



Amino Acid Profile of Biodegraded Brewers Spent Grains (BSG)

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ABSTRACT: The amino acids profiles of biodegraded brewers spent grains (BSG) were determined. The analysis revealed the presence of 17 amino acids including the major amino acids (cysteine, lysine and methionine) required in poultry nutrition. The concentrations of the amino acids however varied with the microbial species used in the degradation process. After 10 days of solid state fermentation at room temperature glutamine, alanine and phenylalanine were detected at very high concentration in *Aspergillus niger* TF-4 degraded BSG. Glutamine, alanine and proline were the major amino acid components of BSG degraded by *Saccharomyces cerevisiae* FP-4, while glutamine, alanine and leucine were found to be the major components of BSG degraded by *Streptomyces* sp UU-2. The concentrations of the required amino acids except lysine were short of the standard requirement for poultry. However their concentrations were positively affected over time by biodegradation. @JASEM

In bioconversion of organic wastes, the protein constituent of the complex substrate is usually broken down into amino acids by the proteolytic activity of the degraders (Singh *et al.*, 1988 and Barimalaa *et al.*, 1994). Farm animals build their body protein primarily from the amino acids absorbed from the digested dietary protein (Sastry and Thomas 1976). In order to synthesize a particular protein all the amino acids required by the animal body must be simultaneously present in sufficient quantities. The body has the capacity to convert surplus of some of the simpler amino acids into certain others. On the other hand, the large animal tissues cannot form certain amino acids

from any other source. The later groups of amino acids are called the essential amino acids (Ranjhan *et al.*, 1974, Sastry and Thomas, 1980). The essential amino acids are necessary for life and must be supplied through feeds in required quantities (Portsmouth, 1978). Our preliminary investigation on the bioconversion of brewers spent grains (BSG) into poultry feed revealed a general increase in dietary protein of BSG degraded by *Aspergillus niger* TE-4, *Saccharomyces cerevisiae* FP-4 and *Streptomyces* sp-UU-2 over time (Essien *et al.*, 2007). This study focuses on the qualitative and quantitative amino acids profile of the biodegraded spent grains.

MATERIALS AND METHODS

Sample Collection: Brewer's spent grains (BSG) samples used in this study were collected from Golden Guinea Brewery PLC. Umuahia, Nigeria. The biodegraders, namely *Aspergillus niger* TE-4, *Saccharomyces cerevisiae* FP - 4 and *Streptomyces* sp. UU-2 were isolated from textile effluent, fresh palmwine and Uyo ultisol, respectively. The isolates were characterized and their identities confirmed according to the procedures described by Samson *et al.*, (1984), Kreger-Van Rif (1984), Buchannan and Gibbons (1974) and Cowan (1985). The fungal isolates were purified and maintained on Sabouraud dextrose agar (Difco) plates, while pure cultures of the bacterium, *Streptomyces* sp. UU-2, was maintained on slightly acidic (pH 5.6) nutrient agar (Difco) plates. None of the biodegraders have been associated with poultry illnesses. Thirty (30) grams sample of the BSG were sterilized by autoclaving at 121°C and then degraded with 5 day old pure cultures of *Aspergillus niger* TF-4, *Saccharomyces cerevisiae* FP-4 and *Streptomyces* sp. UU-2. The modified solid state fermentation technique was adopted. In this procedure 30g of sterile BSG samples were aseptically transferred to sterile 500ml Erlenmeyer flasks, and inoculated separately with 10ml spores/cell (10^6 spores/cells per ml) suspension obtained from 7 day old pure cultures of the test

organisms. The inoculated flasks in replicates of three were allowed to ferment for 10 days at room temperature ($28 \pm 2^\circ\text{C}$). A set of uninoculated flask containing 30g of sterile BSG served as the control. This technique has previously been used by Singh *et al.*, (1988) and Barimalaa *et al.*, (1994) for bioconversion of organic wastes.

Amino Acid Analysis: During the biodegradation process, subsamples of 3 and 10 day old fermented BSG samples were subjected to amino acid analysis. About 50mg of the biodegraded BSG samples were homogenized and hydrolysed with 10ml of 6N HCl containing 1% thioglycolic acid under vacuum at 110°C for 24 hr. Prior to amino acid analysis both the acid and alkaline hydrolysates were neutralized. The solution of the hydrolysates were injected into a Hitach 1835 automatic amino acid analyser and the amino acid quality determined by ion-exchange chromatography on a cationic exchange as described by Glazer *et al.*, (1976).

The amino acid mixtures were often taken up in an acid buffer and loaded on the ion exchange column. The more basic amino acids were most tightly bound than those with acidic groups. The column was eluted gradually into buffers of increasing pH and ionic strength. The acidic amino acids were removed readily from the resin followed by neutral

amino acids and finally by basic amino acids. Each amino acid was clearly separated from the other. The individual amino acids were quantified by reaction with ninhydrin and the resultant colour

density was measured by spectrophotometry. The concentration of the amino acids was expressed in g/16gN (Glazer *et al.*, 1976).

RESULTS AND DISCUSSION

The analysis revealed the presence of variable concentrations of lysine (Leu), histidine (His), arginine (Arg), asparagine (Asp), threonine (Thr), serine (Ser), glutamine (Glu), proline (Pro), glycine (Gly) alanine (Ala), cysteine (Cys) valine (Val), methionine (Met), isoleucine (Ileu), leucine (Leu) tryptophan (Try) and phenylalanine (Phe) in biodegraded BSG (Table 1). Of all these amino acids the most important amino acids in poultry nutrition are cysteine, lysine and methionine (Portsmouth 1978, Ranjhan *et al.*, 1974, Sastry and Thomas 1988). Other amino acids necessary in

poultry feeds, also detected in the fermented BSG were histidine, arginine, asparagine, glycine, valine, isoleucine leucine, tryptophan and phenylalanine. But their concentrations were remarkably higher than the values recommended for poultry nutrition. The results also revealed that the concentrations of lysine, histidine, asparagine, threonine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine decreased with increase in fermentation period while the concentrations of arginine, serine, glutamine and cysteine increases with the fermentation period.

Table 1: Summary of concentration (g/16gN) of amino acids in biodegraded BSG.

Amino acid	* Poultry requirement	Control		<i>A. niger</i>		<i>S. cerevisiae</i>		<i>Streptomyces sp</i>	
		3	10	3	10	3	10	3	10
Lysine	1.1 - 1.2	2.6	2.1	0.9	1.1	0.9	1.1	2.0	1.5
Histidine	0.4 - 1.4	3.1	2.2	0.2	1.2	1.9	1.0	2.8	1.0
Arginine	1.2 - 1.4	1.0	2.2	1.2	0.7	1.0	1.5	1.0	1.3
Asparagine	0.8 - 0.9	4.3	3.9	3.9	2.8	4.0	3.5	3.9	3.2
Threonine		3.1	1.9	3.8	1.5	2.3	3.8	2.6	2.1
Serine		2.8	3.6	3.0	2.9	3.1	3.8	2.9	3.4
Glutamine		1.1	9.4	7.7	6.1	7.8	7.7	6.7	9.6
Proline		5.4	4.0	3.9	1.2	7.0	4.4	4.2	2.2
Glycine	1.0 - 1.1	4.7	3.4	3.5	2.3	2.9	2.8	2.6	3.0
Alanine		7.4	3.9	5.6	5.0	5.3	5.4	5.0	7.0
Cysteine	0.3 - 0.4	0.4	0.7	0.2	0.3	0.9	0.3	0.4	0.7
Valine	0.8 - 1.0	4.8	4.2	2.4	3.2	3.6	2.4	3.2	3.1
Methionine	0.7 - 0.8	2.3	1.8	1.5	1.5	1.6	1.2	1.6	1.8
Isoleucine	0.8 - 0.8	2.1	1.5	1.4	0.9	1.7	1.2	1.5	1.1
Leucine	1.4 - 1.6	5.4	4.5	3.9	3.4	4.2	4.2	4.2	4.7
Tryptophan	0.6 - 0.7	5.5	5.4	4.6	2.9	5.2	3.8	4.4	3.2
Phenylalanine	0.7 - 0.8	6.2	6.0	5.8	3.8	5.7	2.8	6.2	3.8

*(Portsmouth, 1978., Sastry and Thomas, 1980)

A summary of the amino acids composition (g/16gN) also shows a reduction in the lysine level of the spent grain from 2.17g to 1.10g/16N by *Aspergillus niger*, to 1.16g /16gN by *Saccharomyces cerevisiae* and to 1.54g/16gN by *Streptomyces sp* within 10 days of fermentation at room temperature. Same was observed for the concentration of cysteine which was reduced from its initial content of 0.74g/16N to 0.30g/16gN by *A. niger*, 0.36/16gN by *S. cerevisiae* and to 0.71g/16gN by *Streptomyces sp* within 10 days. Similarly, the concentration of methionine was reduced from 1.86g/16gN to 1.52g/16N, 1.25g/16gN and 1.83g/16gN respectively by *A. niger*, *S. cerevisiae* and *Streptomyces* species.

Apart from the values obtained for lysine, the concentrations of cysteine and methionine recorded after 10 days of fermentation were short of the requirement for poultry (Portsmouth 1978, Sastry and Thomas 1980). The higher values recorded for these amino acids after 3 days of degradation is an indication that if the spent grains are fermented for a longer period, the biodegraders would successfully convert them to levels necessary for

poultry. Other amino acids, namely, histidine, arginine, proline, glycine, valine, isoleucine, tryptophan and phenylalanine were also positively affected by the fermentation process but will require a much longer period to be degraded to the recommended standards for poultry nutrition. On the other hand fermentation by *S. cerevisiae* and *Streptomyces* had little effect on the concentrations of threonine, serine, glutamine, proline, alanine, and leucine in BSG, although the biodegraders are of high proteolytic potential.

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