Effects of Varying Fermentation Period on the Chemical Properties of Tropical Sikcle Pod (*Senna obtusifolia*) Seed Meal

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Abstract: A study was conducted to evaluate the proximate composition, amino acid profile and levels of anti-nutritional factors of *Senna obtusifolia* seeds subjected to varying fermentation periods. The seeds of *Senna obtusifolia* were naturally fermented for 0, 3, 5, 7 and 9 days, respectively. Each representative sample was analysed in triplicates in a complete randomized design for dry matter, crude protein, crude fibre, ether extract, nitrogen free extract, amino acids and levels of anti-nutritional factors using standard laboratory procedure. The results indicated an increasing trend as the fermentation period progresses for the protein (26.95 to 28.29%), ash (4.50 to 5.31%) and some amino acid content. Lysine and methionine increased from 1.19 to 2.97g/100g and 2.20 to 2.88g /100g. As the fermentation period progressed, the crude fibre, nitrogen free extract, ether extract and anti-nutritional factors were observed to decrease. Crude fibre decreased from 11.17 to 4.23%, NFE 41.85 to 37.31% and ether extract 3.65 to 1.98%. Similarly, Tannins and oxalates decreased from 5.42 to 1.17 g/100 g and 1.95 to 0.36 g/100 g, respectively. It can be concluded that fermenting *Senna obtusifolia* seeds for up to 9 days has significantly reduced the levels of ant-nutritional factors and improved the protein and amino acid profile of *Senna obtusifolia* with less depreciation in some of the proximate components. There is need to conduct further studies to investigate the effect of fermenting *Senna obtusifolia* seeds beyond nine (9) days. Furthermore, studies should be conducted using fermented *Senna obtusifolia* seed meal in a feeding trial with a view to evaluating its feeding value.
[Augustine, C., Waba, Y.E., Abdullahi I.A and Nyalas, B.P. **Effects of Varying Fermentation Period on the Chemical Properties of Tropical Sikcle Pod (*Senna obtusifolia*) Seed Meal.** *Academ Arena* 2017;9(12):52-56]. ISSN 1553-992X (print); ISSN 2158-771X (online). http://www.sciencepub.net/academia. 9. doi:10.7537/marsaaj091217.09.

Keywords: Sickle pod, chemical properties, varying fermentation period.

Introduction

The utilization of lesser-known legumes will go a long way in addressing the feed crises that has engulfed the Nigerian livestock industry. Adegbenro *et al*. (2011) further buttressed the need to exploit some under-utilized seeds which could argument the costly conventional feed resources. One of such under-exploited legumes is *Senna obtusifolia* seeds**.** *Senna obtusifolia* is a pantropical weed that belongs to the family *leguminosae caesapinioideae*. It is an erect bushy annual shrub that grows up to 90 cm tall and propagates through seed. The leaves are obovate and the flowers are yellow in colour (Akobundun and Agyakwa, 1998). Most seeds of legumes contain toxic components which may limit their utilization (Parul, 2014; Adebowale and Maliki, 2011). The chemical composition of the seeds as revealed by Ingweye *et al*. (2010) and Ismaila *et al*. (2011) indicated that they have good nutritional properties (29.54 and 18.46% crude protein) but also contained some anti-nutritional factors (tannins 388.50 mg/100g, phytates 240.50 mg/100g and oxalate 83.25 mg/100g) which may adversely affect the performance of animals that may consume the seed meal. Processing treatments have been known to reduce anti-nutritional factors and improve nutrient utilization (Effiong *et al*., 2011; Tuleun *et al*., 2011; Emiola, 2013). Fermentation has been reported to modify some physical characteristics of cereals and legumes, increase the level of some nutrients, digestibility and bioavailability of nutrients (Brown *et al*., 1998), decrease levels of anti-nutrients, increase nutrient density (Tomkins *et al*., 1987; Nnam, 1998), and imparts some antimicrobial properties (Mensah *et al*., 1990; Mensah *et al*., 1991). Fermentation holds promise as a food processing method that can be used to diversify the food uses of some under-exploited plant foods (Anthony and Babatunde, 2014). Fermentation period has been documented to influence the chemical properties of seeds (Yashim *et al*., 2009 Adebowale and Maliki, 2011). At the moment, base-line information on the effects of varying fermentation periods on the proximate composition, amino acid profile and levels of anti-nutritional factors of *Senna obtusifolia* seeds seems to be meager. Therefore, more studies are needed to bridge such information gap. It was in of view of the above that this study was conducted to investigate the effects of varying fermentation periods on the chemical properties of *Senna obtusifolia* seeds.

Materials and methods

Seed collection and processing

The seeds were harvested from bushes around Mubi area of Adamawa State, Nigeria. The plant and seeds were authenticated at the Department of Biological Sciences, Adamawa State University, Mubi, Nigeria. The seeds were boiled, washed, drained and placed in an air tight container and allowed to naturally ferment for 3, 5, 7, and 9 days, respectively. At the end of each respective fermentation period, each representative sample was removed from the fermenting container and properly sun-dried, ground to meal and used for the chemical analysis.

Chemical analysis

The proximate composition of the seed meal and levels of the anti-nutritional factors were determined in triplicates in a complete randomized design using the standard procedure of Official Association of Analytical Chemists [AOAC] (2004). The dry matter content was determined using the oven-dry method and crude protein was determined using the Kjeldahl procedure. Soxhlet extraction method was used for the determination of ether extract, while the fibre content was evaluated using the trichloroacetic method. The ashing procedure was used to determine the ash content, while nitrogen free extract (NFE) was computed using the formula:

NFE = 100 – (% Moisture + CP + CF +EE +ASH)

Where:

CP = Crude Protein

CF = Crude Fibre

EE = Ether Extract

Similarly, the energy values were computed using the formula of Pauzenga (1985) which is expressed as ME= 37 × %CP + 81 × % EE + 35.5 × % NFE. The energy values obtained in Kcal/kg were converted to mega joules per kilogram (MJ/kg). The amino acid profile was analyzed using High Performance Liquid Chromatograph (HPLC) Buck Scientific BLC 10/11 model equipment.

Data analysis

Data obtained were subjected to Analysis of Variance (ANOVA) of the completely randomized design (CRD). Duncan Multiple Range Test (DMRT) was used to separate the treatment means where significant difference occurred. The results were expressed as means with their standard error of the means of triplicate determinants.

Results and discussion

The proximate composition of *Senna obtusifolia* seeds subjected to varying fermentation periods is presented in Table 1. The proximate composition were significantly ($P<0.05$) affected by the different treatments. The crude protein was observed to increase with progressive increase in fermentation period. Fermentation period on the 9th day recorded the highest crude protein content. The crude protein content was observed to increase from 26.95 to 28.79%. An increase in the protein content after fermentation was attributted to the activities and increase number of lactic acid bacteria present during fermentation. The increase in protein contents agreed with the finding of Afoakwa *et al*. (2004). This finding is also in line with the report of Adebowale and Maliki (2011) who obtained similar results for pigeon pea (*Cajanus cajan*) seed flour. They attributed such increase in protein content to net synthesis of protein by fermenting seeds which might have resulted in the production of some amino acids during protein synthesis (Uwagbute *et al*., 2000). Some Researchers (El Hag *et al*., 2002; Ali *et al*., 2003) reported that fermentation can be effectively used to improve the nutritional quality of cereal grains by increasing protein content and digestibility which is in line with the findings of this study.

The ash content was observed to significantly ($P<0.05$) increase with increase in the fermentation period. The ash content increase from 4.50 to 5.31% from day 0 to 9. This result concurred with the findings of Adebowale and Maliki (2011) and Anthony and Babatunde (2014). The latter explained that the increase in the ash content after fermentation could be due to the incomplete utilization of minerals by the fermenting organisms during their metabolism.

The ether extract and nitrogen free extract (NFE) significantly ($P<0.05$) decreased as the fermentation period advances. The decrease in the ether extract was due to the breakdown of fatty acids and glycerol by lipolitic organisms during fermentation (Anthony and Babatunde, 2014). The NFE decreased from 41.85 to 37.31% with fermentation at the 9th day, recording the lowest value of 40.18%. This was due to the utilization of sugars in the seeds by the fermenting microbial mass which is in line with the report of Anthony and Babatunde (2014).

The crude fibre similarly indicated a decreasing trend with progressive increase in the fermentation period. Fermentation at the 9th day recorded the least crude fibre content of 4.23% which is in agreement with the findings of Anthony and Babatunde (2014) who attributed such decrease to the enzymatic breakdown of fibre by the fermenting microbes which utilized them as carbon source and convert them to microbial biomass thereby reducing the fibre content (Rainbault, 2001).

Table 1: Proximate Composition of *Senna obtusifolia* Seeds Subjected to Varying Fermentation Period

Fermentation period (days)						
Nutrients (%)	**(T1)**	**T2(3)**	**T3(5)**	**T4(7)**	**T5(9)**	**SEM**
Dry matter	90.72	90.33	90.27	90.18	90.23	21.43^{NS}
Crude protein	26.95^{c}	26.49^{c}	27.34^{b}	27.19^{ab}	28.79^{a}	3.74*
Crude fibre	11.17^{a}	8.64^{b}	8.51^{b}	6.41^{c}	4.23^{d}	1.66*
Ether extract	3.65^{a}	3.42^{b}	3.39^{b}	2.52^{c}	1.98^{d}	0.32*
Ash	4.50^{b}	4.54^{b}	5.01^{a}	5.11^{a}	5.31^{a}	0.44*
NFE	41.85^{a}	40.99^{a}	40.92^{a}	39.89^{b}	37.31^{c}	5.94*
*Energy (MJ/Kg)	10.40	10.20	10.32	10.30	10.01	3.15^{NS}

a, b, c, d = Means in the same row with different superscripts are significantly different (P<0.05) * = Significant at 5% level of probability, SEM = standard error of mean.

The amino acid profile (Table 2) also indicated a significant (P<0.05) increasing trend as the fermentation period progresses. Fermentation was reported to cause biological enrichment of food substrate with protein, essential amino acids and vitamins (Skeinkraus, 1998) which is in agreement with the finding of this study. The highest lysine (2.97g/100g) and methionine (2.88g/100g) contents were recorded in the seeds fermented for 9 days. Net synthesis of protein by fermenting seeds resulted in the production of some amino acids during the protein synthesis (Marero *et al*. 1989; Uwagbute *et al*., 2000).

Table 2: Amino acid Profile of *Senna obtusifolia* Seeds Subjected to Varying Fermentation Periods (g/100g)

Fermentation period (days)						
Amino acids	**T1(0)**	**T2(3)**	**T3(5)**	**T4(7)**	**T5(9)**	**SEM**
Lysine	1.19^{c}	1.80^{c}	1.94^{c}	2.37^{ab}	2.97^{a}	0.31*
Methionine	2.20^{c}	2.31^{c}	2.37^{c}	2.48^{ab}	2.88^{a}	0.44*
Threonine	2.25^{c}	2.34^{c}	2.45^{b}	2.69^{ab}	2.78^{a}	0.10*
Isoleusine	2.32^{b}	2.23^{b}	2.44^{b}	2.43^{b}	2.96^{a}	0.61*
Leucine	3.59^{c}	3.77^{b}	3.78^{b}	3.92^{a}	4.01^{a}	0.12*
Alanine	0.97^{e}	1.15^{c}	1.22^{b}	1.38^{a}	1.39^{a}	0.63*
Phenylalanine	1.82^{d}	2.22^{c}	2.52^{b}	2.52^{b}	2.68^{a}	0.32*
Valine	1.63^{c}	2.34^{b}	2.39^{b}	2.45^{a}	2.65^{a}	0.71*
Arginine	1.19^{d}	1.17^{d}	1.49^{c}	2.16^{b}	2.59^{a}	0.13*
Glutamic acid	0.94^{e}	1.35^{d}	1.43^{c}	1.61^{b}	1.73^{a}	0.32*
Proline	2.15^{c}	2.44^{b}	2.53^{b}	2.59^{ab}	2.69^{a}	0.46*
Glycine	1.32^{c}	1.44^{c}	1.51^{bc}	1.60^{a}	1.67^{a}	0.92*

a, b, c, d, e = Means in the same row with different superscripts are significantly different (P<0.05) * = Significant at 5% level of probability, SEM = standard error of mean

The levels of anti-nutritional factors (Table 3) showed a decreasing pattern as the fermentation period increases. Tannins and oxalates decreased from 5.42 to 2.02% and 1.95 to 0.28%, respectively. Anthony and Babatunde (2014) made similar observation for soya bean (*Glycine max*) flour; Magdi (2011) for pearl millet; Lasekan and Shabnam (2013) for Rambutan (*Nephelium lappaceum*). This reduction may be due to some enzymatic reaction in addition, microorganisms' breakdown the carbon and nitrogen sources and use them for production of energy and their activity during fermentation (Hemingway, 1988). The decrease in tannin has been associated to microbial activity during fermentation (Dhankher and Chauhan, 1987; Ikemefuna *et al*., 1991; Elhag *et al*., 2002) or to abstraction of hydride ions and rearrangement of the phenolic structures due to the acidic environment caused by the fermenting microbes (Towo *et al*., 2006). The decrease in oxalate content was linked to reasons reported by Simpson *et al*. (2009) that a reduced pH caused by microbes during fermentation, can change insoluble oxalate bound ions to soluble oxalate content which will be used as energy source by oxalotrophic bacteria. The phytates level was similarly observed to decrease as the fermentation period progresses with fermentation period at the 9th day indicating the lowest level of phytate. This reduction might be due to low pH caused by fermenting microbes which has enhanced phytase activity resulting to lowering of the phytate content. The reduction in pH of fermented foods caused by the production of various organic acids such as lactic acid and acetic acid favours the activity of the enzymes phytase which is able to dephosphorylate phytate effectively. (Marfo *et al*., 1990; Sanberg and Andlid

2002; Reale *et al*., 2007; Ab-deland *et al*., 2011). This might be responsible for the reduction of the phytates content of *Senna obtusifolia* seed meal.

Table 3: Levels of Ant-nutritional Factors of *Senna obtusifolia* Seeds Subjected to Varying Fermentation Periods

Fermentation period (days)						
Anti-nutrients (100g/100g)	**T1(0)**	**T2(3)**	**T3(5)**	**T4(7)**	**T5(9)**	**SEM**
Oxalates	1.95[a]	1.28[b]	1.01[b]	0.36[c]	0.28[c]	0.06*
Tannins	5.42[a]	3.21[b]	2.73[b]	2.02[c]	1.17[d]	0.07*
Flavonoids	3.86[a]	3.13[b]	1.28[c]	0.30[d]	0.01[e]	0.05*
Phytates	4.61[a]	3.41[b]	2.52[bc]	1.71[d]	0.25[e]	0.01*
Saponins	2.37[a]	1.78[b]	1.90[b]	1.94[b]	0.35[c]	0.10*

a, b, c, d, e = Means in the same row with different superscripts are significantly different (P<0.05) * = Significant at 5% level of probability, SEM = standard error of mean.

Conclusion

In conclusion, fermenting *Senna obtusifolia* seeds for up to 9 days has effectively detoxify and improved the protein and amino acid content of the seeds with little depreciation in some of the proximate composition. There is need to conduct further studies to investigate the effect of fermenting *Senna obtusifolia* seeds beyond nine (9) days. The fermented seeds should be used in a feeding trial with a view to evaluating its feeding value.

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12/25/2017