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Microscopic Analysis of Extruded and Pelleted Barley and Sorghum Grains

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Abstract

Scanning Electron Microscopy (SEM) technique has been used in food and feed industry to characterize the final product with the main focus on changes that occur to starch material. This study demonstrates the use of SEM for examining the changes that occur to starch granules after exposing sorghum and barley grains to mild (pelleting) and intensive (extrusion) feed processing methods. In this study, SEM images analysis is proved to be a useful tool to recognize the changes that occur to grains after a different processing method. The present study showed that the swelling and melting of starch granules are influenced by the severity of the processing method. In case of pelleting process, oval shape appearance of starch granules remained intact after the pelleting process; however, absence of intact oval shape of starch granules occurred after the extrusion process.

Keywords: Grains, Scanning Electron Microscopy, Gelatinization, Starch, Feed Processing.

1. Introduction

Grains usually represent the main ingredient component in both ruminant and monogastric animals feed and are considered the primary energy source (Svihus et al., 2004). Before feeding animals, grains are ground to increase the digestibility and to improve mixing with other feed ingredients (Al-Rabadi et al., 2009). Due to the incomplete starch digestion, grains are further processed to enhance starch gelatinization and thus digestibility (Svihus et al., 2005). Excellent positive correlations have been reported between extent of starch gelatinization and digestibility among animal feed (Svihus et al., 2005). Extent of starch gelatinization is dependent on the processing method (pelleting, steam flaking, expanding, extrusion) and operating variables within the processing method (level of water addition, temperature and retention time) (Gilpin et al., 2002). The pelleting process is the most conventional method in producing animal feed where feed material is exposed to steam and then forced through a die (Thomas et al., 1997). The extrusion process is defined as a high temperature short time treatment where feed material is exposed to friction and shearing forces. Different microscopy techniques, such as scanning electron microscopy (SEM) have been reported to be applied into food and feed industry for quality control purposes and particularly in cereal products to determine the extent of starch gelatinization and characteristics in final product (Lee *et al.*, 2000; Srikaeo *et al.*, 2006; Srikaeo, 2008; Olav and Svihus, 2011). The objective of this study was to use microscopic analysis to examine the influence of two different feed processing methods (steam pelleting and extrusion) on starch structural changes of processed sorghum and barley grains used for animal feed.

2. Materials and Methods

2.1. Grain Processing (Steam Pelleting)

Sorghum and barley grains were obtained from the Queensland Department of Primary Industry and Fisheries, Australia. Grains were milled under steady state conditions using 4 mm hammer mill screen size (i.e., when there is no change in motor load or ampere meter reading) before being steam pelleted (Ring Die 520 diameter, Munch Edelstahl, Hilden, Germany) under constant motor load. For both barley and sorghum grains, steam conditioning temperature was 85 °C and moisture added as a steam at pre-conditioner at rate of 1.9 and 2.5%. Pellet diameter and length were 4.0 and 6.0 mm,

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respectively. After the pellets were prepared, they were sealed into 25 kg plastic bags and stored at 4 °C before examination using scanning electron microscopy.

2.2. Grain Processing (Extrusion)

High-temperature short-time (HTST) extrusion cooking was conducted using a co-rotating twin-screw model Prism Eurolab KX16 (Thermo Prism, Staffordshire, UK). The barrel diameter was 16 mm with a length/diameter ratio of 40:1. The die had two openings each 2 mm in diameter and 8 mm in length. Melt pressure was measured with a pressure transducer fitted to the die block (Terwin, Nottinghamshire, UK). Motor torque, screw speed, barrel temperatures and melt pressure were monitored with Prism software (Sysmac-SCS version 2.2; Omron Corporation, Milton Keynes, UK). Die temperature ranged from 90-100 °C and die pressure ranged 4.85-16.61 bar. Liquid feed rate and dry feed rate were recorded manually after being calibrated before processing. Dry feed was fed through a single screw volumetric feeder (KX16 Powder feeder; Brabender Technology, Duisburg, Germany). Water was injected through a port 150 mm from the start of the barrel using a peristaltic pump (L/S 7523) with a Tygon Lab tubing 13 (0.8 mm internal diameter, Masterflex; Cole-Parmer Instrument Company, Vernon Hills, IL, USA). The dry feed rate for barley and sorghum was 20 g/min and 25 g/min, respectively, and the amount of water added at the extruder barrel was adjusted to compensate for moisture differences in the samples to have a dough moisture content of 55% for barley and 50% for sorghum (wb). Barley fractions were extruded at lower feed rate and higher moisture content, compared to sorghum, to avoid any possible blockage during extrusion. High barrel temperature settings (140 °C) and constant screw speed of 200 r.p.m. were used.

Samples were collected when the extruder was running at a steady state (i.e., stable values for both torque and die pressure). The samples were collected over 15–20 min., placed in an aluminium tray, and dried in a hot air oven (50 °C for 24 h) (Ballogou *et al.*, 2011). After drying, they were sealed into plastic bags and stored at -18 °C pending visual examination by using scanning electron microscopy.

2.3. Scanning Electron Microscopy (SEM)

Specimens were mounted onto aluminium stubs with carbon tabs and sputter coated with a 10-15 nm layer of

platinum using an EIKO IB-5 Platinum Sputter Coater. Samples were viewed by field emission scanning electron microscope (JEOL 6300 or JEOL 6400, Japan). Representative Scanning Electron Micrographs were selected by taking many 5 to 10 pictures for the selected sample. The selected sample contains many grain fragments on the carbon tabs. For each grain fragment, many pictures were taken at different magnifications (range from 25-1500X) to explore any major structural difference at the grain fragment level and starch granule level. To solve the challenge of selecting the representative sample, a random micrograph from many micrographs with similar features and appearance was selected as a representative picture.

3. Results and Discussion

The SEM images for steam pelleted sorghum and barley samples are shown in Figure 1 and 2, respectively. These images show the difficulty of characterizing grain fragment borders in the pellet due to the union of fragments caused by the high compaction force between particles and the die surface during pelleting process. Union of grain fragments is considered extremely important in determining pellet quality (pellet durability) which has been reported to be related to the extent of starch gelatinization which enhances the binding properties of grain fragments (Thomas et al., 1996). When starch granules in pelleted grains are compared with unprocessed starch granules in sorghum (Figure 3) and barley grains (Figure 4), it seems that there was no swelling in starch granules after pelleting process has occurred. This may be due to low level of moisture addition during pelleting process. From a process prospective, Leaver (1988) reported that the maximum inclusion level of water during the pellet process should not exceed 6%. It has been reported that water addition above this level can cause die blockage and increase the energy required for the pelleting process (Thomas et al., 1996). Svihus et al. (2005) reported that the extent of starch gelatinization that occurs after pelleting process ranges from 1-20% of the total starch content. Stevens (1987) reported that conditioning the mash corn caused a limited gelatinization and that most of the starch gelatinization occurred when the feed material passed through the die.



Figure 1. SEM image for sorghum pellets at different magnification levels Figure 1A show cross section of sorghum pellet. Figure 1B and 1C shows no changed occurred to starch granules after pelleting process.

These findings may suggest that starch gelatinization occurs on pellet surface when the feed material pass through the die which keeps the integrity of pellet after pressing through the die.

The SEM images of extruded sorghum and barley are shown in Figure 5 and Figure 6, respectively. The SEM images provide a clear view of changes of the intact starch granules in the extruded sorghum and barley samples. In raw unprocessed.



Figure 2. SEM image for barley pellets at different magnification levels. Figure 1A show cross section of barley pellet. Figure 1B and 1C shows no changed occurred to starch granules after pelleting process.



Figure 3. SEM image for raw unprocessed sorghum grain.



Figure 4. SEM image for raw unprocessed barley grain



Figure 5. SEM image for sorghum extrudate at different magnification levels. White arrows in Figure 5A show formation of air sacs after extrusion process.



Figure 6. SEM image for barley extrudate at different magnification level. White arrows in Figure 6A and 6B indicate formation of air sacs.

grains, SEM images for sorghum (Figure 3) and barley grains (Figure 4) show clearly the oval-shaped and circular starch granules and protein matrix that is attached to starch granules. However, the extrusion process for both grain types resulted in swelling and melting of starch granules and consequently changed the microstructure of starch granules of being shapeless and mud-like structure as described previously by Srikaeo (2008) and to scatter protein matrix that surround starch granules as shown clearly and more pronounced in sorghum extruded samples micrograph (Figure 5-C). Due to high shearing force and thermal temperature, an extrusion process has been reported to achieve complete starch gelatinization and to completely rupture starch granules (Skoch et al., 1983). SEM images of cross sections of sorghum and barely extrudate showed the presence of small numbers of air sacs of irregular size

(Figure 5-A and Figure 6-A, respectively). These air sacs resulted from the rapid reduction in pressure once extrudate exposed to atmospheric pressure and consequently rapid evaporation of internal moisture (Berrios *et al.*, 2004).

In comparison to extrusion process, commercial processes (such as pelleting) are usually operated under less energy-intensive conditions and thus are considered as less expensive compared to extrusion process. In addition, nutrient digestibility (such as protein and starch digestibility) after extrusion process seems to be grain dependent (Ezeogu et al., 2005). For example, exposing sorghum to high thermal treatments has been reported to form disulphide bond cross-linked prolamin proteins and extensive polymerisation of the prolamins which limit protein digestibility (Ezeogu et al., 2005). The presence of tight protein matrix that surrounds starch granules within densely packed endosperm cells in sorghum can also reduce the extent of starch digestion (Hamaker et al., 1987; Rooney and Pflugfelder, 1986). A recent study showed that the extant and the rate of starch digestibility were higher in barley extruded grains compared to sorghum grains (Al-Rabadi et al., 2009). These factors may make the extrusion process as not the best processing method to adopt in feed industry for sorghum grains.

4. Conclusion

It can be concluded from micrograph images that starch granules in both grain types retain its integrity after the pelleting process. However, exposing grain fragments of barley and sorghum to extrusion process can eliminate the integrity of starch granules by inducing swelling and melting of starch granules. Scanning electron microscopy could be practical for feed industries for the quality control in cereal based diets where starch gelatinization is the most influential factor on starch digestibility.

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