Nig J. Biotech. Vol. 21 (2010) 12 – 17
ISSN: 0189 17131
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Effects of Natural Fermentation on the Nutrient Composition of a Mixed Substrate of Spent Sorghum Grain and Sweet Potato Leaves

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Abstract
A 1:1 mixture of spent sorghum grain and sweet potato leaves was fermented naturally for a period of five weeks at room temperature with a view to determining the effects of fermentation on its nutrient composition. Fermentation brought about an increase in the Crude Protein, Total Lipid and Nitrogen Free Extract contents by 25.43%, 81.29% and 24.57% respectively. The Crude Fibre content of the mixed substrate dropped significantly ($P<0.05$) while Calcium and phosphorous values were found to increase. A fermentation period of three weeks was observed to be optimum for enhancement of the protein content of the substrate. Two amino acids including cystine and glutamic acid were found to increase while histidine, arginine, aspartic acid, threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine were found to be decreased.

Key words: Spent grain, Sweet potato leaves, Nutrient composition, Fermentation

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Introduction

Feeds usually contain a high proportion of cereal grains and legumes which happen to be part of the staple food of Nigerians. The availability of these feed ingredients is not adequate to meet the demands of a rapidly growing human population. With the greater proportion of these farm products being directed towards meeting human nutritional needs, and the needs of other industrial sectors, little is left for animal feed production. This competition often pushes the price of finished feeds upwards (Iyayi and Aderolu, 2004). It has become necessary to search for alternative cheaper sources of feed ingredients as continued dependence on conventional sources such as maize, wheat offal, fish meal and groundnut cake may not be the solution to the problems facing the Nigerian livestock industry. Agro-industrial by-products and agricultural residues are in abundance in Nigeria (Iyayi, 2004). These have been major sources of alternative feed ingredients. One of such by-products is brewer's grain, a by-product from the brewing industry. The brewing industry is among the leaders in the Nigerian industrial sector and possibly the largest consumer of sorghum grains in the country. Every year thousands of tons of spent grain is generated as a by-product of the brewing of beer. In the past the disposal of this waste was a significant part of costs for the breweries. Today, however, the by-product is sold by the breweries to local animal farmers due to the discovery of the feeding value of brewery spent grain for livestock animals.

Sweet Potato (*Ipomoea batatas),* (L.) Lam is a dicotylodonous plant belonging to the morning glory family (Convolvulaceae). The plant is cultivated in Jos and other Northern parts of Nigeria mainly for its tubers. After harvesting the tubers, large quantities of leafy vines are left behind. The plant produces a large amount of biomass with foliage production at 4.3 – 6.0 DM/ha/crop (Ruiz *et al*., 1980). According to Ishida *et al*., (2000), the leaves are of high nutritive value.

In spite of the nutritional advantages of sweet potato leaves and brewers spent grain, they are high in crude fibre content.

The high fibre content of agricultural residues and by-products limit their value in the feeding of monogastric animals. Even for the feeding of ruminants, they have to be processed to enhance voluntary intake and to maximize the utilization of available nutrients (Fetuga and Tewe, 1980). This paper investigates the possibility of enhancing the nutrient composition of a 1:1mixture of brewers spent sorghum grain and sweet potato leaves through natural fermentation, so as to determine its potential for use as an animal feed or feed supplement.

Materials and Methods

Fermentation Procedure: A 1:1 mixture of spent sorghum grain and sweet potato leaves (MS) were fermented naturally. No special sterility measures were taken as effort was being made to simulate the environmental conditions obtainable in food fermentations by local people. A mixture of 10g of spent sorghum grain and 10 g of ground sweet potato leaves was introduced into each of twelve 250ml conical flasks. Fifty millilitres of clean tap water (just enough water to appropriately wet the substrates but not to submerge them) was added. The contents were thoroughly mixed using a spatula. The mouths of the flasks were plugged with rubber bungs containing two glass tubes to allow the movement of air into and out of the fermentation flasks. The outer ends of the tubes were plugged with cotton wool to prevent the possible loss of spores from the fermenting substrates. The flasks were divided into four sets of three flasks each. The first set of three flasks were labelled 'week three', the second set, 'week 4' and the third set, 'week 5'. The last set of three flasks served as the controls. The Control flasks were prepared in the same way as the other flasks except that they were sterilized by autoclaving at 121^{o}C for 15 minutes. The fermentation was carried out at 25^{o}C over a period of five weeks Weekly pH values and temperature readings were determined during the fermentation period. At the end of each specific period of fermentation, the flasks for that period were disconnected and their contents were dried to constant weight in a hot air oven at a temperature of 60^{o}C. The dried fermented substrates were milled, stored in clean dry bottles and were later subjected to proximate and amino acid analyses.

Proximate Analysis: The samples were analyzed for their Crude Fibre (CF), Crude Protein (CP), Ash, Total Lipids (TL), Nitrogen Free Extract (NFE), Calcium (Ca) and Phosphorous (P) contents using the method of Association of Official and Analytical Chemists (AOAC, 1980).

Amino Acid Analysis: The amino acid compositions of non-fermented and 3-week fermented grain leaf samples were determined using the method of Spackman *et al*. (1958). This included the defatting of the samples; hydrolysis of the defatted samples under nitrogen; and the amino acid of the hydrolysate using a Technicon Sequential Multisample (TSM) Amino Acid Analyzer.

Statistical Analysis: One-way ANOVA was used to determine significant changes in the crude protein level. When the F test was significant, LSD test was used to compare means.

Results and Discussion

The result of the proximate analysis is presented in Table 1. Crude protein value increased from 24.62 to 29.38 by the third week of fermentation, and afterwards rose non-significantly. Total percentage increase was 25.43%. Apart from a slight drop in week four, total lipid (TL) rose as a result of fermentation with an overall increase of 81.29%. Ash, calcium and phosphorous levels increased all through the fermentation period. While ash recorded an increase of 52.3%, calcium recorded an overall increase of 114%. The value of phosphorous increased by 195% within the period of degradation. A total decline of 72.16% was observed in the crude fibre level. There was profuse utilization up to the end of 'week four and a stabilization at week five. Nitrogen free extract (NFE) continued to rise until week five where it dropped slightly. Interestingly this rise in the NFE coincided with the period of major drop in the crude fibre.

The pH of the grain-leaf substrate continued to drop through the first three weeks of fermentation and then started to rise in the 4th week (Table 3).

The temperature of the fermenting substrate rose over the ambient temperature during the first and second weeks of fermentation, with the highest rise being recorded at the end of 'week 2' (Table 3).

Fermentation of the mixed substrates caused a reduction of the lysine, histidine, arginine, aspartic acid, threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine contents. The cystine and glutamic acid content were slightly increased (Table 2).

The results obtained showed that natural fermentation brought about changes in the nutrient composition of the 1:1 mixture of spent grain and sweet potato leaves (SGSPL) substrate. Since significant reduction ($P<0.05$) in the crude fibre level continued up to week 4, it can be deduced that a period of four weeks is optimum time for crude fibre degradation. Crude fibre is basically cell walls consisting majorly of cellulose and lignin. The cellulose component has been put at between 22-25 percent in young plants and 30-40 percent in mature plants (Bodgan, 1977). The reduction observed in the crude fibre content was due to the action of cellulolytic microorganisms present in the fermenting substrates. These microorganisms bring about the breakdown of the cellulose component to utilizable sugars. This increases the energy value of the substrate increasing its potential for use in feeding animals, especially monogastrics that usually are not able to break down cellulose. Different groups of microorganisms may have contributed to the crude fibre reduction but the fungi are probably the favourites considering that this fibre-degrading ability is more widespread among them (Ofuya and Nwajiuba, 1990; Iyayi and Losel, 2001). Successful degradation of cassava peel (a fibrous by-product of cassava processing) by *Rhizopus* sp has been achieved by Ofuya and Nwajiuba (1990). They reported that over 35.00% of the original cellulose content of the substrate was lost during solid state fermentaion. *Aspergillus niger* grown on rye grass straw yielded similar results (Han, 1978). The results of Ogbonna and Popoola (1997) working with maize straw also corroborate this finding.

The Nitrogen Free Extract (NFE) content rose in the fermenting sample with the highest percentage rise being during the first three weeks of fermentation. This increase in NFE coincided with the period of profuse fibre utilization. Higher NFE values indicate higher levels of soluble or near soluble carbohydrates such as sugars resulting from the degradation of cellulose. The drop in the NFE values in Week 5 was due to the assimilation of the breakdown products by the microorganism especially at a time when there was no further degradation of fibre.

The crude protein content of the SGSPL sample rose significantly ($P<0.05$) as a result of fermentation. A non significant increase in crude protein level was recorded after Week 3, showing that a three-week fermentation period is optimum for the enhancement of the crude protein content of the experimental substrate. This increase in the crude protein content of the fermented SGSPL was as a result of protein synthesis during the fermentation process. Different workers have attempted to elevate the total protein content of biomass through solid state fermentation (Rodriguez *et al*., 1985; Iyayi and Losel, 2001; Iyayi, 2004; Iyayi and Aderolu, 2004). . The crude protein increase of 25.43% obtained from the natural fermentation of the 1:1 mixture of sweet potato leaves and spent grain was not as high as the figures obtained (leaves - 27.18% and grain - 34.80%) when the leaf and grain samples were separately fermented (Onyimba et al., 2007, 2009). Considering the significant increase in the protein content of fermented SGSPL, a fermented combination of sweet potato leaves and spent sorghum grain should prove useful as a cheap alternative source of protein for monogastrics and ruminant especially where both spent grain and sweet potato leaves are in short supply. The Total Lipid (TL) content of SGSPL was highly built

up especially within the first three weeks of fermentation. This increase in the lipid content means additional calories for animals using the fermented substrate as feed or feed supplements.

Ash values for SGSPL rose all through the fermentation period. Calcium content of the substrate was built up. Phosphorous content was also found to increase. The increase observed in the mineral content is desirable as it can help in meeting the mineral requirement of specific animal species.

Weekly measurements of the pH of the fermenting sample showed a progressive decrease up to Week 3 and an increase from Week 4. As the sugars in the substrate are being fermented, organic acids accumulate resulting in the lower pH values observed. The subsequent increase in pH observed from Week 4 indicates that less acid is being produced and that the products of protein fermentation which are alkaline are being accumulated in the fermentation medium. The fermentation of free amino acids from protein metabolism causes the release of ammonia resulting in increase in the pH values (Achi, 2005).

The temperature of the fermenting sample was found to increase slightly. This was due to heat being generated as a result of exothermic reactions catalysed by microbial enzymes. This increase in temperature was highest in Week 2 and is probably as a result of higher microbial activity during the second week of fermentation.

Fermentation of the SGSPL resulted in increases in the values of two of the amino acids - cystine and glutamic acid (Table 2). There were, however, drops in the values of a greater number of amino acids including histidine, arginine, aspartic acid, threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine. The increases observed were due to the synthesis of amino acids during the fermentation process (Nester et al., 1973). A number of workers have reported the production of amino acids as a result of fermentation. Tosaka et al. (1983) reported the production of lysine as a result of substrate fermentation with *Candida glutamicum* and *Escherichia coli*. Delgado et al. (1982) also reported the over-production of threonine from substrates inoculated with *E. coli*, *Candida* sp and *Saccharomyces cerrevisiae* respectively. During the fermentation process, some of the free amino acids in the system are utilized in building up the protein sector of the substrates. This probably explains the drop observed in the values of a number of amino acids in the fermented substrate. Another possible reason for the drop in the content of some amino acid is the fermentation of deaminated proteins (amino acid fermentation). This view is supported by Winarno and Reddy (1986) who stated that fermentation of proteins causes an increase in the level of free amino acids but longer fermentations result in losses of lysine or other essential amino acids. A shorter fermentation period may need to be employed as this may avoid losses of amino acids thereby ensuring a better amino acid profile.

Table 1 The Effects of Natural Fermentation on the Chemical Composition of the Mixture of Spent Sorghum Grain and Sweet Potato Leaves (% dry matter)

Time(wks)	Moisture	CP	CF	TL	Ash	NFE	Ca	P
0	13.38	24.62	28.20	2.94	7.33	36.91	1.04	0.22
3	10.26	29.38	13.81	5.81	8.69	42.31	2.23	0.65
4	11.49	30.86	7.95	5.05	10.16	45.98	2.32	0.66
5	11.23	30.88	7.85	5.33	11.17	44.77	2.31	0.84

wks = weeks CP = Crude Protein CF = Crude Fibre TL = Total Lipid. NFE = Nitrogen Free Extract Ca = Calcium P = Phosphorous

Table 2 The Effects of Natural Fermentation on the Amino Acid Contents of the Mixed Substrates (SGSPL) (g/100g of protein)

Amino Acid	Non-Fermented	Fermented
Lysine	2.95	1.87
Histidine	2.04	1.81
Arginine	3.35	2.52
Aspartic Acid	11.05	7.37
Threonine	2.75	2.13
Serine	3.90	2.00
Glutamic Acid	11.42	11.84
Proline	1.81	1.18
Glycine	1.24	0.97
Alanine	4.35	3.63
Cystine	0.48	0.50
Valine	4.01	2.11
Methionine	1.67	1.26
Isoleucine	5.87	3.25
Leucine	10.30	4.83
Tyrosine	3.45	1.78
Phenylalanine	4.73	2.09

Table 3. The weekly pH and Temperature Changes of the Fermenting Mixed Substrate

Fermentation Time	pH	Ambient Temperature (^oC)	Substrate Temperature (^oC)
0 week	6.7	27.0	27.0
1 week	5.35	27.5	28.0
2 weeks	4.9	28.5	30.5
3 weeks	4.15	28.0	28.5
4 weeks	4.8	27.0	27.0
5 weeks	5.4	27.5	27.8

Conclusion

In conclusion, fermentation brought about a number of desirable changes in the nutrient composition of the mixture of spent sorghum grain and sweet potato leaves. With the reduction of the fibre content and the enhancement of other nutrients like protein, nitrogen free extract, lipid and minerals including calcium and phosphorus, the potential for using the fermented grain-leaf substrate as a feed or feed supplement for livestock is increased. Where shorter fermentation time does not improve the amino acid profile, supplementation with cheap synthetic essential amino acids is recommended. Animal feeding trials with the fermented material would be a further step in determining its actual place as a feed or feed supplement.

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