Densification of Heat Sensitive Protein/Fibre Biomass

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Emami, S., L.G. Tabil, L. Campbell, E. George and P. Adapa. 2013. **Densification of heat sensitive protein/fibre biomass**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **55**: 3.19-3.28. Production of feed enzyme from biomass material is growing as it is used in the poultry industry to improve feed utilization efficiency. Biomass from agricultural residues have low bulk density and irregular shape and size which makes them difficult to handle, transport, store and utilize in their original form and wastage of material is an issue. Therefore, there is need to densify biomass containing feed enzyme with the aim of preserving its activity intact. In this study, grain-based fungal biomass containing heat sensitive enzyme was mixed with ingredients like binder and fat at different levels. Pellets were made at die temperatures of 45, 70, and 85°C by applying a pre-set load of 4400 N (138.9 MPa) using single-pelleter unit (SPU), which was attached to the Instron testing machine, to study their effect of pellet enzyme activity. Pellet quality was determined by measuring pellet density, durability and enzyme activity to optimize conditions most suitable to produce high density and durability pellets having the highest enzyme activity. Pellets added with bentonite, cornstarch, and FFSG (fructooligosaccharide, fructose, sucrose, glucose) as binders showed the highest durability. Increasing pelleting temperature from 45 to 85°C did not affect enzyme activity markedly and pellets made at 85°C had acceptable enzyme activity (77.8% in FFSG treatment and 100.0% in bentonite treatment). From the results obtained using the SPU, three binders were chosen to make pellets in the pilot-scale pellet mill by employing three different oils as lubricant. The binder and lubricant types had significant effect on bulk density, pellets density, and durability. Overall, pellets made with FFSG and hydrogenated golden flake had high bulk density (815 kg/m^3), pellet density (1282 kg/m^3), and durability (98.9%). Enzyme activity of pellets manufactured in the pilot-scale pellet mill was not reduced with values ranging from 90 to 100%. From a production economics standpoint, pelletizing grain-based fungal biomass containing feed enzyme using bentonite and hydrogenated golden flake could be used in industrial scale with minor loss in enzyme activity. **Keywords:** Biomass, Enzyme activity, Feed enzyme, Binder, Lubricant

La production d'enzymes alimentaires qui proviennent de la biomasse est en croissance car celles-ci sont utilisées pour améliorer l'efficacité alimentaire des moulées pour la production de volailles. La biomasse provenant de résidus agricoles a une masse volumique faible et une granulométrie irrégulière. Ces caractéristiques la rendent difficile à manipuler, transporter, entreposer et utiliser dans son état d'origine, et les pertes sont problématiques. Par conséquent, la concentration de la biomasse contenant des enzymes alimentaires devient nécessaire et le procédé se doit de préserver leur activité. Dans cette étude, de la biomasse fongique d'origine céréalière qui contient des enzymes sensibles à la chaleur a été mélangée à des ingrédients tels que des liants et des gras, et ce à différentes concentrations. Les granules étaient pressées à des températures de 45, 70 et 85°C en utilisant une presse unitaire à granules exerçant une contrainte prédéfinie de 4400 N (138,9 MPa). Cet équipement était relié à une machine Instron pour tester l'activité enzymatique des granules ainsi formées. La qualité des granules était déterminée en mesurant la masse volumique, l'intégrité et l'activité enzymatique des granules. Le but était d'optimiser les conditions pour produire des granules durables et de haute densité qui ont l'activité enzymatique la plus élevée. Les granules auxquelles étaient ajoutées des liants tel que de la bentonite, de la fécule de maïs et des FFSG (fructooligosaccharide, fructose, sucrose, glucose) avaient l'intégrité la plus élevée. Une augmentation de la température de pression de 45 à 85°C n'avait pas d'effet marqué sur l'activité enzymatique et les granules formées à 85°C avaient une activité enzymatique satisfaisante (77,8 % pour le traitement FFSG et 100,0 % pour le traitement bentonite). À la lumière des résultats obtenus avec la presse unitaire à granules, trois liants ont été choisis pour faire les granules à l'aide d'une presse à granules expérimentale et trois différentes huiles ont été utilisées comme lubrifiant. Les types de liants et de lubrifiants avaient un impact significatif sur la masse volumique, la densité et l'intégrité des granules. En général, celles faites avec des FFGS et du gras hydrogéné « golden flake » avaient une masse volumique de vrac (815 kg/m^3), une masse volumique de granules (1282 kg/m^3) et une intégrité (98,9 %) élevées. L'activité enzymatique des granules produites par la presse à granules expérimentale n'était pas diminuée et variait de 90 à 100 %. D'un point de vue économique, la production à l'échelle industrielle de granules à base de biomasse fongique d'origine céréalière, de bentonite et de gras hydrogéné « golden flake » est intéressante et présente peu de pertes d'activité enzymatique. **Mots clés:** biomasse, activité enzymatique, enzyme alimentaire, liant, lubrifiant.

INTRODUCTION

Poultry feed containing cereal biomass (wheat, barley, oats, triticale, and rye), which is high in non-starch polysaccharides (NSP), is supplemented with enzymes to optimize the digestibility of the diet. Feed enzymes are effective additives providing options for feed producers to select raw materials and to improve efficiency of current diet formulations by improving digestibility, growth, and feed conversion. Utilization of enzymes reduces feed cost resulting in benefits to both feed producers and farmers (GNC Bioferm Inc. 2012; Campbell and Bedford 1992).

NSPs are relatively high molecular weight compounds, which dissolve in the intestine and increase the viscosity of gut contents. Consequently, the animal growth rate decreases and the ratio of feed to weight gain increases (Silversides and Bedford 1999). Adding enzymes to poultry feed results in higher feed conversion ratio and greater yield than in feeds without enzymes. Application of enzymes in swine and poultry has the most interest since their digestive systems do not produce enzymes to fragment and digest plant cell walls. Therefore, enzymes break down the feed to digestible form to be absorbed by animal digestive tract and increase the nutritional value of the feed (GNC Bioferm Inc. 2012; Campbell and Bedford 1992).

Recently, there has been high interest in the production of bio-products, such as feed enzyme from biomass, which has its own challenges to overcome. The biomass-based feed enzyme contains about 30% protein. Inherently, biomass has low bulk density, and irregular shape and size which makes it difficult to handle, transport, store, and utilize in its original form. Therefore, an efficient solution is to densify low bulk density biomass into pellets and cubes with higher bulk density (Kaliyan and Morey 2009). However, during pelleting operation, the ground material is heated due to friction between the die and material. The heat is of concern because the activity of granulated and powdered enzymes is adversely affected, and the moisture content of enzyme has been found to be inversely proportional to the enzyme activity at high temperatures (Slominski et al. 2007). This overheating could result in an increase in resistance to digestion of protein and starch, inactivation of bio-compounds such as vitamins and endogenous enzymes, and increased intestinal viscosity caused by high processing temperatures (Silversides and Bedford 1999). Pre-treatments such as steam explosion or microwave heating could destroy enzymes. However, application of binders during pelleting at low temperature and moisture content could enhance pellet strength and durability while circumventing biomass overheating and the resulting enzyme inactivation.

Ground agricultural biomass is composed of lignocellulosic matrix (Adapa et al. 2009a); the durability of resulting pellets depends on thermo-physical forces that bond the ground biomass particles together (Kashaninejad and Tabil 2011; Kaliyan and Morey 2009). There are primarily three types of binders that when mixed with ground biomass forms different types of bonds: a) matrix type binders such as dry starch and dry sugar; b) film type binders such as bentonite, lignosulfonates, and gum; and c) chemical binders such as $Ca(OH)_2$ and NaOH (Pietsch 1976). The binding reactions that improve durability and strength of a biomass pellet are starch gelatinization, protein denaturation and solubilization, and subsequent recrystallization of sugars and salts. Starch is gelatinized in the presence of moisture and heat, which happens during steam conditioning or application of shearing force on high moisture biomass in the die. The durability of pellet increases with an increase in gelatinized starch content (Kaliyan and Morey 2009). Application of pre-gelatinized starch results in higher durability than raw starch (Wood 1987).

Protein acts as a plasticizer in the presence of heat and results in enhanced pellet durability. Exposure to heat, moisture, and shearing forces results in protein denaturation particularly in cereals, such as wheat, rye, and barley, which have potential to make dough. Raw protein results in higher pellet durability as opposed to denatured protein (Wood 1987).

Fibre is not considered as a good binder of particles since it has high resilience (Kaliyan and Morey 2009). Biomass is composed of lignocellulosic matrix containing cellulose, hemicellulose, and lignin (Adapa et al. 2009a). The role of lignin is to fill the spaces in the cell wall between cellulose and hemicellulose. It is linked to hemicellulose and crosslinks different plant polysaccharides, increasing mechanical strength of the cell wall and subsequently of the whole plant structure (Chabannes et al. 2001; Adapa et al. 2009b). Pretreating biomass with chemicals such as NaOH, CaO, or urea degrades the cell wall structure and separates lignin from cellulose. Therefore, the resilience characteristics of fibers in biomass are reduced with application of pretreatment resulting in higher pellet durability values. Incorporation of water (19-23%) also lowers resilience characteristics of the fibers.

Fat and oil reduce pellet durability since fat acts as a lubricant between particles and die wall, which facilitates its ejection through the die. During compaction process, the natural fat in the cell wall could be exposed and act as a bridge between particles to increase durability. Nevertheless, fat is not recognized as a binder but rather as a lubricant (Kaliyan and Morey 2009).

There are variety of ingredients and products, which are being used as binder. Tabil et al. (1997) reported that the durability of pellets made from low quality alfalfa chops improved when collagen protein (0.2%), lignosulfonate (1.25%), bentonite (5.0%), hydrated lime (1.9%), or pea starch (0.74%) were used as binders. Starch, molasses, natural paraffin, plant oil, lignosulfonate, and synthetic agents have been used in fuel pellets to increase durability and reduce dust and fines generated in storage, handling, and transportation (Kallio 2011). However, Hill and Pulkinen (1988) reported that addition (by weight) of 4% bentonite, 1.5% Perma-Pel (lignosulfonate), 1.5% lignosite 458, 4% of neutralized liquid lignosite, 4% of liquid molasses, or 40% of ground barley grain did not improve the durability of alfalfa pellet over the control.

Therefore, there is a need to conduct a study on the densification of enzyme containing biomass product having low dust emissions, high quality (density and durability), and easier and efficient to handle. Also, there is a need to study the effect of different binders, pelleting temperatures, and lubricants on durability and enzyme activity of

biomass pellets using the single-pelleting unit and the pilot-scale pellet mill. Consequently, two primary objectives were identified as part of this study: 1) to enhance the physical quality (bulk density and durability) of feed enzyme-containing biomass pellets; and 2) to preserve the enzyme activity of pelletized product by optimizing pelleting conditions.

MATERIALS AND METHODS

Materials

The material, a grain-based fungal biomass containing feed enzyme was obtained from GNC Bioferm Inc. (Bradwell, SK) in the fall of 2012 and was subsequently stored in an airtight bag to retain its initial moisture content (8.5%). Bentonite (Canadian Clay Products Inc., Wilcox, SK), corn starch (Ingredion Canada Inc., Pointe-Claire, QC), FFSG (acronym of fructooligosaccharide, fructose, sucrose, glucose) (Ingredion Canada Inc., Pointe-Claire, QC), lignosulfonate (Lignotech USA Inc., Rothschild, WI), pea starch (called Starlite, Parrheim Foods, Saskatoon, SK), waxy barley (Parrheim Foods, Saskatoon, SK), and maltodextrin (Ingredion Canada Inc., Pointe-Claire, QC) were used as binders. Mineral oil (Swimco Canada Inc., Georgetown, ON), hydrogenated fats, namely, silver prills (Trident Feeds, UK) with melting point of 55°C and golden flake fat (Trident Feeds, UK) with melting point of 50-55°C, were used as lubricant additives in the pilot-scale pellet mill.

Particle Size Analysis

Tests for particle size analysis of biomass were conducted using ASAE Standard S319 (ASABE 2011). The U.S. sieve numbers of 20, 40, 60, 100, 200 and 270 (sieve opening sizes: 0.841, 0.420, 0.250, 0. 149, 0.074 and 0.053 mm, respectively) were arranged on the Ro-Tap sieve shaker (W. S. Tyler Inc., Mentor, OH) where 100 g of biomass sample was shaken for 10 min. The test was repeated three times. The geometric mean diameter (dgw) and geometric standard deviation (Sgw) were calculated.

Bulk Density and Particle Density

Bulk density of biomass and pellets made by pilot-scale pellet mill was determined using a 0.5-L cylindrical container (SWA951, Superior Scale Co. Ltd., Winnipeg, MB) filled using a funnel, with its discharge opening located 55 mm above the top edge of the container. Once filled, the funnel was removed from top and the container was tapped on a wooden table for approximately 10 times to allow the material to settle down. Rolling a cylindrical stainless steel bar across the container in two perpendicular directions levelled the container. Subsequently, the container was weighed. The mass per unit volume yielded the bulk density of the biomass in kg/m3. The bulk density was determined in five replicates for each sample.

Moisture Content

The moisture content of sample was determined in three replicate using AACC standard 44-15A (AACC 2005), where 2-3 g of material was oven-dried at 130°C for 90 min in duplicates.

Chemical Analysis

Crude protein, crude fat, and total ash were determined according to AOAC standard 984.13, 920.39 and 942.05, respectively (AOAC 2005) in duplicate. Acid detergent fiber (ADF) and lignin were measured using AOAC standard method 973.18 (AOAC 2005) in three replicates. Neutral detergent fiber (NDF) content was determined using AOAC standard method of 2002.04 (AOAC, 2005) in three replicates. Cellulose and hemicellulose contents were calculated indirectly as explained by Mani et al. (2006). Cellulose content was calculated by subtracting lignin from ADF content. Hemicellulose was calculated by subtracting ADF from NDF content.

Enzyme Activity

Enzyme survival calculations were based on beta-glucanase activity as representative of stability of the enzyme complex. Enzyme quantification was determined as reducing sugars (glucose equivalents) by direct comparison with a reference enzyme standard (in house). Enzyme activity was expressed as a percentage of the standard. The samples (30 mg) were added to 8 ml buffer (0.1 M sodium acetate-HCl, pH 4.0). Substrate solution (1.0 ml of 0.5% lichenan, Icelandic moss, Sigma-Aldrich Canada Co., Oakville, ON) in the same buffer was added and incubated at 30 ^{o}C for 0, 5, or 10 min. The enzyme reaction was stopped by addition of 1 ml DNS (3,5-dinitrosalicyclic acid solution (16 g NaOH, 300 g NaKtartrate, 10 g 3,5-dinitrosalicyclic acid in one l deionized water). The samples were cooled followed by dilution with 8 ml deionized water, and subsequently, the optical density (OD) was determined at 540 nm. OD readings for unknown enzyme samples were expressed as a percentage of the reference enzyme standard.

Sample Preparation and Densification

All samples were adjusted to 12% moisture content, except for the treatment with FFSG since it was in liquid form and provided sufficient moisture for densification. All re-moistened samples were stored in airtight bags at room temperature and mixed every 12 h for at least 72 h to ensure moisture equilibrium has been attained. Seven different types of binders were added to the re-moistened biomass at 12% moisture content as follows: bentonite 1.5% (by mass), corn starch 5.0%, lignosulfonate 1.0%, pea starch 5.0%, waxy barley 5.0% and maltodextrin 5.0%. Samples at original moisture content (8.5%) were added with FFSG at 5.0%.

Single Pelleting Unit (SPU)

The biomass was pelleted in a SPU as shown by Kashaninejad and Tabil (2011) and used in previous studies (Tabil and Sokhansanj 1996, 1997; Adapa et al. 2002; Mani et al. 2006; Shaw et al. 2009). The device consisted of a steel cylindrical die with internal diameter of 6.35 mm and a length of 125 mm. The die was coiled with a heating element to maintain the die temperature at 45, 70 and 85^{o}C to simulate frictional heating encountered by biomass in commercial pelleting operations (Adapa et al. 2006; Mani et al. 2006; Kashaninejad and Tabil 2011).

A plunger was mounted to the upper moving crosshead of Instron testing machine (3360 Dual Column Tabletop Testing Systems, Instron Corp. Norwood, MA) to apply compressive force on biomass. The cylindrical die sat on a raised base equipped with sliding gate at the bottom. On the base, there was an opening allowing the densified sample to be discharged from the die when sliding gate was opened. The biomass sample (0.5-0.6 g) was loaded into the die and compressive force of 4400 N (138.9 MPa) was applied using the Instron machine fitted with a 5000 N load cell to densify the material. The crosshead speed of plunger was maintained at 50 mm/min and was retained in place for 60 s once the pre-set compressive force was achieved to start the relaxation test (Kashaninejad and Tabil 2011) and also to avoid spring-back of biomass (Mani et al. 2006). Subsequently, the plunger was retracted to release compression force and the sliding gate at the bottom was opened. After 30 s, the plunger was lowered to eject the pellet through the bottom of die and base. The force-deformation and force-time data during compression and relaxation were logged in the computer.

Pilot-scale Pelleting

The pilot-scale CPM CL−5 pellet mill (California Pellet Mill Co., Crawfordsville, IN) was used for processing of biomass grinds into pellets. The pellet mill consisted of a corrugated roller (diameter of 85.0 mm) and ring die assembly. The diameter of ring die was 190.5 mm having a thickness of 32 mm. The pelleting die had internal diameter of 126.5 mm. The pellet-die hole diameter and l/d ratio were 6.5 mm and 6.9, respectively. The rotational speed of the pellet mill was maintained at 250 rpm.

According to preliminary test, 12% moisture content was not adequate to manufacture pellet in the pilot-scale pellet mill. Therefore, the moisture content of biomass grinds was adjusted to 14% (instead of 12% used in SPU), except for the treatment with FFSG since it was in liquid form and provided sufficient moisture for densification. Also based on the experimental outcomes of SPU tests, it was decided to use only three of the seven binders for pilot-scale pellet test. The binder and amounts used were bentonite 1.5%, cornstarch 5.0%, FFSG 5.0%. Subsequently, the mixture was placed in a sealed plastic bucket mounted on a concrete mixer and mixed for 2 h. The FFSG binder was added to biomass without any moisture adjustment. In addition, mineral oil (1.5%) and hydrogenated fats, namely golden flake (1.5%) and silver prill (1.5%), were used as lubricants. The hydrogenated fat was preheated in an oven at 50°C to melt it and was sprayed over the sample to obtain a homogenous mixture. The mixture having combinations of binders and lubricants was fed to the pellet mill without any steam conditioning to protect the enzyme in biomass against overheating. Uniform flow of material from the hopper to the pellet mill was controlled using a vibratory feeder. Material passed through the pilot-scale pellet mill for an average period of 10 min. Pellets were collected and weighed to calculate the pellet mill throughput (kg/h). A data logger connected to a laptop computer was employed to record pellet mill energy consumption (kWh) in actual time and to calculate the specific energy (MJ/t) required for making pellets from each treatment. Temperature of pellets was determined at the discharge die using an infrared thermometer (Model 42530, Extech Instruments, Nashua, NH). Pellets were cooled down by spreading on a sheet of paper at ambient temperature (20ºC). Once cooled, the pellets were stored in plastic bags for further tests.

Pellet Durability

Durability of pellets obtained from SPU was measured in ten replicates using the drop test method (Al-Widyan and Al-Jalil 2001; Khankari et al. 1989; Sah et al. 1980; Shrivastava et al. 1989), where a single pellet was dropped from a 1.85 m height on a metal plate. The ratio of the weight of the larger portion of the pellet retained intact to the initial weight of pellet was expressed as the percentage durability of the pellet.

Durability of pellets made by the pilot-scale pellet mill was measured following the ASABE Standard S269 (ASABE 2011), where 100 g pellets were placed in a dust-tight chamber and tumbled for 10 min at 50 rpm. Fine and broken pellets were separated from coarse ones using a sieve with hole opening of 7.93 mm and weighed to determined percentage of broken pellets respect to the initial pellet weigh during tumbling, as durability value.

Statistical Analysis

The effects of binder type and temperature were determined using a completely randomized experimental design with factorial treatment structure. There were two variable factors, the binder type (control, bentonite, corn starch, FFSG, lignosulfonate, pea starch, waxy barley, and maltodextrin) and the pelleting temperature (45, 70, and 85°C). Analysis of variance (ANOVA) and comparison of means (Duncan's multiple range test at P = 0.05) were performed using the Statistical Analysis System (Version 9.3, SAS Institute Inc., Cary, NC) by the GLM procedure to evaluate the effect of each variable and their interactions.

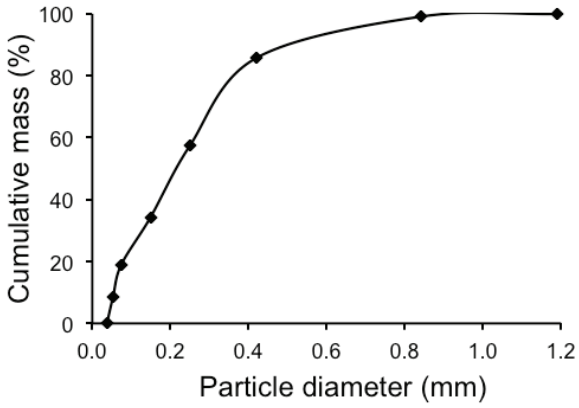

Fig. 1. Typical particle size distribution of grain-based fungal biomass.

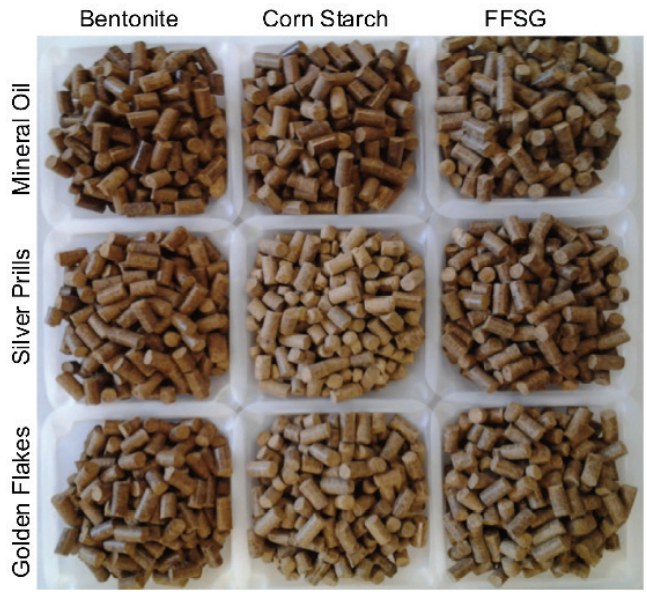

Fig. 2. Photograph of pellets manufactured using grain-based fungal biomass with bentonite, corn starch and FFSG as binder and mineral oil, golden flake and silver prills as lubricant in pilot-scale pellet mill.

RESULTS AND DISCUSSION

Physical and Chemical Properties of Biomass

Figure 1 presents the particle size distribution of biomass. More than 80% (by mass) of particles had diameter of 0.42 mm (passing U.S. sieve number 40) or smaller, with all particles having a diameter of 1.20 mm or less. The grain-based fungal biomass in this study had geometric mean diameter (d_{gw}) of 0.185 mm and a geometric standard deviation (S_{gw}) of 0.174 mm. The d_{gw} of grain-based fungal biomass was smaller than that of the biomass samples (from agricultural straw) ground by different hammer mill screen size (6.4, 3.2, 1.6 and 0.8 mm) to make pellet reported by Adapa et al. (2010). Particle density and bulk density of biomass in the current study were 1447 ± 17 and 501 ± 4 kg/m^3, respectively. Adapa and co-workers' (2010) reported particle density and bulk density of 1288 and 180 kg/m^3, respectively for barley straw ground with hammer mill (screen size of 0.8 mm), lower than the values of the sample in this study.

Table 1 shows the chemical composition of the biomass used in this study. The crude protein content of the biomass in this study (29%) was significantly higher than that of whole barley grain and pearled barley in the study of Faraj et al. (2004) (13.5 and 10.4%, respectively). This is because of the presence of fungal material containing enzyme in our biomass. The cellulose and lignin contents (17.9 and 20.0%, respectively) of our biomass were higher than the values reported by Žilić et al. (2011) for hulless barley (1.01-1.58% and 0.28-0.77%, respectively).

Pellet Density, Durability and Enzyme Activity of Pellets made by SPU

The effect of binder, temperature and their interaction effects on pellet density was significant ($P < 0.01$, Table 2). The highest pellet density was observed in the pellets with bentonite (average of 1338 kg/m^3) and corn starch (average of 1316 kg/m^3) and the lowest was obtained in control samples (no binder) (average of 1169 kg/m^3) and FFSG (average of 1062 kg/m^3). In this study, the density of all pellets made with binders, except FFSG, increased compared to the control pellets (average 1169 kg/m^3, Table 3). This trend was in agreement with values reported by Tabil (1996) for pellets made from low and intermediate quality of alfalfa using bentonite, lignosulfonate, or cornstarch. The density of pellets made with bentonite and lignosulfonate at 85°C (1355 and 1341 kg/m^3, respectively) was close to density reported by Tabil (1996) for alfalfa pellets using the same binders (1366-1382 and 1333-1363 kg/m^3, respectively). However, the density of biomass pellets made with pea starch (1256-1286 kg/m^3) in this study was lower than alfalfa pellet made with the same binder (1366-1383 kg/m^3) (Tabil 1996). Increasing pelletizing temperature resulted in higher density as shown in Table 2. Particles of biomass would undergo more compaction at high temperature, as the particles undergo more plastic deformation as a result of applied heat. Lignin as thermoset binder starts to soften at highly elevated temperatures and pressures. This phenomenon improves binding properties between particles (Adapa et al. 2009b).

The effect of binder, temperature, and their interaction effects on pellet durability were significant ($P < 0.01$, Table 2). The durability of pellets using different binders (Table 3) was in the descending order of bentonite > corn starch > FFSG > waxy barley > maltodextrin > pea starch > lignosulfonate > control. The durability of pellets added with bentonite at all temperatures (average 96%, Table 3) and lignosulfonate at 70 and 85°C was higher than the durability reported by Tabil et al. (1997) for alfalfa pellets made by the same binder types (78.2-88.8 and 74.1-85.8%, respectively). The durability reported by Tabil et al. (1997) was based upon the Kapt-al pellet durability tester as reported by Larsen et al. (1996). Furthermore, the difference between the nature and composition of sample (alfalfa) used by Tabil et al. (1997) and that used in the current study (grain-based biomass) could be the reason for this durability difference. Nevertheless, comparison between results of the current study and the study of Tabil et al. (1997) was conducted to show the effect of binders when used during pelleting. Pellet durability increased as pelletizing temperature increased in this study. This increased durability with pelleting temperature may be caused by starch gelatinization and protein plasticizing (Wood 1987; Kaliyan and Morey 2009).

Enzyme activity of pellets compared to unprocessed biomass did not decrease markedly. The retention of enzyme activity may be related to the low moisture content

Table 1. Chemical composition of grain-based fungal biomass presented as means (standard deviations).

Component	Content (% db)
Crude protein	29.0 (0.1)
Crude fat	2.1 (0.0)
Total ash	7.8 (0.0)
ADF	22.8 (0.1)
NDF	42.8 (0.1)
Lignin	4.9 (0.0)
Cellulose	17.9 (0.0)
Hemicellulose	20.0 (0.0)

db: dry basis, ADF: acid detergent fibre, NDF: neutral detergent fibre.

in the mixture during processing (Slominski et al. 2007). Enzyme activity of some treatments (such as bentonite and pea starch) was even lower at higher pelleting temperature, which could be related to error of assay. The lowest enzyme activity was observed when FFSG was used as binder (76.8-79.5%). This was related to the presence of high moisture content in this binder; treatments with higher moisture content have lower temperature resistivity of its enzymes. According to the opinion of GNC Bioferm Inc., an enzyme activity of at least 80% is an acceptable value.

Physical Properties and Enzyme Activity of Pellets Made by the Pilot-scale Pellet Mill

Figure 2 shows pellets manufactured from grain-based fungal biomass using bentonite, cornstarch, and FFSG as binder and mineral oil, golden flake and silver prills as lubricant in the pilot-scale pellet mill. Biomass pellets made with bentonite had darker color followed by those made with cornstarch and FFSG. All pellets had smooth external appearance without any sign of crack on the surface. Temperature of pellets discharged from the pilot-scale pellet mill die is presented in Table 4. Biomass pellets manufactured with bentonite or cornstarch as binder discharged at temperature from 46.2 to 55.2°C. However, biomass pellets with FFSG discharged at markedly higher temperature (84.3 to 87.6°C). This may be due to the change in viscosity of FFSG caused by chemical reactions such as caramelization and polymerization during heating in the pellet mill both in the conditioning chamber and pellet mill die. The effect of binder and lubricant type on bulk density was significant ($P < 0.01$, Table 5). The highest bulk density was observed in biomass pellets added with FFSG (average of 821 kg/m^3) followed by those added with cornstarch and bentonite, respectively. All three types of lubricant provided enough lubricity to eject the pellets through the die. Biomass pellets with golden flake had significantly higher durability than those with silver prills and mineral oil (Table 6). Overall, biomass pellets added with FFSG as binder and golden flake or silver prills as lubricant resulted in the highest bulk density (815-837 kg/m^3).

Biomass pellet density was significantly affected by the binder and lubricant ($P < 0.01$, Table 7). Application of FFSG in biomass increased pellet density significantly (Table 6, 1244-1282 kg/m^3) and resulted in higher density compared to other binders when identical lubricant was used. Biomass pellets added with silver prills and golden flake resulted in significantly ($P < 0.05$, Table 6) higher pellet density than those added with mineral oil. This result was in agreement with what was concluded for bulk density, confirming that the combination of FFSG as binder and golden flake or silver prills as lubricant resulted in high bulk and pellet density, which is beneficial for handling, transportation, and storage of feed enzyme-containing biomass.

The effect of binder and lubricant type on durability of pellets was significant ($P < 0.01$, Table 5), in that using binder improved the durability of pellets and using lubricants did not adversely affect pellet durability. Our result did not agree with Hill and Pulkinen (1988) who reported that adding bentonite (4%) did not improve durability of alfalfa pellets. The highest average of durability in this study was observed in pellets with FFSG (94.9%) followed by bentonite and cornstarch. Similar to the result of bulk density, the highest durability was obtained when golden flake was employed as lubricant. Although pellets with corn starch as binder and mineral oil as lubricant had high durability (97.4%), they had relatively low bulk density (782 kg/m^3) and pellet density (1151 kg/m^3). Overall, golden flake as a lubricant resulted

Table 2. Effect of binder type (B) and temperature (T) on pellet density and durability of pellets made by the single pelleting unit.

Source of variation	DF	Pellet density		Durability	
		SS	P-value	SS	P-value
B	7	1734938	<0.01	17383	<0.01
T	2	357833	<0.01	13542	<0.01
B × T	14	349157	<0.01	12064	<0.01
Residuals	216	1149327	---	23906	---
Total	239	3591255	---	66895	---

DF: degrees of freedom, SS: Sum of squares, P: probability.

Table 3. Pellet density, durability, and enzyme activity of pellets made by the single pelleting unit using different binders at different temperatures (n = 10) means (standard deviations).

Binder	Temperature (°C)	Pellet density (kg/m^3)	Durability (%)	Enzyme activity[1] (%)
Control (no binder)	45	1119 (41)^{j} [2]	39 (8)^{e}	89.7
	70	1180 (25)^{hij}	73 (13)^{d}	80.4
	85	1207 (13)^{ghi}	91 (5)^{abc}	95.5
Bentonite (1.5%)	45	1331 (12)^{abc}	89 (10)^{abc}	98.6
	70	1328 (19)^{abcd}	100 (0)^{a}	100.0
	85	1355 (25)^{a}	100 (0)^{a}	100.0
Corn starch (5.0%)	45	1249 (23)^{efgh}	88 (7)^{bc}	97.8
	70	1343 (22)^{a}	99 (0)^{a}	97.8
	85	1356 (28)^{a}	99 (1)^{a}	95.4
FFSG (5.0%)	45	891 (178)^{k}	88 (6)^{abc}	79.5
	70	1117 (163)^{j}	92 (9)^{abc}	76.8
	85	1177 (79)^{hij}	94 (9)^{abc}	77.8
Lignosulfonate (1.0%)	45	1215 (95)^{fghi}	64 (13)^{d}	89.3
	70	1316 (128)^{abcde}	91 (15)^{abc}	81.2
	85	1341 (42)^{ab}	94 (17)^{abc}	88.0
Pea starch (5.0%)	45	1285 (11)^{abcdef}	85 (14)^{c}	93.4
	70	1256 (20)^{defg}	84 (15)^{c}	92.1
	85	1286 (15)^{abcdef}	84 (14)^{c}	100.0
Waxy barley (5.0%)	45	1234 (78)^{fgh}	85 (14)^{bc}	93.1
	70	1269 (43)^{bcdefg}	89 (13)^{abc}	89.0
	85	1264 (61)^{cdefg}	89 (17)^{abc}	88.3
Maltodextrin (5.0%)	45	1155 (55)^{ij}	70 (14)^{d}	100.0
	70	1239 (38)^{fgh}	96 (4)^{ab}	83.9
	85	1207 (118)^{ghi}	93 (9)^{abc}	89.7

FFSG: fructooligosaccharide, fructose, sucrose, glucose

[1] The enzyme activity percentage was calculated relative to the feed enzyme before adding any binder or pelletizing.

[2] Mean values with at least one common letter are not significantly different at P = 0.05

Percentages indicated above for binders are based on total mass of the sample (biomass plus binder).

in higher biomass pellet durability compared to using silver prills or mineral oil as lubricant in the pellets with identical binders (Table 6). Pellets containing FFSG and golden flake had durability of 98.9%. This treatment possessed high bulk density (815 kg/m^3) and pellet density (1244 kg/m^3).

The enzyme activity values of pellets are shown in Table 6. The enzyme activity in the majority of the treatments ranged from 96 to 100%, except in pellets made from bentonite as binder and silver prills as lubricant, which had enzyme activity of 89.5%. The enzyme activity even in the latter treatment was acceptable in the feed industry.

Pellet mill throughput of all treatments lubricated by mineral oil was significantly higher than other lubricants except when pellets made by FFSG; throughput of biomass lubricated by silver prills was higher than other lubricants. Durability of pellets was negatively correlated to the total specific energy in pellets with bentonite and corn starch (Table 6) which was contrary to the results of Adapa et al. (2010) who reported that durability was positively correlated to specific energy required to make pellet from biomass at different mill screen size. However, pellet durability in this study was positively correlated to the total specific energy in biomass pellets with FFSG.

Table 8 presents moisture content and ash content of biomass and pellets manufactured by the pilot-scale pellet mill. Moisture content of different treatments was different depending on the binder type and lubricant used to be manufactured. Adding binders did not increase the ash content markedly, even in pellets added with bentonite. This is related to the low concentration of binder in the mixture. Kashaninejad and Tabil (2011) reported that ash content increased from 6.3% in untreated biomass to 17.3 and 34.8% when 1 and 2% NaOH solution, respectively were used in microwave pretreatment of biomass.

Addition of binders for improving the pellet quality should also take the production economics into consideration. Even though the usage of binders tends to reduce the feed production costs, generally only those binders are selected which can improve production economics significantly. In this study, the tests of the physical properties and enzyme activity of the pellets showed that the combination of pellets having FFSG and golden flake resulted in higher pellet quality. However, FFSG is an expensive product (approx. CAD$ 350-500/t), which can adversely affect the production economics. Among the three binders, which gave higher pellet density and durability values, bentonite has the lowest cost (approx. CAD$ 97/t), whereas cornstarch is the most

Table 4. Composition and temperature of pellets discharged from pilot-scale pellet mill die.

Binder	Lubricant	Discharge temperature (°C)
Bentonite (1.5%)	Mineral oil (1.5%)	52.4
	Golden flake (1.5%)	46.2
	Silver prills (1.5%)	50.4
Corn starch (5.0%)	Mineral oil (1.5%)	55.2
	Golden flake (1.5%)	51.5
	Silver prills (1.5%)	50.0
FFSG (5.0%)	Mineral oil (1.5%)	85.6
	Golden flake (1.5%)	84.3
	Silver prills (1.5%)	87.6

FFSG: fructooligosaccharide, fructose, sucrose, glucose
Percentages indicated above for binders and lubricants are based on total mass of the sample (biomass plus binder and additive).

expensive (approx. CAD$ 500-700/t). In the pilot-scale pellet unit, fats and oils (hydrogenated golden flake, hydrogenated silver prills, and mineral oil) were also added to provide lubrication during pelleting. Hydrogenated golden flake is commercially available for approximately CAD$ 1890-1990/t and hydrogenated silver prills fat is commercially available for approximately CAD$ 2400-2650/t. It depends on the feed manufacturer to select binders and oils for feed production based on the combination of product quality and production costs.

Table 5. Effect of binder (B) and lubricant (L) type on bulk density and durability of pellets made by the pilot-scale pellet mill.

Source of variation	DF	Bulk density		Durability	
		SS	P-value	SS	P-value
B	2	13786	<0.01	713	<0.01
L	2	3880	<0.01	1907	<0.01
B × L	4	5252	<0.01	1330	<0.01
Residuals	18	2993	---	15	---
Total	26	25911	---	3964	---

CONCLUSION

The effect of different binders and pelleting temperature on density, durability, and enzyme activity of pellets made in single-pelleter unit (SPU) from feed enzyme-containing biomass was successfully investigated. Among all the tested binders, bentonite, corn starch, and FFSG (fructooligosaccharide, fructose, sucrose, glucose) resulted in the highest values of pellet density and durability with very minor enzyme activity loss at pelleting temperature of 85°C. These three binders in combination with three different lubricants (mineral oil, hydrogenated golden flake, and hydrogenated silver prills) were mixed with biomass and were pelleted using the pilot-scale pellet mill. Pellets manufactured in the pilot-scale pellet mill had high bulk density, pellet density and durability. Grain-based fungal biomass mixed with FFSG and golden flake resulted in the most durable pellets with low moisture

Table 6. Bulk density, pellet density, durability, throughput, total specific energy and enzyme activity of pellets made by the pilot-scale pellet mill using different binders and 1.5% of different lubricants presented as means (standard deviations).

Binder	Lubricant	Bulk density[(1)] (kg/m^3)	Pellet density[(2)] (kg/m^3)	Durability[(1)] (%)	Throughput (kg/h)	Total specific energy[(3)] (MJ/t)	Enzyme activity[(5)] (%)
Bentonite	Mineral oil	741 (21)[(4)e]	1136 (73)^{c}	90.6 (0.7)^{d}	21.18	197	96.8
(1.5%)	Golden flake	800 (9)^{bc}	1229 (48)^{b}	95.8 (1.6)^{b}	9.44	352	100.0
	Silver prills	760 (7)^{de}	1264 (54)^{ab}	79.2 (0.4)^{e}	8.54	410	89.5
Corn starch	Mineral oil	782 (24)^{cd}	1151 (57)^{c}	97.4 (0.6)^{a}	25.74	178	100.0
(5.0%)	Golden flake	804 (12)^{bc}	1238 (62)^{ab}	91.7 (0.8)^{cd}	12.35	259	100.0
	Silver prills	764 (1)^{de}	1226 (45)^{b}	57.9 (1.3)^{f}	5.56	726	100.0
FFSG	Mineral oil	810 (3)^{b}	1282 (46)^{a}	92.7 (1.1)^{c}	14.46	344	99.4
(5.0%)	Golden flake	815 (7)^{ab}	1244 (31)^{ab}	98.9 (0.1)^{a}	10.53	430	100.0
	Silver prills	837 (11)^{a}	1272 (20)^{ab}	93.2 (0.3)^{c}	19.03	350	96.3

FFSG - fructooligosaccharide, fructose, sucrose, glucose
(1) n = 3
(2) n = 10
(3) Total specific energy was calculated for running pellet mill plus energy required for manufacturing pellet.
(4) Mean values with at least one common letter are not significantly different at P = 0.05
(5) The enzyme activity percentage was calculated relative to the feed enzyme before adding any binder or pelletizing.
Percentages indicated above for binders and lubricants are based on total mass of the sample (biomass plus binder and additive).

Table 7. Effect of binder (B) and lubricant (L) type on pellet density of pellets made by the pilot-scale pellet mill.

Source of variation	DF	Pellet density	
		SS	P-value
B	2	68601	<0.01
L	2	65946	<0.01
B × L	4	72350	<0.01
Residuals	81	208954	---
Total	89	415851	---

content and high enzyme activity. Taking production economics into account, the high cost and addition rate (5%) of FFSG may make it uneconomical for use. Bentonite may be an economical binder alternative resulting in pellets having reasonable durability and enzyme activity.

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Table 8. Moisture and ash contents of pellets made by the pilot-scale pellet mill using different binders and lubricants (n = 2), means (standard deviations).

Binder	Lubricant	Moisture content (% wb)	Ash (% wb)
Grain-based fungal biomass	---	8.5 (0.1)	7.8 (0.0)
Bentonite	Mineral oil (1.5%)	10.9 (0.1)^{a}	8.3 (0.0)^{d}
(1.5%)	Golden flake (1.5%)	8.4 (0.3)^{cd}	7.6 (0.0)^{e}
	Silver prills (1.5%)	7.3 (0.1)^{e}	8.9 (0.0)^{c}
Corn starch	Mineral oil (1.5%)	10.0 (0.0)^{b}	7.4 (0.2)^{f}
(5.0%)	Golden flake (1.5%))	8.7 (0.2)^{c}	9.1 (0.1)^{b}
	Silver prills (1.5%)	6.2 (0.1)^{g}	7.6 (0.0)^{e}
FFSG	Mineral oil (1.5%)	6.7 (0.3)^{f}	9.3 (0.1)^{a}
(5.0%)	Golden flake (1.5%)	8.1 (0.0)^{d}	7.3 (0.1)^{f}
	Silver prills (1.5%)	6.4 (0.1)^{fg}	7.7 (0.0)^{e}

Mean values with at least one common letter are not significantly different at P = 0.05
FFSG - fructooligosaccharide, fructose, sucrose, glucose
wb: wet basis
Percentages indicated above for binders and lubricants are based on total mass of the sample (biomass plus binder and additive).

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