SHORT COMMUNICATION

Chemical composition of raw and processed sorrel (*Hibiscus sabdariffa* L.) seed as a potential feed resource

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Abstract

Monogastric animals primarily rely on legumes such as soybean as sources of plant protein. However, these ingredients are often in short supply, costly, and heavily taken by humans. Over recent years, the availability of these traditional feed sources, once considered the primary plant protein for monogastric diets, has been reduced due to high production costs and increased competition for human consumption. This situation resulted in growing interest in the use of alternative feed ingredients to reduce production costs. An alternative protein concentrate worth exploring is sorrel seed meal. Sorrel (Hibiscus sabdariffa) seed have a strong nutritional profile, containing 38.57% protein, 20.50% ether extract, 16.50% crude fibre, and 11.63% nitrogen-free extract. Sorrel seed - processed in three forms: boiled in 100°C water for 30 min, soaked in water for 12hours and boiled 30 min in 100°C water and fermented for 3 days. Processed seeds were analysed for crude protein (%), amino acid profile (%) and phytochemicals (mg/g) using standard procedures. Proximate, amino acid profile, qualitative and quantitative data were subjected to analysis of variance (ANOVA) using SAS (2003). Means were separated using Duncan Multiple Range Test. Processing sorrel seed- improved crude protein (CP) in soaked sorrel seed meal (26.34%) was significantly higher than in raw sorrel seed meal (22.06%) and fermented sorrel seed meal (23.84%) and the amino acid profile improved. Alkaloid level was reduced in boiled and fermented, while saponins and flavonoids were absent in all the processed methods. The quantitative analysis of phytochemicals in processed sorrel seeds were significantly decreased in alkaloids and cyanides, while saponins reduced in boiled and fermented sorrel seed, oxalates also reduced when soaked. In conclusion, processed sorrel seed (Boiled, Soaked and fermented) enhanced nutrients availability, improved amino acid profile and reduced anti-nutritional factors to tolerable levels at α0.05. Therefore, this study was carried out to evaluate the chemical composition of raw and processed sorrel seeds, and observed that sorrel seed has feed resource potential.

Keywords: Chemical composition; Amino acid profile; Phytochemical factors; Sorrel seeds.
Running title: Chemical composition of raw and processed sorrel seed

Composition chimique des graines crues et transformées d'oseille (*Hibiscus sabdariffa L.*) en tant que ressource alimentaire potentielle

Résumé

Les animaux monogastriques dépendent principalement des légumineuses, comme le soja, comme sources de protéines végétales. Cependant, ces ingrédients sont souvent en quantité limitée, coûteux et largement consommés par les humains. Ces dernières années, la disponibilité de ces sources traditionnelles d'alimentation, autrefois considérées comme les principales protéines végétales pour les régimes *monogastriques, a diminué en raison des coûts de production élevés et de la concurrence accrue pour la consommation humaine. Cette situation a suscité un intérêt croissant pour l'utilisation d'ingrédients alternatifs afin de réduire les coûts de production. Une source concentrée de protéines alternative méritant d'être explorée est la farine de graines d'oseille. Les graines d'oseille (Hibiscus sabdariffa) présentent un profil nutritionnel intéressant, contenant 38,57 % de protéines, 20,50 % d'extrait éthéré, 16,50 % de fibres brutes et 11,63 % d'extrait non azoté. Les graines d'oseille ont été transformées sous trois formes : bouillies dans de l'eau à 100?C pendant 30 minutes, trempées dans l'eau pendant 12 heures puis bouillies 30 minutes dans de l'eau à 100?C, et fermentées pendant 3 jours. Les graines transformées ont été analysées pour leur teneur en protéines brutes (%), leur profil en acides aminés (%) et leur composition en composés phytochimiques (mg/g) selon des procédures standard. Les données proximales, le profil en acides aminés, ainsi que les analyses qualitatives et quantitatives ont été soumis à une analyse de variance (ANOVA) en utilisant SAS (2003). Les moyennes ont été comparées à l'aide du test de Duncan. Le traitement des graines d'oseille a amélioré leur teneur en protéines brutes (PB) : la farine de graines trempées (26,34 %) était significativement plus élevée que celle des graines crues (22,06 %) et fermentées (23,84 %), et le profil en acides aminés s'est également amélioré. Les niveaux d'alcaloïdes ont diminué dans les graines bouillies et fermentées, tandis que les saponines et les flavonoïdes étaient absents dans toutes les méthodes de transformation. L'analyse quantitative des composés phytochimiques dans les graines transformées a montré une réduction significative des alcaloïdes et des cyanures, tandis que les saponines ont diminué dans les graines bouillies et fermentées, et les oxalates ont également été réduits lors du trempage. En conclusion, les graines d'oseille transformées (bouillies, trempées et fermentées) ont amélioré la disponibilité des nutriments, optimisé le profil en acides aminés et réduit les facteurs anti-nutritionnels à des niveaux tolérables à a0,05. Ainsi, cette étude a été menée pour évaluer la composition chimique des graines d'oseille crues et transformées, et a révélé que ces graines présentent un potentiel en tant que ressource alimentaire.*

Mots-clés : Composition chimique ; Profil en acides aminés ; Facteurs phytochimiques ; Graines d'oseille

Introduction

Monogastrics depends on groundnut and soyabean as their plant protein source, these conventional feed materials are short in supply, expensive and highly consumed by humans, this necessitated an alternative plant protein resource and one of such alternative is sorrel (*Hibiscus sabdariffa* L.) seed. Sorrel belongs to the family *Malvacea* locally known as "Isapa" in Yoruba, it is a well- adapted crop in the semi-arid zone of West Africa including Nigeria and it is generally planted as a boarder crop, sorrel seeds contained high amount of protein, dietary fibre and minerals such as phosphorus, calcium and magnesium (Ismail *et al*., 2008). Raw sorrel seeds are known to have a bitter taste which is attributed to presence of phytochemicals. The raw seed has been reported to contain total phenols, tannins and phytic acid and these have been shown to have detrimental effects on the health and performance of animals (Dairo *et al*., 2011; Keyembe, 2011). However, sorrel plant (*Hibiscus sabdariffa* L.) produces seeds that has potential to serve as one of the alternative feed resources. The sorrel plant thrives on a wide range of tropical soil conditions and perform well on relatively poorer soil (Adanlawo and Ajibade, 2006). The sorrel seed contains about 35.90% crude protein, 10.14% ether extract,10.09% ash and 15 -17% crude fibre (Dashak and Nwanegbo, 2002). It was reported by Kwari *et al.* (2011) that sorrel seeds contain 5.18% arginine,16.5% crude fibre,13.5% ether extract and 38.57% crude protein.Sorrel seeds are relatively cheap, readily available and less competitive between humans and animals. In Nigeria, sorrel seed is less than two-fifth the cost of soyabean and about half the price of groundnut cake, hence justifying the need to investigate its use in feeding animals.

However, raw sorrel seed possesses a bitter taste, which is attributed to presence of anti-nutritional factors (Kwari,2011).S The unprocessed seed has also been reported to contains phenols, tannin and phytic acid as common anti-nutrients and these have been shown to have detrimental effects on the health and performance of animals (Dairo *et al*., 2011 and Keyembe, 2011). The effective utilisation of sorrel seeds by non-ruminant animals necessitates processing such as boiling, fermentation, sprouting, etc. to inactivate the anti –nutritional factors (Soetan and Oyewole, 2009). Therefore, this study was aimed at evaluating the chemical composition, amino acid profile and phytochemicals of raw and differently processed sorrel seeds meal.

Materials and Methods

This study was carried out at the Central Laboratory, Animal Science, University of Ibadan, Ibadan, Oyo State, Nigeria.

Sources and processing of sorrel seeds

The sorrel seeds were purchased from Gajigana Farm, Borno State during the harvesting period. The debris in the raw sorrel seeds were carefully removed and the processing techniques adopted were according to (Ari *et al*., 2012). Sorrel seeds were poured into boiled water (100°C) for 30 min at the rate of 500g/L, sieved, sundried, milled and tagged as boiled sorrel seed meal (BSSM). Sorrel seeds were soaked in water for 12hr (Duwa *et al*., 2012) sieved, sundried, milled and tagged as soaked sorrel seed meal (SSSM). fermented sorrel seed meal, (FSSM) was done by pouring the sorrel seed into boiled water (100°C) for 30 min, sieved and covered in an airtight covered bucket for three days (Kwari *et al*., 2011). All the processed sorrel samples were sundried, tagged and chemically analyzed appropriately.

Chemical Analysis

Determination of proximate analysis

Proximate analysis of the raw and processed sorrel seeds were determined using the method of AOAC (2010). Metabolizable energy (ME) was calculated from the proximate composition data using the formular of Pauzenga (1985):

ME(Kcal/kg) = 37+%CP+81.1+%EE+35.5+%NFE

Phytochemical analysis

Raw and processed sorrel seeds were taken to the Biochemical Laboratory for phytochemical analysis, for qualitative or quantitative analysis.

Determination of Phytochemicals

The method used for phytates analysis was as described by (Lopez-Moreno *et al*.,2022), saponins (Akinmutimi, 2001), oxalates (Liu,2004); Petroski and Minich, 2020), flavonoids, tannins and alkaloids (Sofowora, 2008), trypsin inhibitor (Salas *et al*., 2018), phenols (Kalla *et al*., 2015.

Determination of amino acid profile

Amino acids profile was done as described by Fanar *et al.* (2021)

Statistical analysis

Proximate, amino acid profile, qualitative and quantitative data were subjected to analysis of variance (ANOVA) using SAS (2003). Means were separated using Duncan Multiple Range Test.

Results and Discussion

Chemical compositions of raw and processed sorrel seeds

The result of the chemical composition of raw and processed sorrel seeds was presented in Table 1. There were no significant differences in crude protein content of boiled and soaked sorrel seed meal (26.21 and 26.34%), respectively, the values were similar to the value recorded for fermented sorrel seed meal (23.84 %),but lower compared to the raw sorrel seed meal (22.06%). It shows that processing methods has proved to be effective by improved the crude protein level of the seed, the higher crude protein content observed in the processed sorrel seeds could possibly be due to the modification effect of the processing methods that leads to crude protein improvement (Soetan and Oyewole, 2009). Similarly, the crude fibre levels of raw sorrel seeds (7.77%) did not differ significantly (P<0.05) with processed sorrel seeds (BSSM and SSSM) (7.64% and 7.30%, respectively), except in fermented sorrel seed meal (FSSM) (4.21%).The presence of an adequate level of fibre in the processed sorrel seed reveals that the seed can be utilized as a better source of fibre for animals(Aliyu, 2020). The ether extract levels of raw sorrel seeds (16.23%) did not differ significantly to processed sorrel seed, boiled, soaked and fermented, 12.37%, 13.47% and 11.60%, respectively. The ash contents recorded in this study, did not differ significantly across the groups. However, the nitrogen free extract recorded in this study did not differ significantly among the processed sorrel seed meal. The result obtained was in line with report of (Aliyu, *et al*.,2020; Abu El Gasim *et al*., 2008) who observed that the boiling, soaking or sprouting of sorrel seeds is accompanied by a significant increase in protein, fat and crude fibre content.

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Table 1: Proximate composition of raw and processed sorrel seeds

Parameters (%)	RSSM	BSSM	SSSM	FSSM	SEM	P-value
Dry matter	91.97^{c}	94.29^{a}	93.09^{b}	95.02^{a}	0.38	0.001
Crude protein	22.06^{b}	26.21^{a}	26.34^{a}	23.84^{ab}	0.68	0.045
Crude fibre	7.77^{a}	7.64^{a}	7.30^{a}	4.21^{b}	0.50	0.005
Ether extract	36.23^{a}	12.37^{b}	13.47^{b}	11.6^{b}	2.91	0.014
Ash	4.67	5.67	4.67	4.00	0.43	0.652
Nitrogen free extract	29.27^{b}	51.14^{a}	48.23^{a}	56.36^{a}	2.97	0.008

^{a,b,c} = Means with different superscripts on the same row significantly differs (P<0.05) RSSM: Raw Sorrel Seed Meal; BSSM: Boiled Sorrel Seed Meal; SSSM: Soaked Sorrel Seed Meal; FSSM: Fermented Sorrel Seed Meal

Amino acids profile of raw and processed sorrel seed

The result of amino acid profil of raw and processed sorrel seed meal is presented in Table 2.

It was observed that processing methods improved amino acid profile of raw sorrel seeds. The sulphur amino acids (methionine, cysteine and threonine) increased and decreased in the lysine contents of the soaked compared to the raw seed, this was in line with the report of (Abu El Gasim *et al*., 2008) who reported that the seed was soaked in sodium azide solution for 12hr,despite the medium were differ, the same duration was exploit, and this could be the reason for the uniquiness of the results. Which was in contrast with the report of (Kwari *et al*., 2011) who observed that soaking reduced the methionine but had no effect on lysine contents of sorrel seed. The results obtained in this study might be due to the duration of soaking of sorrel seeds. In the present study, the sorrel seeds were soaked for 12hr while (Kwari *et al*., 2011) soaked for 24hr in water.

Table 2: Amino acid profile (%) of raw and differently processed sorrel seeds

	Raw SS	Boiled SS	Soaked SS	Fermented SS	SEM	P-value
Essential Amino Acids						
Histidine	4.25^{c}	4.39^{b}	4.54^{a}	4.55^{a}	0.00	0.000
Isoleucine	6.33^{b}	6.54^{a}	6.69^{a}	6.62^{a}	0.01	0.011
Leucine	8.04	8.15	8.31	8.22	0.01	0.083
Lysine	7.08^{a}	7.14^{a}	2.31^{b}	2.35^{b}	0.00	0.000
Methionine	2.12^{c}	2.24^{b}	2.42^{a}	2.43^{a}	0.01	0.000
Phenyalalanine	6.23^{c}	6.29^{b}	6.45^{a}	6.42^{a}	0.00	0.000
Threonine	4.08^{d}	7.10^{c}	7.23^{b}	7.31^{a}	0.00	0.000
Tryptophan	2.21^{c}	2.25^{b}	2.42^{a}	2.42^{a}	0.00	0.000
Valine	6.11^{d}	6.18^{c}	6.47^{a}	6.44^{b}	0.00	0.000
Non-essential Amino Acids						
Arginine	5.89^{c}	6.13^{b}	6 .26^{a}	6.33^{a}	0.01	0.000
Alanine	5.09^{c}	5.16^{b}	5.30^{a}	5.34^{a}	0.00	0.000
Aspartic acid	8.12	8.04	8.19	8.12	0.01	0.249
Cysteine	2.19	2.25	2.23	2.36	0.01	0.249
Glutamine	7.79^{c}	7.83^{c}	7.90^{b}	8.03^{a}	0.00	0.000
Glycine	5.19	5.27	5.38	5.41	0.01	0.053
Proline	6.15^{b}	6.15^{b}	6.50^{a}	6.52^{a}	0.01	0.000
Serine	4.03^{c}	4.09^{b}	4.39^{a}	4.34^{a}	0.00	0.000
Tyrosine	3.37^{b}	3.44^{b}	3.65^{a}	3.59^{a}	0.00	0.000

$^{a, b, c}$ = Means with different superscripts on the same row significantly differs ($P<0.05$).

SS = sorrel seed; SEM = standard error of mean

Phytochemical factors of sorrel seeds

The results of qualitative and quantitative analysis of raw and processed sorrel seed is presented in tables 3 and 4. Despite the rich nutritional composition of sorrel seed, there were reports of the presence of anti-nutritional factors (Aliyu *et al*., 2020). The results recorded in qualitative analysis of this study revealed that processing methods did not changed the presence of phytates and cyanides in sorrel seeds. However, saponins and flavonoids were absent when boiled, soaked and fermented quote reference. Phenols and oxalates also reduced when soaked and fermented, while alkaloids absent when boiled and fermented, however, tannins did not appear at all in both qualitative and quantitative analysis, which was in line with the report of Okereke *et al*. (2015) who reported that *Hibiscus sabdariffa* was negative for tannins. In quantitative analysis, it was observed that, processing methods has proved to be effective in alkaloids and cyanides when boiled, soaked and fermented. Alkaloids reduced from 7.06 g/100g to 5.62, 5.97 and 2.92 g/100g respectively while cyanides also reduced from 19.31 mg/L to 7.49, 17.82 and 13.99 mg/L, respectively. Reduction was also observed in oxalates when soaked from 16.37mg/g to 10.57mg/g. This could be as a result of leaching, where by some oxalates might have leached in to the soaking water, which cause the reduction. The results recorded in this study were in line with the finding of (Aliyu *et al*., 2020) who reported that the decrease in the levels of these anti-nutrients indicates their non -appearancee to a minimum level and rendering the diet appetizing for animals to consume. The percentage reduction in alkaloids, saponins, oxalates and cyanides observed in sorrel seeds subjected to boiling, soaking and fermented, agreed with the report of Yagoub and Abdullah (2007) and Udensi *et al.* (2008) who observed that processing of leguminous seeds either by cooking, soaking, autoclaving, roasting, or fermentation significantly improved the nutritional and functional properties of legume seeds. Akinmutimi (2004) had also observed that most processing methods employed in improving the food value of non-conventional feedstuffs do not completely eliminate anti-nutritional factor substances but only reduce their concentration to tolerable levels in the feedstuff.

Table 3: Qualitative analysis of phytochemicals in sorrel seeds

Phytochemicals	Raw	Boiled	Soaked	Fermented
Alkaloids	++	+	+++	+
Saponins	+	-	-	-
Tannins	-	-	-	-
Flavonoids	+	-	-	-
Phenols	+	++	+	+
Trypsin inhibitors	+++	_	+++	+
Cyanides	++	++	++	++
Oxalates	++	++	+	+
Phytates	+++	+++	+++	+++

+++highly present ++moderately present + slightly present -absent

Table 4: Quantitative analysis of phytochemicals in sorrel seeds

Phytochemical	Raw SS	Boiled SS	Soaked SS	Fermented SS	SEM	P-value
Alkaloids (g/100g)	7.06^{a}	5.62^{b}	5.97^{ab}	2.92^{c}	0.48	0.000
Saponins (g/100g)	1.03	0.53	1.13	1.00	0.14	0.456
Oxalates (mg/g)	16.37^{b}	20.00^{a}	10.57^{c}	16.50^{b}	1.04	0.000
Cyanides (mg/L)	19.31^{a}	7.49^{c}	17.82^{a}	13.99^{b}	1.40	0.000

$^{a, b, c}$ means with different superscripts on the same row significantly differs (P<0.05)

SS = Sorrel seed

Conclusion

It is concluded that processing methods (boiled,soaked and fermented) explored in this study enhanced nutrients availability such as crude protein, crude fibre and ether extract and also improved the amino acids profile and reduced the anti-nutrients which resulted to the improvement of the nutritional and functional properties of the seeds. The processing methods also significantly improved the amino acids profile and reduced the anti-nutrients which resulted to the improvement of the nutritional and functional properties of the seeds.

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Date received: 16th January, 2025

Date accepted: 14th April, 2025