PROXIMATE COMPOSITION, QUANTITATIVE AND QUALITATIVE ANALYSIS OF PROCESSED COTTONSEED CAKE

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ABSTRACT

Cottonseed cake (CSC) is one of the promising non-conventional protein feed ingredients but its use in livestock feed is limited due the presence of antinutrients such as gossypol, phytate, and alkaloids, which can impede the efficiency of feed utilisation and performance of animals. Processing of CSC could reduce its antinutritional contents. Hence, this study evaluated the effects of different processing methods on nutritional content of CSC. The CSC was subjected to four processing methods as follows: Treatment 1: Raw (unprocessed) cottonseed cake; Treatment 2: Cottonseed cake fermented with distilled water; Treatment 3: Cottonseed cake fermented with corn starch steep liquor; Treatment 4: Acetone extracted cottonseed cake; and Treatment 5: Ethanol extracted cottonseed cake. Data were subjected to descriptive statistics and ANOVA at α0.05. Fermentation of CSC with distilled water and corn starch steep liquor significantly decreased crude protein content (21.81% and 21.61%, respectively). Furthermore, total gossypol was lower in acidified ethanol extraction of CSC (3.26%) compared to other treatment groups. Whereas oxalate content was lower in distil water fermented CSC (0.62%) than other treatments. In conclusion, processing of CSC reduced its antinutrients (alkaloids, gossypol and phytate), and increased its phytonutrients (flavonoids, saponins and total phenolic compound), where ethanol extracted CSC was the best amongst the processing methods used.

Keywords: Cottonseed meal, Fermentation, Solvent extraction, Phytochemicals, Antinutrients

INTRODUCTION

The high inflation rate has impacted the livestock business negatively through high cost of protein feed ingredients, hence, the need to look into non-conventional protein feed ingredients, that has a balanced nutrient profile, less expensive and readily available (Wanapat *et al*., 2013). Cottonseed cake (CSC) is one of the non-conventional protein feed ingredients that has a balanced nutrient profile (such as crude protein content, fat content, metabolisable energy, amino acids etc.) less expensive and readily available, but its use is limited due to some anti-nutritional factors that can impede growth performance and nutrient utilisation in livestock. Some of the anti-nutritional factors present in CSC are gossypol, tannins, cyclopropenoid fatty acid and some others but, its gossypol has been reported to be the highest in concentration and it is of great concern if CSC is to be used as a feed ingredient in monogastric nutrition. Cottonseed cake has been regarded as one of the most promising sources of plant proteins due to its high crude protein content (40.67-59.63%) and balanced amino acid profile (Zhou *et al*., 2015; Kadam *et al*., 2021) but contains anti-nutritional factors like gossypol (0.02 to 6.64%) which reduces the efficiency of nutrient utilisation (Price *et al.*, 1993).

Feed processing is one of the methods adopted by livestock farmers to reduce the anti-nutritional factors in feed ingredients, and improve feed efficiency and utilisation in livestock. One of the processing methods that have been employed is fermentation. This has been shown to improve the efficiency of feed utilisation in broilers (Khempaka *et al*., 2014). Duodu *et al*. (2018) discovered that using the soaking and autoclaving methods greatly decreased the amount of phytic acid while also reducing the gossypol levels by 34.48% and 27.59%, respectively. Therefore, the objective of this study was to evaluate the effects of different processing methods on the proximate composition and phytochemicals of cottonseed cake.

MATERIALS AND METHODS

Experimental site

This processing method was conducted in the Department of Animal Science and chemical compositions of the processed test ingredient was carried out at the Faculty of Pharmacy Central Laboratory, University of Ibadan, Ibadan, Nigeria.

Sources of test ingredients

The cottonseed cake used for the study was purchased from JT Essentials feed mill Abuja, Nigeria.

Processing of cottonseed cake

Two processing methods of cottonseed cake were adopted: solvent extraction (ethanol and acetone) and submerged fermentation (distilled water and cornstarch steep liquor. The solvent extraction method used was according to the methods of Pelitire *et al*. (2014), while submerged fermentation was as described by Folkard *et al*. (2001), The treatments evaluated were as follows: Treatment 1: Raw (unprocessed) cottonseed cake; Treatment 2: Cottonseed cake fermented with distilled water; Treatment 3: Cottonseed cake fermented with corn starch steep liquor; Treatment 4: Acetone extracted cottonseed cake; and Treatment 5: Ethanol extracted cottonseed cake.

Chemical analysis

The proximate composition was determined using the standard methods described by AACC (2000), while the phytochemical analysis of the sample was determined using the standard method described by Banu and Cathrine (2015).

Data analysis

Data was subjected to descriptive statistics and ANOVA using SPSS (2016). Means were separated using Tukey's HSD at 5% probability level.

RESULTS AND DISCUSSION

The proximate composition of raw and processed cottonseed cake (CSC) is shown in Table 1. Significant ($p<0.05$) differences were observed in all the parameters analysed. The dry matter contents of the raw and processed CSC obtained in this study varied between 92.70 and 95.82%. These were within the range of 87.36-95.25% reported by Zhuo *et al*. (2023). Fermentation of CSC with distilled water and corn starch steep liquor significantly decreased crude protein content. Similarly, de Olmos *et al*. (2022) reported that subjecting soybean to fermentation decreased crude protein value. The observed effect could be due to the fact that microbes were unable to degrade CSC protein, but rather consumed the free amino acids during the fermentation process. Furthermore, fermentation of CSC with distilled water and corn starch steep liquor significantly decreased crude fibre content. This is consistent with the report of Mageshwaran et al. (2024) who observed that fermentation of CSC decreased crude fibre value. Processing of CSC decreased ash contents. This could be as a result of solubilisation of some vitamins and minerals like phosphorus in water (Agume *et al*., 2017).

Table 1: Proximate composition of processed and unprocessed cottonseed cake

Parameters (%)	RCSC	DCSC	CCSC	ACSC	ECSC	SEM	P-value
Dry matter	92.70^{d}	95.04^{bc}	94.33^{c}	95.60^{ab}	95.82^{a}	0.31	<0.001
Ether extract	9.95^{a}	2.45^{c}	3.38^{b}	1.10^{d}	0.85^{e}	0.89	<0.001
Crude protein	26.12^{a}	21.81^{cd}	21.61^{d}	22.27^{c}	22.84^{b}	0.44	<0.001
Crude fibre	13.19^{a}	8.74^{b}	6.80^{c}	6.37^{d}	6.19^{e}	0.70	<0.001
Ash content	1.38^{a}	1.02^{d}	1.27^{ab}	1.25b^{c}	1.14^{cd}	0.03	<0.001
Nitrogen Free Extract	42.06^{c}	61.02^{b}	61.28^{b}	64.61^{a}	64.80^{a}	2.27	0.001

^{a,b,c,d,e}Means on the same row with different superscripts are significantly ($p<0.05$) different. RCSC= raw cottonseed cake,, DCSC= Distilled water fermented cottonseed cake, CCSC= Corn starch steep Liquor fermented cottonseed cake (Omi Ogi), ECSC =Ethanol extracted cottonseed cake ACSC= Acetone extracted cottonseed cake

Table 2 shows the qualitative analysis of the phytochemicals present in the raw and processed cottonseed cake. Saponin was abundant in solvent extracted CSC whereas, there was reduced steroids and alkaloids with the absence of phenols and tannins. The reduced flavonoids recorded in solvent extracted CSC might be due to the fact that flavonoids are sensitive to heat and high exposure of CSC to heat during extraction process can reduce their concentration in CSC. Flavonoids were found to be heat sensitive (Chaaban *et al*., 2017) and direct heating of flavonoids to 75 °C may impair enzyme activity and impede the flavonoid production pathway, reducing the total flavonoid concentration (Zhang *et al*., 2018).

Table 2: Qualitative analysis of the phytochemicals in unprocessed and processed cottonseed cake

Phytochemicals	RCSC	DCSC	CCSC	ACSC	ECSC
Saponins	+ve	+ve	+ve	++ve	++ve
Tannins	+ve	+ve	+ve	-ve	-ve
Flavonoids	++ve	++ve	++ve	+ve	-ve
cardiac glycosides	+ve	+ve	+ve	+ve	+ve
Anthraquinones	+ve	+ve	+ve	+ve	+ve
Terpenoids	+ve	+ve	+ve	+ve	+ve
Steroids	++ve	++ve	++ve	+ve	-ve
Alkaloids	++ve	++ve	++ve	+ve	+ve
Phenols	+ve	+ve	+ve	-ve	-ve

RCSC= raw cottonseed cake, DCSC= Distilled water fermented cottonseed cake, CCSC= Corn starch steep Liquor fermented cottonseed cake, ECSC =Ethanol extracted cottonseed cake ACSC= Acetone extracted cottonseed cake +ve = present ++ve = abundant -ve = absent

Table 3 shows the quantitative analysis of phytochemicals present in unprocessed (raw) and processed CSC. High concentrations of phytate and oxalate were recorded in acidified acetone and ethanol solvent extracted CSC compared to fermented CSC. However, acidified ethanol solvent extraction of CSC significantly reduced total gossypol content. However, the process was laborious and expensive. Pelitire *et al*. (2014) made similar observation. Whereas, this report revealed that acidified acetone did not reduce the total gossypol. Fermentation of CSC with corn starch steep liquor significantly reduced total gossypol content compared to fermentation with distill water. This could be attributed to the increased activities of microbes, such as *Lactobacillus bulgaricus*, which could aid in the degradation of gossypol. Corn starch steep liquor could serve as substrate for *Lactobacillus casei*, thereby facilitating their proliferation (Thakur *et al*., 2019).

Table 3: Quantitative analysis of the phytochemicals in unprocessed and processed cottonseed cake

Parameters (%w/w)	**RCSC**	**DCSC**	**CCSC**	**ACSC**	**ECSM**	SEM	P-value
Alkaloids	2.80^{a}	2.40^{ab}	$2.13b^{c}$	1.78^{c}	1.70^{c}	0.11	0.001
Terpenoids	$0.70b^{c}$	0.85^{b}	0.85^{b}	1.25^{a}	0.45^{c}	0.07	0.001
Saponin	0.37^{b}	1.10^{a}	0.50^{b}	1.25^{a}	0.43^{b}	0.11	0.001
Tannins	1.86^{a}	1.85^{b}	1.49^{c}	0.00^{d}	0.00^{d}	0.23	0.001
Flavonoids	2.47^{c}	5.12^{a}	2.74^{b}	2.12^{d}	0.00^{e}	0.44	0.001
Total phenolic compound	1.54^{b}	2.17^{a}	1.46^{c}	0.00^{d}	0.00^{d}	0.24	0.001
Oxalate	0.09^{b}	0.07^{b}	0.07^{b}	0.12^{a}	0.13^{a}	0.01	0.001
Phytate	0.74^{b}	0.62^{c}	0.71^{b}	1.30^{a}	1.30^{a}	0.08	0.001
Total gossypol	19.10^{ab}	16.10^{b}	12.24^{c}	19.52^{a}	3.26^{d}	1.62	0.001

[a,b,c,d,e]Means on the same row with different superscripts are significantly ($p<0.05$) different. RCSC= raw cottonseed cake, DCSC= DCSC= Distilled water fermented cottonseed cake, CCSC= Corn starch steep Liquor fermented cottonseed cake, ECSC =Ethanol extracted cottonseed cake ACSC= Acetone extracted cottonseed cake

CONCLUSION AND RECOMMENDATION

The result of this study revealed that processing of cottonseed cake reduced its anti-nutrients and improve the phytonutrients. Furthermore, solvent extraction using ethanol reduced the gossypol content by 83%. However, it is laborious and expensive and may not be recommended for smallholder farmers. Nevertheless, the use of corn starch steep liquor in the reduction of gossypol content in cottonseed cake is highly encouraged.

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