheless, the availability of lysine was the same in corresponding samples (unheated and steamed).

Antinutritional factors

Some antinutritional factors were assayed on flour samples. Residual contents are shown in Table 5. CF and CFHT did not contain protease inhibitors (trypsin and chymotrypsin) nor haemagglutinating activity. However, SF and SFHT showed slight inhibitor contents; this was not surprising since heat treatments occurring during and after flour preparation are known to destroy these compounds (Liener & Kakade, 1969). Similarly, heat labile antiamylase activity was never detected. On the other hand, phytate content was twice higher in cottonseed flours than in soybean seed flours; flour preparations and heat-treatments did not improve the phytate content.

Biological assays (Table 6)

Mean initial weight of rats was 80 g. The growth was about 6·3 g/day in the control rats fed casein and ranged from 6·2–6·9 g/day in the groups receiving the cottonseed flour samples. However, rats fed the SF and SFHT showed a slower growth (4·3–5·5 g/day). PER and growth reflected an excellent nutritional utilization of CF and CFHT which was slightly lower in SF- and

TABLE 4
Essential Amino Acid Equilibrium and Chemical Score[a]

Amino acids	*CF*	*CFHT*	*SF*	*SFHT*	*Casein*
Ile	83	83	110	110	125
Leu	92	93	110	110	124
Lys	85	80	107	96	136
Met + Cys	60[b]	58[b]	53[b]	51[b]	55[b]
Phe + Tyr	150	146	153	155	168
Thr	88	90	98	100	108
Try	130	—	120	—	150
Val	94	96	90	88	124
Chemical score	60	58	53	51	55

[a] Chemical score $= \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in reference pattern}} \times 100.$

[b] Limiting amino acids.

SFHT-fed rats. Dry matter and nitrogen digestibility were a little reduced in cottonseed-fed groups; this can be explained by the higher dietary fibre contents in these flours. The biological value (BV) of CF is consistent with the slight lysine deficiency. This deficiency was increased by a loss in available lysine after steaming. The net protein utilization (NPU) only decreased significantly in the group fed CFHT and confirmed the preceding data.

Intestinal parameters

After killing, the pancreas was removed and weighed; any enlargement was observed and confirmed the lack of protease inhibitors in the flours (Table 7).

TABLE 5
Antinutritional Factors

	CF	*CFHT*	*SF*	*SFHT*
Trypsin inhibited units (TIU/mg protein)	0	0	0·97	0·65
Chymotrypsin inhibited units (CIU/mg protein)	0	0	0·80	0
Lectins	0	0	0	0
Antiamylase	0	0	0	0
Phytate (g/100 g dry matter)	4·43	4·42	1·95	2·01

TABLE 6
Results of Biological Assays

Groups	*CF*	*CFHT*	*SF*	*SFHT*	*Casein*
Growth (g/day)[1]	$6{\cdot}89 \pm 0{\cdot}71^{a}$	$6{\cdot}18 \pm 1{\cdot}42^{a}$	$4{\cdot}30 \pm 0{\cdot}53$	$5{\cdot}51 \pm 0{\cdot}55^{a}$	$6{\cdot}29 \pm 1{\cdot}45^{a}$
PER[2]	$3{\cdot}27 \pm 0{\cdot}24^{ac}$	$3{\cdot}07 \pm 0{\cdot}34^{bc}$	$2{\cdot}76 \pm 0{\cdot}32^{b}$	$3{\cdot}03 \pm 0{\cdot}32^{bc}$	$3{\cdot}64 \pm 0{\cdot}21^{a}$
Nitrogen digestibility[3]	$84{\cdot}0 \pm 2{\cdot}0^{b}$	$79{\cdot}5 \pm 2{\cdot}6^{c}$	$82{\cdot}5 \pm 2{\cdot}3^{b}$	$85{\cdot}0 \pm 2{\cdot}4^{b}$	$92{\cdot}0 \pm 1{\cdot}9^{a}$
Dry matter digestibility	$83{\cdot}8 \pm 1{\cdot}8^{c}$	$82{\cdot}8 \pm 1{\cdot}4^{c}$	$87{\cdot}5 \pm 1{\cdot}0^{b}$	$86{\cdot}0 \pm 1{\cdot}1^{b}$	$90{\cdot}0 \pm 1{\cdot}2^{a}$
Biological value[4]	$79{\cdot}0 \pm 4{\cdot}2^{a}$	$69{\cdot}4 \pm 4{\cdot}5^{b}$	$84{\cdot}3 \pm 7{\cdot}9^{a}$	$83{\cdot}3 \pm 5{\cdot}3^{a}$	$82{\cdot}0 \pm 2{\cdot}9^{a}$
NPU[5]	$66{\cdot}4 \pm 3{\cdot}9^{a}$	$55{\cdot}1 \pm 4{\cdot}3^{b}$	$71{\cdot}9 \pm 7{\cdot}7^{a}$	$70{\cdot}7 \pm 5{\cdot}1^{a}$	$72{\cdot}9 \pm 3{\cdot}5^{a}$

[1] Mean value ± SEM. Values with same superscript letter in a row are not significantly different ($p < 0{\cdot}05$).
[2] Calculated as weight gain versus protein intake.
[3] Calculated as absorbed N versus ingested N corrected by endogenous N. Expressed as %.
[4] Calculated as fixed N versus absorbed N. Expressed as %.
[5] Calculated as N digestibility × Biological value. Expressed as %.

Intestinal hydrolase levels failed to show any noxious effect from the flours either at the duodenal or at the jejunal level (Table 7); these data signify that eventually, lectins present in the flours were destroyed by heat-treatments (Boufassa *et al.*, 1986). Light microscopic studies, i.e. villus and crypt lengths (Table 8) corroborated the preceding data; these studies failed to show any difference in morphological parameters nor any impairment of the proliferative compartment.

TABLE 7
Pancreas Weight and Intestinal Hydrolase Activities[1]

Groups	*CF*	*CFHT*	*SF*	*SFHT*	*Casein*
Pancreas weight[2]	$0{\cdot}26 \pm 0{\cdot}05^{a}$	$0{\cdot}27 \pm 0{\cdot}03^{a}$	$0{\cdot}29 \pm 0{\cdot}05^{a}$	$0{\cdot}25 \pm 0{\cdot}03^{a}$	$0{\cdot}25 \pm 0{\cdot}06^{a}$
Alkaline phosphatase[3]	$8{\cdot}38 \pm 2{\cdot}66^{a}$	$7{\cdot}19 \pm 1{\cdot}56^{a}$	$7{\cdot}38 \pm 1{\cdot}72^{a}$	$7{\cdot}86 \pm 2{\cdot}08^{a}$	$8{\cdot}67 \pm 1{\cdot}20^{a}$
Maltase[2] ($\times 10^{2}$)	$3{\cdot}1 \pm 0{\cdot}2^{a}$	$3{\cdot}0 \pm 0{\cdot}1^{a}$	$3{\cdot}0 \pm 0{\cdot}4^{a}$	$2{\cdot}8 \pm 0{\cdot}1^{a}$	$2{\cdot}8 \pm 0{\cdot}2^{a}$

[1] Mean value ± SEM; values with same superscript letter in a row are not signficantly different ($p < 0{\cdot}05$).
[2] Relative to 100 g body weight.
[3] Expressed as μmol of substrate hydrolysed min^{-1} mg $protein^{-1}$.

TABLE 8
Morphological Parameters of Small Intestinal Mucosa

Dietary groups	*Villi height*	*Crypts height*	*Villus/Crypt ratio*
CF	715 ± 70^a	307 ± 34^b	$2{\cdot}35 \pm 0{\cdot}23^c$
CFHT	724 ± 63^a	315 ± 19^b	$2{\cdot}31 \pm 0{\cdot}27^c$
SF	729 ± 58^a	320 ± 25^b	$2{\cdot}29 \pm 0{\cdot}23^c$
SFHT	708 ± 67^a	317 ± 20^b	$2{\cdot}26 \pm 0{\cdot}30^c$

Measurements are expressed as μm. Values with same letter in a column are not significantly different ($p < 0{\cdot}05$).

CONCLUSION

The data shown in this work allow us to draw up a promising balance; biological tests showed a good nutritional intrinsic value for cottonseed flour, which is placed at the same level and is competitive with soybean flour. However, our results do not allow us to prejudge effects of chronic feeding of the cottonseed flour on the digestive tract, protein equilibrium and mineral balance (phosphorus, calcium and oligoelements). Longer assays are imperative. Technological treatments (i.e. protein concentrate and isolate) may allow one to decrease fibres and phytate contents since these compounds, when provided in excess, must be considered as antinutritional factors, especially if the cottonseed flours are expected to be blended in infant formulas. Finally, relative deficiency of essential amino acids, particularly lysine, could be easily compensated by supplementations in food products.

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