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Effect of Supplementation with Soybean Flour and Cooking on Anti-nutritional factors and in vitro protein digestibility of corn flour

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Abstract:
The objectives of this study are to evaluate the effect of supplementation by differences ratios of defatted soya bean cake and cooking on nutritional quality, Anti-nutritional factors and minerals contents of corn flour. The effect of supplementation of corn flour with different levels of soybean cake and cooking on Anti- nutritional factors, total minerals contents (K, Ca, Mn, Fe, Cu and Pb), was investigated. Supplementation with 3, 5 and 7% Soya bean flour insignificantly ($P \leq 0.05$) increased the protein content, anti-nutritional factors (phytic acid and total polyphenols). Cooking of supplemented flour increased the protein content and decreased the minerals contents (K, Ca, Mn, Fe, Cu and Pb).

Keywords: Supplementation, Anti-Nutrients, Cooking, Soy Bean, Corn Flour

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Introduction

Cereals are the most widely cultivated and consumed crops on globally. In Nigeria, specifically in the Northern part of the country, cereal provides a major food resource for man. It is the major source of energy and protein in the diet of many people. Maize is the second most important cereal crop in Nigeria ranking behind sorghum in the number of people it feeds. Maize is a multipurpose crop, providing food and fuel for human being and feed for animals (poultry and livestock). Its grain has great nutritional value and can be used as raw material for manufacturing many industrial products. (Afzal,M et al, 2009).In most countries, lime-treated corn provides 31% of the total protein and 45% of the energy intake, and beans 24% of the protein and 12% of the calories. Such diet is low in protein quality and quantity, as well as in energy (US National Library of Medicine Institutes of Health 2003).

To overcome these deficiencies, corn can be supplemented either with its limiting amino acids, lysine and tryptophan, or better still, with whole soybeans which improve not only the amount and quality of the protein consumed but, because of their high oil content, the energy intake as well. The soybean (Glycine max) is one of the most important food plants of the world, and seems to be growing in importance. It is an annual crop, fairly easy to grow, that produces more protein and oil per unit of land than almost any other crop. It is a versatile food plant that, used in its various forms, is capable of supplying most nutrients. It can substitute for meat and to some extent for milk. The soybean (Glycine max) is the seed of the leguminous soybean plant. It has high protein content and is not very expensive. Therefore, it has been pro- posed as an ideal source for protein supplementation of starchy foods. It is a crop capable of reducing protein malnutrition. In addition, soybeans are a source of high value animal feed. Higher levels of soybean products, however, may affect the consistency of the lime-treated corn dough and, therefore, the tortilla acceptability. At a level of 15 parts of whole soybean or 8 parts soybean-derived products, to 85--92 parts of corn there were no significant changes in the rheological or organoleptic characteristics of the tortilla prepared thereof. (US National Library of Medicine Institutes of Health 2003). Since corn is usually cooked, but not ground, at home, the soybean supplement can be successfully added at the wet--milling stage of dough preparation or whole soybeans and corn may be cooked together, when a nutritional intervention is desired at the village level. (US National Library of Medicine Institutes of Health 2003). At an industrial scale, if whole soybeans are used, they may be cooked together with corn, and if soy flour is used, this can be mixed at the end of the process when the cooked corn is ground to flour. (US National Library of Medicine Institutes of Health 2003).

Material and methods

Corn grains:

A Grain of corn was obtained from the department of agronomy, faculty of agriculture, university of Khartoum, Shambat, Sudan.

Preparation of grains sample:

The grains were cleaned, freed from foreign seeds, broken and shrunken ones, then was milled into fine flour using house blender and mortar to pass through 0.4mm screen and stored in polyethylene bags at 4℃for further analysis.

Soy beans seeds:

Soybean seeds were brought from Omdurman local market.

Preparation of soy bean sample:

The seeds were defatted using hexane solvent and dried in a hot air oven at 70℃for 3-4 hours, then milled in to fine flour using house blender and mortar to pass through 0.4mm and stored in polyethylene bags at 4℃ for further use. All chemicals used in this study were being of reagent grade.

Supplementation:

Defatted soybean seeds flour was added using Pearson square to increase the nutritive value of corn flour by 3, 5 and 7%, respectively. The number of composite flour samples after supplementation were three samples.

Cooking:

Cooking of the sample was performed by suspending the flour of each sample in distilled water in the ratio of 1:2 (flour: water, w/v) and the slurry will be shaken to avoid lumps while boiling in a water bath for 20 min. The viscous mass was spread out thinly in dishes and oven dried at 70℃. The dried flakes were milled into fine flour by house blender and mortar to pass through 0.4mm screen and stored at4℃ for further analysis.

Determination of Tannin Content

Quantitative estimation of tannins was carried out using the modified vanillin – HCl methanol, according to Price, M et al, (1978). The vanillin HCl reagent was prepared by mixing equal volumes of 8% concentrated HCl in methanol and 1% vanillin in methanol, the two solvents of the reagent were mixed just prior to use, it was discarded if a trace of colour appeared.

Determination of phytic acid content:

Phytic acid content of samples was determined according to the modified method of Wheeler ,E and Ferrel ,R (1971). One gram of finely ground sample was weighed into 100 ml conical flask, and then 50 ml of 3% of TCA solution (W/V); containing 10% (W/V) sodium sulphate; was added, after shaking for one hour, the slurry obtained was centrifuged at 3000 rpm for 15 minutes. Ten milliliter of supernatant was transferred into 50 ml boiling tube. Then 4 ml of $FeCL_3$ solution (2 mg Fe^{3+}/ ml 3% TCA), was centrifuged at 3000 rpm for 15 minutes and the clear supernatant was carefully decanted. The precipitate was then be washed twice by dispersing well into 25 ml 3% TCA, heating in the boiling water bath (10 minutes) and centrifuged. Washing was repeated once with water. The precipitate was dispersed in few millilitres distilled water enriched with (3) ml 15 NaOH with mixing. The volume was made approximately (30) ml with distilled water and was heated in the water bath for (30) minutes. The content of the tube was filtered hot through whattman paper No. 1 filter paper and filtrate were discarded. The precipitate from the paper was dissolved with (40) ml hot 3.2 N HNO_3 into a (100) ml volumetric flask. The paper was washed with the several portions of distilled water. The content of the flask was cooled and diluted to volume with distilled water. Five millilitres' aliquots were transferred into another 100 ml volumetric flask and diluted to approximately 70 ml distilled water. Then, 20 ml of 1.5 M KSCN (Potassium thiocyanate) were added; completing the volume up to the mark. The intensity of colour was immediately read at 480 nm (corning 259). A blank probe was run each set of samples. The iron content was calculated from prepared standard curve of $Fe(NO_3)_3$. The phytate was estimated from the assumption that it contains 28.2 % P (De Boland, A et al., 1975) and phytate phosphorous from a molar ratio of 4:6 Fe: P.

Determination Total Polyphenols:

Total Polyphenols present in flour sample, from seed was estimated using Prussian blue assay, as described by (Price, M et al (1978). Ground sample (60 mg) was extracted with 3 ml absolute methanol in the test tube, by constant shaking for one minute, and then was poured into filter paper. The tube was quickly rinsed with additional 3 ml of methanol and the content was poured at once into filter paper. The filtrate was diluted to 50 ml with distilled water, mixed with 3 ml 0.1 M $FeCL_3$ IN 0.1 N HCL for 3 minutes, followed by the timed addition of 3 ml 0.008 M $K_3Fe(CN)_6$. The absorption was read after 10 minutes at 720 nm on a spectrophotometer (Coring, 259).

Protein digestibility:

The protein digestibility was carried out according by Monjula et al. (1991).

A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 ml of 0.1 MHCL at 37oC for 2 hours. The reaction was stopped by the addition of 15 ml 10% trichloro-acetic acid (TCA). The mixture was then filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-kjeldahl method. Digestibility was calculated using the following equation:

Protein digestibility (%) = (N in supernatant ×N blank)/ (N in sample

Statistical analysis

All data were subjected to statistical analysis, each determination was carried out and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of Variance (ANOVA) Gomez,k and Gomez,A (1984). Duncan Multiple Range Test (DMRT) was used to separate means. Significance was accepted with at $P \leq 0.05$.

Results and discussion

Anti-nutritional factors of Corn flour and Soya bean flour:

The anti-nutritional factors of corn flour and Soya bean flour were seen in Table 1. The phytic acid content of Soya bean flour was 253.167mg/100g which was higher than the value reported by (*Joyce,O et al., .2004*) who reported that phytic acid of soya bean was 138.92 mg/100g, while that of corn flour was found to be 183.920mg/100g, which was very higher than the value ranged (61–78) given by(*Aaron,J.Cowieson.,2005*) .The tannin content of Soya bean flour was found to be 328.727 mg/100g which was very higher than the value 0.71 mg/100g tannin content of Soya bean (Brown color) reported by(*Malenčića,D. et al.,2008*) and that of Maize was 282.020 mg/100g which was lower than that reported by(*Oseni O.A and Ekperigin M, 2007*) who reported 576 mg/100g tannin content of Corn flour , The total polyphenol of Soya bean was found to be 215.410mg/100g which was lower than the value .2 mg/100g type Soya bean flour (Alisa × Meli) obtained by (Malenčića,D. et al.,2008), and that of Corn flour was fund 216.037 mg/100g.

Table 1: Anti-nutritional factors (mg/100g) of Soya bean flour and Corn flour

Samples	Phytic acid	Tannin (%)	Total polyphenols
Soya bean	253.167a ±18.93	328.727c ±6.64	215.410b ±1.91
Corn flour	183.920c ±13.77	282.020e ±9.30	216.037b ±4.90

** Means not sharing a common letter in the same column are significantly different (p≤0.05) according to least significant test (LSD)*
* Values are means (±SD) of three replicates.

Effect of supplementation with 3, 5and7% Soya bean and cooking on anti-nutritional factors of Corn flour:

Phytic acid content

As shown in Table 1. The phytic acid content of corn flour was found to be 183.920 mg/100g. Supplementation with 3, 5 and7% Soya bean flour significantly (P≤0.05) increased the phytic acid to214.163, 235.803and 261.490mg/100g, respectively. Cooking of Corn flour supplemented with3, 5 and 7% Soya bean flour significantly (P≤0.05) decreased the phytic acid to 189.35, 98.297, and 58.130mg/100g, respectively. This result was agreed with the findings reported by (*Fageer A S M et al., 2004*) who reported that the effect of malt pre-treatment and/or cooking decreased the phytate content of Corn flour.

Tannin Content

Tannin content of corn flour supplemented with 3, 5 and 7% Soya bean flour (Table 1) was found to be 320.683. 362.097and 386.080 mg/100g, respectively, insignificant (P≤0.05) increase of tannin content in corn flour was observed. Cooking of Corn flour supplemented with3, 5 and 7% Soya bean flour insignificantly (P≤0.05) decreased the tannin content to 213.713, 168.247 and 118.403%, respectively. The reduction in tannin content of control and composite flours after cooking may be due to the heat degradation of these molecules as well as changes in their chemical reactivity or the formation of insoluble complexes.

Total Polyphenols:

The total polyphenols contents of corn flour (Table 1) were seen 216.037mg/100g, it was significantly (P≤0.05) increased after supplementation with 7% Soya bean flour to 236.163amg/100g, while significantly (P≤0.05) decrease was observed after supplementation with 3and 5% Soya bean flour (182.643) and (197.993) mg/100g, respectively. Cooking of corn flour supplemented with 3% and 7% Soya bean flour significantly (P≤0.05) decreased the total polyphenols content to 94.443, 68.037and38.153gmg/100g, respectively. The loss in polyphenols during cooking might be due to the fact that phenols react with protein forming poorly extractable protein- phenolic complexes. (*Mohammed nour, AA et al., 2015*).

Table 2: Effect of cooking on anti-nutritional factors (mg/100g) of Corn flour supplemented with different ratios of Soya bean flour.

Supplementation levels (%)	Treatment	Phytic acid	Tannin (%)	Total polyphenols
0	Raw	183.920c ±13.77	282.020e ±9.30	216.037b ±4.90
3	Un cooked	214.163b ±3.73	320.683d ±4.90	182.643d ±3.22
	Cooked	189.35c ±0.55	213.713f ±4.19	94.443e ±5.05
	Un cooked	235.803b	362.097b	197.993c

5		±1.28	±0.69	±2.03
	Cooked	98.297d ±0.58	168.247g ±0.24	68.037f ±0.55
7	Un cooked	261.490a ±3.52	386.080a ±0.61	236.163a ±0.43
	Cooked	58.130e ±1.70	118.403h ±0.65	38.153g ±0.89

* Mean ±SD values having same superscript within a column are insignificantly different (P≤0.05) according to DMRT.

Effect of supplementation with3, 5 and 7% Soya bean and cooking on in vitro protein digestibility of Corn flour.

In vitro protein digestibility (IVPD%) of corn flour was found to be 40.178%

(Table 3). Supplementation with 3, 5 and 7% Soya bean flour insignificantly (P≤0.05) increased the IVPD% of Corn flour to 56.099, 53.425and 46.837%, respectively. IVPD %; there is improvement in IVPD% of 3% and 5% composite corn flour. Cooking of corn flour supplemented with 3%soya bean flour insignificantly (P≤0.05) increased the IVPD% to 50.471%, while significant (P≤0.05) decrease in IVPD %was observed after supplementation with 5 and 7% soya bean flour.

Table 3: Effect of cooking on protein content (%) and in vitro protein digestibility (%) of Corn flour supplemented with different ratios of Soya bean flour.

Supplemented level %	Treatment	IVPD (%)
0	Raw	40.178e ±2.16
3	Raw	56.099a ±1.06
	Cooked	50.471c ±2.14
5	Raw	53.425b ±0.42
	Cooked	33.181f ±0.63
7	Raw	46.837d ±0.80
	Cooked	21.862g 0.00

*Mean ±SD values having same superscript within a column are insignificantly different (P≤0.05) according to DMRT.

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