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Changes in the Nutrient Composition of Brewery Spent Grain Subjected to Solid State Natural Fermentation

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Abstract
Spent sorghum grains were fermented naturally for a period of five weeks at room temperature with a view to determining the effects of fermentation on their nutrient compositions. The chemical analyses of the samples were carried out according to AOAC standard methods (AOAC, 1980). Fermentation brought about an increase in the Crude Protein, Total Lipid and Nitrogen Free Extract contents by 34.80%, 41.44% and 47.81% respectively. The Crude Fibre content of the spent grain dropped to 10.54% while Calcium and phosphorous values were found to increase. A fermentation period of three weeks was observed to be optimum for the significant (P<0.05) enhancement of the protein content of the grains. Amino acids like lysine, leucine and glutamic acid were found to be slightly boosted while histidine, arginine, aspartic acid, threonine, serine, proline, glycine, alanine, cystine, valine, methionine, isoleucine, tyrosine and phenylalanine were found to be depressed.

Key word: Spent sorghum grains, fermentation, Nutrient composition, Brewery spent grain

Introduction

Countries of the world have been in recent times experiencing a rapid growth in population. This is most evident in the developing nations where this increase is rather phenomenal. An increase in population naturally demands an increase in food supply to meet the nutritional demands of this growing population. Despite efforts that have been made to increase food supply in the developing countries food supply has not kept pace with population growth (FAO, 1974).

In Nigeria, there has been a decline in feed supply levels for animal production. Feeds usually contain a high proportion of cereal grains and legumes. The availability of these feed ingredients is not adequate to meet human consumption needs. With the greater proportion of these farm products being directed towards meeting human nutritional needs, little is left for animal feed production. Feed costs are therefore on the high side.

It has become necessary to search for alternative cheaper sources of feed ingredients as continued dependence of 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 are in abundance in Nigeria (Iyayi, 2004). One of such by-products is brewers grain. 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.

Brewers spent grain is a relatively good source of protein and has been used in the feeding of pigs, sheep, poultry and cattle (Stengel, 1993;Westendorf and Wohlt, 2002). The feeding capabilities of brewer's spent grain is, however, limited by its high crude fibre content and low degradability of the crude

protein fraction. This is true, especially in the feeding of monogastrics. This paper investigates the possible enhancement of the nutrient composition brewery spent sorghum grain through solid state natural fermentation.

Materials and methods

Fermentation Procedure: Twenty grams (20g) each of dry spent sorghum grain were introduced into three clean 250ml conical flasks. The contents of each flask was moistened with 50 ml of clean tap water (just enough water to wet the substrate but not to submerge it). A spatula was used in mixing the substrate to ensure adequate moistening. The mouths of the flasks were plugged with rubber bungs fitted with two glass tubes to allow the movement of air into and out of the fermentation flasks. The outer ends of the glass tubes were plugged with cotton wool to prevent the possible loss of spores during the fermentation period. The three flasks were labeled "3 weeks". Similar sets of fermentation flasks were set up to allow weekly monitoring of the fermentation for three to five weeks. The flasks were labeled accordingly. Control flasks were also provided. The control flasks were prepared in the same manner as the other flasks except that the contents of the flasks were sterilized by autoclaving at 121^{o}C for 15 minutes. The fermentation was carried out at ambient temperature (25-28^{o}C) on the laboratory bench. Weekly pH and temperature measurements of the fermenting substrates were recorded. At the end of the fermentation periods, the fermented samples and the controls were dried in an oven at 65 ^{o}C . The dry samples were ground with the aid of a blender and then analyzed for their proximate nutrient and amino acid composition.

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 the non-fermented and the 3-week fermented sweet potato leaves 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

The result of the Proximate analysis is presented in Table 1. The nutrient composition was given as percent of dry matter content. The crude protein (CP) and total lipid (TL) were found to increase as fermentation time increased, with the highest increase being observed during the first three weeks of fermentation. While CP recorded a total increase of 34.80%, TL recorded an overall increase of 41.44%. Nitrogen Free Extract (NFE) rose from 28.97 to 42.82 within the first three weeks of fermentation and stabilized in the fourth and fifth weeks. The total percentage increase in NFE was 47.81%. The ash content had an overall drop of 15.24%. Calcium and Phosphorous values rose and dropped during the fermentation period. At the end of five weeks of fermentation their values had dropped by 7.5% and 18.97% respectively. Crude fibre (CF) recorded an overall drop of 69.18% as a result of fermentation. A profuse utilization was observed within the first three weeks of fermentation corresponding to an average loss of 3.16% per day.

The pH of all the spent grain continued to drop through the first three weeks of fermentation and then started to rise in the 4th and 5th weeks. (Table 3). The temperatures of all 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'. From the third week onward the temperature differences were not as high. The details are shown in Table 3.

The results of the effect of natural fermentation on the amino acid composition of the spent sorghum grain is given in g/100g of protein in Table 2. Fermentation of spent sorghum grain caused a slight boost in the levels of lysine, leucine and glutamic acid. Histidine, arginine, aspartic acid, threonine, serine proline, glycine, alanine, cystine, valine,

methionine, isoleucine, tyrosine and phenylalanine were depressed.

Table 1: The Effects of Fermentation on the Nutrient Composition of the Spent Sorghum Grain (% Dry Matter)

Fermentation Period	Moisture	Crude Protein	Crude Fibre	Total Lipid	Total Ash	Nitrogen Free Extract	Ca	P
0 week	13.45	25.26	31.38	5.14	9.25	28.97	0.40	0.58
3 weeks	11.30	32.51	10.54	7.00	7.13	42.82	0.60	0.65
4 weeks	11.17	33.16	9.73	7.13	7.99	41.99	0.79	0.65
5 weeks	11.30	34.05	9.67	7.27	7.84	41.17	0.37	0.47

Table 2.Amino Acid Composition of the Fermented and Non-Fermented Sweet Potato Leaves (g/100g of protein)

Amino Acid	**Non-Fermented**	**Fermented**
Lysine	5.88	6.52
Histidine	1.45	1.43
Arginine	6.78	4.53
Aspartic Acid	14.43	8.42
Threonine	7.59	3.35
Serine	6.49	5.19
Glutamic Acid	11.48	14.29
Proline	3.93	3.56
Glycine	4.30	2.85
Alanine	6.02	5.39
Cystine	1.24	0.67
Valine	4.30	2.00
Methionine	0.77	0.45
Isoleucine	3.69	4.62
Leucine	10.59	10.77
Tyrosine	4.97	3.21
Phenylalanine	5.90	3.14

Table 3. The weekly pH and Temperature Changes of the Fermenting Spent Sorghum Grain

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

Discussion

Fermentation brought about changes in the nutrient composition of the spent grains. The crude fibre content of the grains had been significantly ($P< 0.05$) reduced by the end of the first three weeks of fermentation. Thereafter, reduction still occurred but non-significantly. A period of three weeks can therefore be said to be the optimum period for crude fibre degradation. The scarcity of literature on the fermentation of spent grains suggests that not much has been done in this area. However, successful degradation of fibrous by- products by microorganisms have been achieved by different workers. Ofuya and Nwajiuba (1990) reported an over 35% loss of cellulose, a major component of crude fibre, during the solid state fermentation of cassava peel with *Rhizopus sp*. *Aspergillus niger* grown on rice straw yielded similar results (Han, 1978). Ogbonna and Popoola (1997) working with maize straw recorded a reduction of 21.49% in crude fibre content. Crude fibre is basically cell walls consisting majorly of cellulose and lignin. The reduction observed in the crude fibre content was due to the action of cellulolytic microorganisms present in the fermenting substrate. These microorganisms bring about the breakdown of the cellulose components to utilizable sugars. The Nitrogen Free Extract (NFE) content rose during the first three weeks of fermentation. This increase in NFE coincided with the period of prolific fibre utilization. Higer NFE values indicate higher levels of soluble carbohydrates such as sugars resulting from the degradation of cellulose. The observed drop in NFE value for 'week 4' was due to the assimilation of breakdown products by the microorganisms especially at a time when there was no further degradation of fibre (Iyayi, 2004). The optimum period of protein content enhancement was three weeks. This is derived from the fact that the observed rise in crude protein was significant ($P<0.05$) only up to the three weeks period of fermentation. After that, the crude protein value rose non-significantly. The increase in the crude protein content of the spent grains was as a result of protein synthesis during the fermentation process. A key reaction links energy metabolism to biosynthesis. Organic acids formed from glucose metabolism reacts with ammonia or the ammonium ion to yield amino acids (Nester et al., 1973). These amino acids are subsequently converted to protein. Different workers have successfully attempted to elevate the total protein content of biomass through solid state fermentation (Reade and Gregory, 1975; Rodriguez et al., 1985; Iyayi and Losel, 2001; Iyayi, 2004; Iyayi and Aderolu, 2004). The 34.80% increase in the crude protein value of the spent grains is comparable to the 31.00% reported by Iyayi (2004). The enhancement of the crude protein contents of the grains makes them potential cheap sources of protein for feeding ruminants and monogastric animals. The increase in the Total Lipid content means more calories for animal using the fermented substrates as feed or feed supplement. Though fermentation reduced the ash content of the grains, the phosphorous and calcium contents of the grains were found to increase. Calcium and phosphorous are important in the diet of animals. These increases would ensure better mineral supply for the production of healthy animals.

Weekly measurements of the pH of the fermenting samples showed a progressive decrease up to Week 3 and an increase from Week 4. As the sugars in the substrates 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 samples were found to increase slightly. This was due to heat being generated as a result of exothermic reactions mediated 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.

The results obtained show that fermentation of the spent grains resulted in increases in the values of some of the amino acids. There were, however, drops in the values of a greater number of amino acids. The increases observed were due to the synthesis of amino acids during the fermentation process. In amino acid biosynthesis, a 5-C intermediate

compound (alpha-ketoglutarate), formed from glucose metabolism reacts with ammonia and is converted to an amino acid which in turn is useful in the formation of other amino acids (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.

In conclusion, fermentation enhanced the nutritive value of spent sorghum grain. With the reduction of the fibre content and the enhancement of other nutrients like protein, NFE, lipid and calcium, the potential for using the spent grain as a feedstuff for livestock is increased. Where shorter fermentation time does not improve the amino acid profile, supplementation with cheap synthetic essential amino acids like methionine should prove useful.

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