Protein Enrichment of Brewery Spent Grains Using *Aspergillus oryzae*

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Abstract

Solid state fermentation of brewery spent grain (BSG) was carried out in this study using Aspergillus oryzae. Fungi isolates obtained from the samples of brewery spent grain collected from Nigerian Brewery Plc., Ibadan, Oyo State, Nigeria, were isolated and identified. Isolate with high percentage of occurrence was tested for the ability to grow and improve protein content of spent grain. The isolate was used to carry out a solid state fermentation on the brewery spent grain to improve the percentage protein of the spent grain. Proximate analyses of the fermented samples were also carried out.

The fungus with the highest frequency of occurrence was identified as Aspergillus oryzae. The proximate analyses result revealed a significant increase in the percentage protein from 18.22% in the unfermented to 28.33% in the solid state fermented spent grain after 35 day of fermentation. The ash content of the spent grain also increased from 3.66 % to 7.4% in the fermented sample and a significant decrease in the carbohydrate from 22.05% to19.05% was observed, ether extract from 5.53% to 3.23%, and the fiber content also decreased from 18.11% to 13.99% in the brewery spent grain with increasing time.

Keywords: *Brewery spent grain, fermentation, proximate analysis, Aspergillus oryzae.*

Introduction

At the present time, there is great need to reduce the pollution resulting from industrial activities. This has become a global concern and all countries, developed and underdeveloped, are trying to adapt to this reality by modifying their processes so that their residues can be recycled. Consequently, most large companies no longer consider residues as waste, but as a raw material for other processes (Joglekar *et al*. 1983; Duru and Uma 2003; SWFN/CAN/BA/NRC 1983). Waste recycling has been advanced as a method for preventing environmental decay and increasing food supplies.

The brewing industry generates quite large amounts of by-products and wastes: spent grain, spent hops and yeast being the most common. However, these can be readily recycled and reused, hence, brewery spent grain (BSG) and other waste from the brewery industries are said to be environmentally friendly (Ishiwaki *et al*. 2000; Mussatto *et al*. 2006).

Brewery spent grain (draff) is a moist by-product/waste of the brewing industry, representing around 85% of the total by-products generated (Mussatto *et al*. 2006). It is obtained from residues of malted or unmalted cereals and other starchy products, after the cereals are wet mashed to extract the malted sugars for the various brewery products. The content of the brewery spent grains may include maize grit, sorghum spent grains and malted barley spent grains. In Nigeria, due to the banning of the importation of malted barley into the country in 1988 by the federal government, local sorghum and maize are used by Nigerian brewers. These cereals could be used individually or as a mixture in an appropriate proportion. Mostly, a mixture consisting of 77% sorghum and 23% maize are used by Nigerian brewers (Adewusi and Ilori 1994).

The chemical composition of BSG varies according to grain variety, harvest time, malting and mashing conditions, and the quality and type of adjuncts added in the brewing process (Huige 1994; Santos *et al*. 2003); but in general, BSG is considered as a lignocellulosic material rich in protein and fiber, which account for around 20 and 70% of its composition, respectively. BSG has also been reported to be rich in water soluble vitamins (Kaur and Saxena 2004). These nutritional qualities of BSG revealed that BSG is high in crude protein and crude fiber content. This makes this by-product a good material for animal feed. The fermentation of waste materials improves their nutritional quality making the material a better animal feed (Rudravaram *et al*. 2006). The crude protein content is increased. Equally, the digestibility (Johnson and Remillard 1983) and palatability (Verma *et al*. 2003) of the waste materials by the animals are increased due to the partial digestion of waste material by the microorganisms.

This research work aims at improving the protein content of the brewery spent grain using the filamentous fungi, *Aspergillus oryzae*, which was isolated from the brewery spent grain.

Materials and Methods

Collection of Samples

The brewery spent grain was collected from the Nigerian Brewery Plc., Ibadan, Oyo State, Nigeria. The spent grain was left to decay on the laboratory bench for 7 days (Ibenegbu 2007).

Isolation and Identification of the Organism

Aspergillus oryzae was isolated from the brewery spent grains using Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA) and Malt Extract Agar (MEA) media. Identification of the isolates was carried out using their macroscopic and microscopic morphology according to Domsch *et al*. (1980). Isolation and identification of the organism was carried out in the Environmental Microbiology Laboratory of the University of Ibadan, Oyo State, Nigeria.

Substrate Preparation for Solid State Fermentation

For solid state fermentation, 50 g of the sample of brewery spent grain was weighed into super bottles. The weighed sample was sterilized by autoclaving at 121°C and 760 mmHg for 15 minutes (Anupama and Ravindra 2001).

Spores Suspension Preparation

Spores were harvested from one week old culture of the isolates. A suspension of spores was obtained by dilution of spores in 10 ml of sterile distilled water. The desired concentration of spores in the suspension was obtained by counting the number of cells in the suspension using heamocytometer and subsequent continuous dilution of the suspension until a desired spore concentration (1×10^6 spores/ml) was obtained (Duru and Uma 2003; Raimbault 1998).

Solid State Fermentation

The super bottles containing the prepared/sterile samples were inoculated with 2 ml of the suspension per 50 g of sample and incubated at 28°C ± 2 for 35 days. Proximate analysis of the waste was carried out before the fermentation and at seven day intervals. Crude protein, ash, fats and oil, carbohydrate and crude fiber were estimated according to AOAC International (Cunniff 1995). This was carried out in replicate of three. Unfermented brewery spent grain was used as control.

Results and Discussion

The proximate analysis of the unfermented brewery spent grain (i.e., the control) revealed the composition of sample of brewery spent grain to be 18.22% crude protein, 18.11% crude fiber, 22.05% carbohydrate, 3.66% ether extract and 5.53% ash. This could be compared to the results obtained by Kaur and Saxena (2004), Duarte *et al*. (2008), Santos *et al*. (2003), and Adewusi and Ilori (1994).

The proximate analysis results revealed that the percentage crude protein (CP) and ash content of the brewery spent grain fermented with *Aspergillus oryzea* increased significantly with time as shown in Figs. 1 and 5. The samples fermented with *Aspergillus oryzea* increased in crude protein from 18.22% in the original unfermented brewery spent grain (i.e., the control) to 28.33% after 35 days of fermentation. The ash content increased significantly from 3.66% in the unfermented BSG to 7.4% after 35 days of fermentation.

Conversely, the percentage crude fiber (CF), carbohydrate, and ether extract decreased with time significantly as shown in Figs. 2, 3 and 4. The crude fiber of the brewery spent grains fermented with *Aspergillus oryzea* decreased from 18.11% of the crude fiber in the unfermented brewery spent grains (i.e., the control) to 13.99% after 35 days of fermentation. The carbohydrate decreased from 22.05% in the control to 19.05% when brewery spent grains were fermented with *Aspergillus oryzae*. Ether extract in the brewery spent grains decreased from 5.53% of the original brewery spent grains to 3.23% after 35 days of fermentation.

These changes in the nutritional content could be attributed to the ability of *A. oryzae* to consume carbohydrate, crude fiber and ether extract to build up their biomass resulting in increased crude protein and ash content (Duarte *et al*. 2008).

The results obtained in this research confirm that fermentation improves the nutritional value of substrates (Carvalheiro *et al*. 2007)

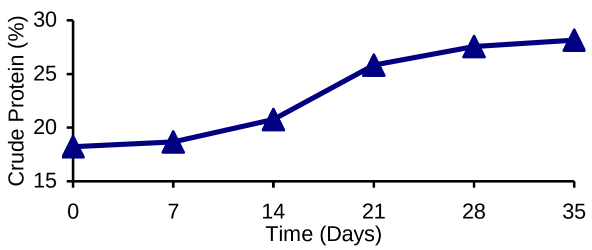

Fig. 1. The change in the crude protein (CP) of brewery spent grains during solid state fermentation with *Aspergillus oryzea* with increasing time.

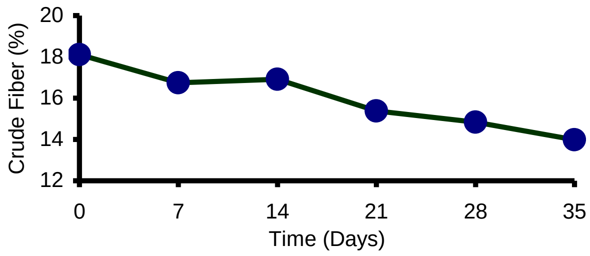

Fig. 2. The change in the crude fiber (CF) of brewery spent grains during solid state fermentation with *Aspergillus oryzea* with increasing time.

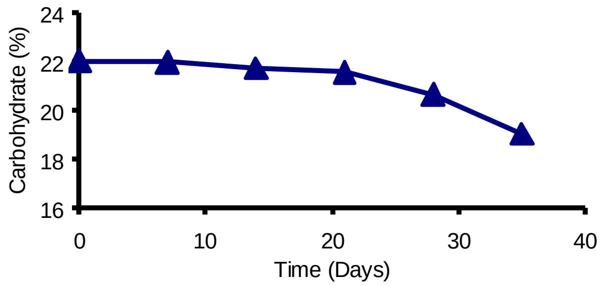

Fig. 3. The change in the carbohydrate of brewery spent grains during solid state fermentation with *Aspergillus oryzea* with increasing time.

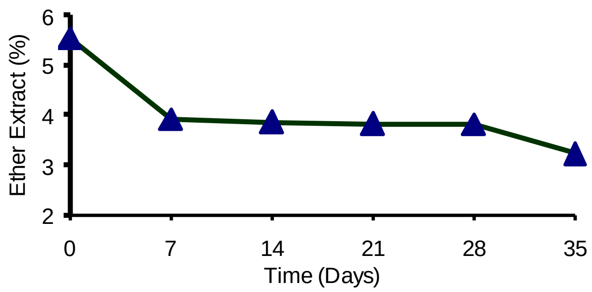

Fig. 4. The change in the ether extract of brewery spent grains during solid state fermentation with *Aspergillus oryzea* with increasing time.

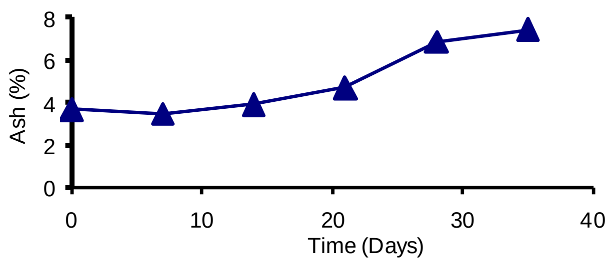

Fig. 5. The change in the ash content of brewery spent grains during solid state fermentation with *Aspergillus oryzea* with increasing time.

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