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Densification of Heat Sensitive Protein/Fibre Biomass

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ABSTRACT Production of feed enzyme, from biomass material, is growing as it is used in the poultry industry to improve feed utilization efficiency. This biomass has low bulk density and irregular shape and size which makes it difficult to handle, transport, store and utilize in its 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, biomass containing heat sensitive enzyme, was mixed with ingredients like binder and fat at different levels. Pellets were made at a pre-set load of 4400 N (138.9 MPa) using single-pelleter (SP) attached to the Instron testing machine at different temperatures to study the effect of pelleting temperature on enzyme activity. Quality of pellets was determined by measuring pellet density, durability and enzyme activity to select the condition resulting in the most durable pellets containing the highest enzyme activity. Pellets added with bentonite, corn starch, 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 SP, three binders were chosen to make pellets in the pilot-scale pellet mill by employing three different oils as lubricant. Binder and lubricant types had significant effect on bulk density, pellets density, and durability. Overall, pellets made by 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 pilot scale pellet mill was not reduced and it ranged from 90 to 100%.

Keywords: Biomass, Enzyme activity, Feed enzyme, Binder, Lubricant.

INTRODUCTION

Poultry feed containing cereal grains (wheat, barley, oats, triticale, and rye), which are high in non-starch polysaccharides (NSP) may be supplemented with enzymes to optimize the results 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, relatively high molecular weight compounds, dissolve in the intestine and increase viscosity of the gut contents. Consequently, the animal growth rate reduces 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. 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 material. Nevertheless, there is a need to overcome challenges in this application. Biomass, naturally, 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 (40-200 kg/m^3) biomass from loose or bale form to pellet and cubes with higher bulk density (600-800 kg/m^3) (Kaliyan and Morey, 2009). The feed enzyme is produced as biomass containing about 30% protein. The activity of granulated and powder enzymes is affected by heat treatment and the moisture content of enzyme has been found to be inversely proportional to the enzyme activity at high temperatures (Slominski et al. 2007). In addition, overheating of the biomass during conditioning or pelletizing in the pellet mill die due to the frictional heating could happen. This overheating could result in an increased 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). Therefore, densification of biomass containing heat-sensitive feed enzyme has some restrictions. Employing some treatments such as steam explosion or microwave heat treatment could destroy enzymes. Application of binders during pelletizing could make up the adverse effect of low temperature and moisture during pelleting on the strength and durability of the densified biomass.

Generally, the structure of agricultural biomass is a lignocellulosic matrix and the durability of pellets depends on thermo-physical forces that used to bond particles together (Kashaninejad and Tabil, 2011; Kaliyan and Morey, 2009). Binders have been categorized to three types: a) matrix type such as dry starch, clay, and dry sugar; b) film type such as bentonite, lignosulfonates, and gum; and c) chemical binders such as $Ca(OH)_2$ and NaOH (Pietsch, 1976). The main binding reactions that improve durability and strength are starch gelatinization, protein denaturation, and solubilization and consequently, the recrystallization of sugars and salts. Starch is gelatinized in the presence of moisture and heat which is provided under steam conditioning or shearing force in die. As gelatinized starch content increases, the durability of pellet increases (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 increases pellet durability. Heat, moisture, and shear force result in protein denaturation particularly in cereals, such as wheat, rye, and barley which have the potential to make dough. A study showed that raw protein increased pellet durability better than denatured protein (Wood, 1987). Fibre is not a good binder between particles since it has high resilience (Kaliyan and Morey, 2009). Biomass has a lignocellulosic matrix containing cellulose, hemicellulose, and lignin (Adapa et al., 2009). 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., 2009). Pretreating biomass with some chemicals such as NaOH, CaO, or urea degrades the cell wall structure and separates lignin from cellulose. Therefore, the resilience characteristics of the fibers are reduced after pretreating the biomass and may increase the durability of pellets. Incorporation of water (19-23%) also lowers resilience characteristics of the fibers. The presence of fat and oil reduces pellet durability since fat acts as lubricant between particles and die wall. However, fat in the material to be pelleted acts as a lubricant in the pellet mill die and facilitates its ejection through the die. The natural fat in the cell wall could come out and play role of a bridge between particles and increase durability. Nevertheless, fat is not recognized as a binder and usually, it is used as lubricant (Kaliyan and Morey, 2009).

There are a 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 binder. 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.

With the desire to manufacture a product which is less dusty, highly densified, and easier and efficient to handle, our group came together to address these issues. The primary objective of this study was to increase the bulk density of feed enzyme-containing biomass to reduce dust and facilitate transportation and handling by pelletizing and preserving the enzyme activity of the

final product. The effect of different binders, pelleting temperatures, and lubricants on durability and enzyme activity was studied in the single pelleting (SP) unit and the pilot-scale pellet mill.

MATERIAL AND METHODS

Materials Feed enzyme containing biomass was obtained from GNC Bioferm (Bradwell, SK) in the fall 2012. The biomass particles were already fine and kept in air-tight bag to keep initial moisture content. 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 binder. Mineral oil (Swimco Canada Inc., Georgetown, ON), hydrogenated silver prills (Trident Feeds, UK) with melting point of 55°C and hydrogenated golden flake fat (Trident Feeds, UK) with melting point of 50-55°C were used as lubricant additives in the pilot-scale pellet mill (CPM).

Sample Preparation and Densification The required amount of water was calculated by mass balance between the original ground sample and the desired sample moisture content of 12%. All treatments were adjusted to 12% moisture content, except treatment with FFSG because FFSG was liquid and provided enough moisture for densification. The sample was re-moistened by adding the required water, mixing it in an air-tight bag. Samples were stored at room temperature and mixed every 12 h for at least 72 h to ensure moisture equilibration. Moisture-adjusted biomass was mixed with the binder at the designed level. The amount of binder for each treatment was as follows: bentonite 1.5%, corn starch 5.0%, FFSG 5.0%, lignosulfonate 1%, pea starch 5%, waxy barley 5% and maltodextrin 5%. In the case of FFSG, the binder was added to biomass without any moisture adjustment as FFSG was liquid.

The biomass was pelleted in a SP unit 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 pf 125 mm. The die was coiled with a heating element maintaining the temperature at appropriate point to simulate frictional heating in commercial pelleting (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 (Model 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 a hole allowing the densified sample to be discharged from the die when sliding gate was opened. Moisture-adjusted sample (0.5-0.6 g) was loaded into the die when the temperature was stable at the set point. The compressive force applied using the Instron machine fitted with a 5000 N load cell and a pre-set load of 4400 N corresponding to a pressure of 138.9 MPa was used to densify the material. The crosshead speed of plunger was set at 50 mm/min. When the compression load achieved the pre-set load, the plunger stopped and was retained in place for 60 s to start the relaxation test (Kashaninejad and Tabil, 2011) and also to avoid spring-back of biomass (Mani et al., 2006). The plunger was then moved up to release compression force, the sliding gate was opened, the plunger moved down after 30 s to eject 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 (d = 85.0 mm) and ring die assembly. The diameter of ring die was 190.5 mm with 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 250 rpm.

According to preliminary test, 12% moisture content was not adequate to manufacture pellets in CPM. Therefore, the moisture content of biomass grinds (2 kg) was adjusted to 14% (instead of 12% used in SP) and the required amount of binder was added, similar to sample preparation for SP experiments, and mixed for 2 h in a sealed plastic bucket mounted on a concrete mixer. According to the preliminary tests conducted using the SP unit, the amount of binder for each treatment was as follows: bentonite 1.5%, corn starch 5.0%, FFSG 5.0%. The FFSG binder was added to biomass without any moisture adjustment. Hydrogenated fat, as binder, preheated in an oven at 50°C to melt, was sprayed over the sample to obtain a homogenous mixture. The mixture was fed to the pellet mill without any 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 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 was employed to record pellet mill energy consumption (kWh) in actual time and to calculate the specific energy (MJ/t) required making pellets from each treatment. Temperature of pellets was determined just at the discharge die using an infrared thermometer (Model 42530, Extech Instruments, Nashua, NH). Pellets were cooled down by spreading on a paper sheet at ambient temperature of the laboratory (ca. 20ºC). Once cooled, the pellets were stored in plastic bag for further tests.

Particle Size Analysis The geometric mean diameter of biomass was determined using ASAE Standard S319 (ASABE, 2011). A Ro-Tap sieve shaker (W. S. Tyler Inc., Mentor, OH) was used for particle size analysis. 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 used to analysis particle sizes. The sieves were placed on a Ro-Tap sieve shaker for 10 min sieve shaking time. The geometric mean diameter (d_{gw}) and geometric standard deviation (S_{gw}) were calculated in three replicates for each sample.

Bulk Density and Particle Density Bulk density of biomass 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. The funnel was removed from top of the container; the container was tapped on a wooden table for approximately 10 times to allow the material to settle down. The container was leveled by rolling a cylindrical stainless steel bar across the container in two perpendicular directions. Subsequently, the container was weighed. The mass per unit volume yielded the bulk density of the biomass in kg/m^3. The bulk density was determined in five replicates for each sample.

Pellet Durability Durability of pellets obtained from SP unit 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). Pellets (100 g) 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 weighed to determined percentage of broken pellets respect to the initial pellet weigh during tumbling, as durability value.

Chemical Analysis The moisture content of sample was determined in three replicate using AACC standard 44-15A (AACC, 2005), where 2 to 3 g of material was oven-dried at 130°C for 90 min in duplicates. The required amount of water was calculated by mass balance between the original sample and the sample with targeting moisture content. The sample was re-moistened by adding required water and mixed in an air-tight bag. Samples were stored at room temperature and mixed every 12 h for at least 48 h to ensure moisture equilibration. 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 of, 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. Quantification of enzyme activity 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°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 1 l deionized water). The samples were cooled, diluted with 8 ml deionized water and optical density (OD) determined at 540 nm. OD readings for unknown enzyme samples were expressed as a percentage of the reference enzyme standard.

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.

RESULTS AND DISCUSSION

Physical and Chemical Properties of Biomass Figure 1 presents the particle size distribution of biomass. The biomass had geometric mean diameter of 0.185 ± 0.174 mm which was smaller than biomass samples ground by different hammer mill screen size (6.4, 3.2, 1.6

and 0.8 mm) to make pellet by Adapa et al. (2010). Particle density and bulk density of biomass were 1446.8 ± 16.5 and 500.8 ± 3.6 kg/m^3, respectively. Table 1 shows the chemical composition of biomass. The crude protein content was significantly higher than biomass in the previous study (1.61 and 1.99% in wheat and barley straw, respectively) reported by Kashaninejad and Tabil (2011). This was related to the presence of enzyme in the biomass. However, the cellulose and hemicellulose were lower than the values reported by previous studies (Kashaninejad and Tabil, 2011). The lignin content (4.9%) was significantly lower than biomasses reported by Adapa et al. (2010) (12.85-17.13%) and Kashaninejad and Tabil (2011) (8.33-11.95%).

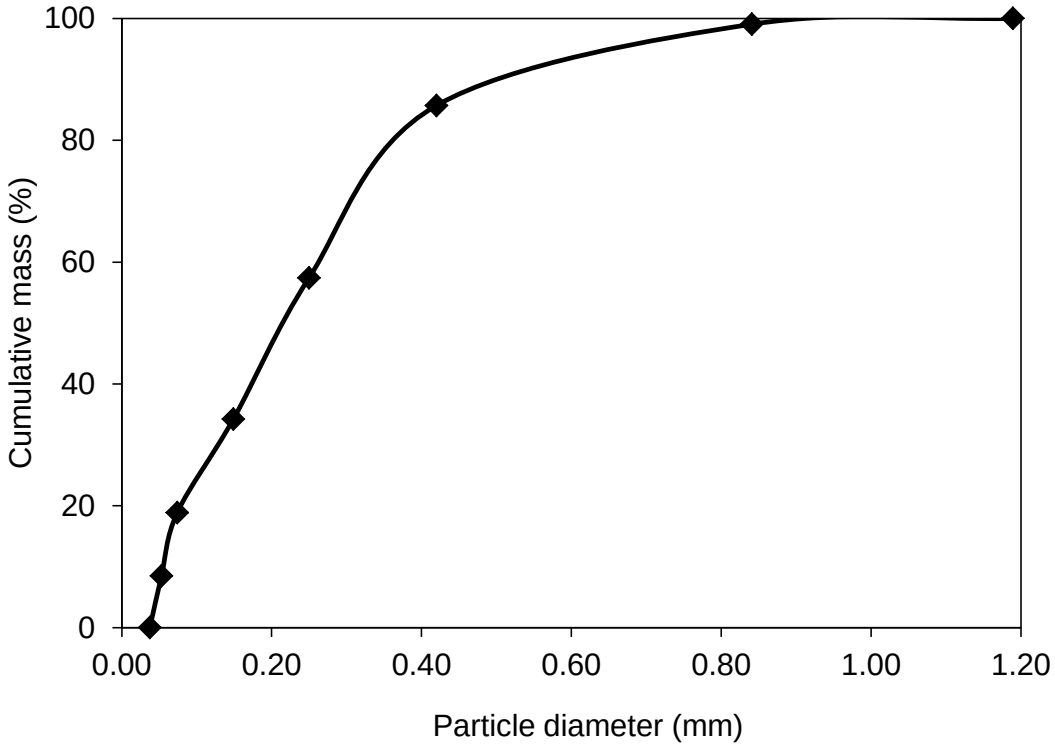

Figure 1. Typical particle size distribution of feed enzyme.

Table 1. Chemical composition of feed enzyme biomass (mean ± standard deviation).

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

Pellet Density, Durability and Enzyme Activity of Pellets made by SP 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). The density of all pellets made by binders, except FFSG, was higher compared to control pellet (average 1169 kg/m^3, Table 3). This trend was in agreement with values reported by Tabil (1996) for pelletizing low and intermediate quality of alfalfa chops using bentonite, lignosulfonate or corn starch. The density of pellets made with bentonite and lignosulfonate at 85°C (1355 and 1341 kg/m^3, respectively) was close to the density reported by Tabil (1996) for pelletizing alfalfa using the same binders (1366-1382 and 1333-1363 kg/m^3, respectively). However, the density of pellets made with pea starch (1256-1286 kg/m^3) was lower than alfalfa pellet made with the same binder (1366-1383 kg/m^3) (Tabil 1996). Increasing the pelletizing temperature resulted in higher density.

The effect of binder, temperature, and their interaction effects on pellet durability was 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 in Tabil et al. (1997) work was based upon the Kapt-al pellet durability tester as reported by Larsen et al. (1996). Pellet durability increased as pelletizing temperature increased. It was related to the effect of heat causing 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 was related to low moisture content in the mixture as stated by Slominski et al. (2007). Enzyme activity of some treatments (such as bentonite and pea starch) was even lower at higher pelleting temperature which was related to error of assay. The lowest enzyme activity was observed when FFSG was used as binder (76.8-79.5%). It was related to the presence of high moisture content in this binder; treatments with higher moisture content

have lower temperature resistibility. Nevertheless, an enzyme activity of 76.8-79.5% is an acceptable range for the feed industry.

Table 2. Effect of binder type (B) and temperature (T) on pellet density, durability, and enzyme activity of pellets made by SP

Source of variation	DF	Pellet density		Durability	
		SS	P-value	SS	P-value
B	7	1734937.85	<0.01	17382.66	<0.01
T	2	357833.13	<0.01	13542.16	<0.01
B × T	14	349156.65	<0.01	12064.26	<0.01
Residuals	216	1149327.35	---	23905.72	---
Total	239	3591254.98	---	66894.79	---

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

Table 3. Pellet density, durability and enzyme activity of pellets made by SP using different binders at different temperatures (n = 10, mean ± standard deviation)

Binder	Temperature (°C)	Pellet density (kg/m^3)	Durability (%)	Enzyme activity[1] (%)
Control	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	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	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	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	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	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	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	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

[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

Physical Properties and Enzyme Activity of Pellets Made by the Pilot-scale Pellet Mill Figure 2 shows the pellets manufactured from biomass using bentonite, corn starch, and FFSG as binder and mineral oil, golden flake, and silver prills as lubricant in the pilot-scale pellet mill. Temperature of pellets discharged from CPM die is presented in Table 4. Pellets manufactured by binder of bentonite and corn starch discharged at temperature from 46.2 to 55.2°C. However, FFSG pellets discharged at markedly higher temperature (84.3 to 87.6°C). It may be related to the change in viscosity of FFSG caused by chemical reactions such as caramelization and polymerization. The effect of binder and lubricant type on bulk density was significant ($P < 0.01$, Table 5). The highest bulk density was observed in pellets made with FFSG (average of 821 kg/m^3) followed by those made with corn starch and bentonite, respectively. All three types of lubricant provided enough lubricity to eject the pellets through the die. Pellets made with golden flake had significantly higher durability than those made with silver prills and mineral oil (Table 6). Overall, treatment of pellets made with FFSG as binder and golden flake or silver prills as lubricant resulted in the highest bulk density (815-837 kg/m^3).

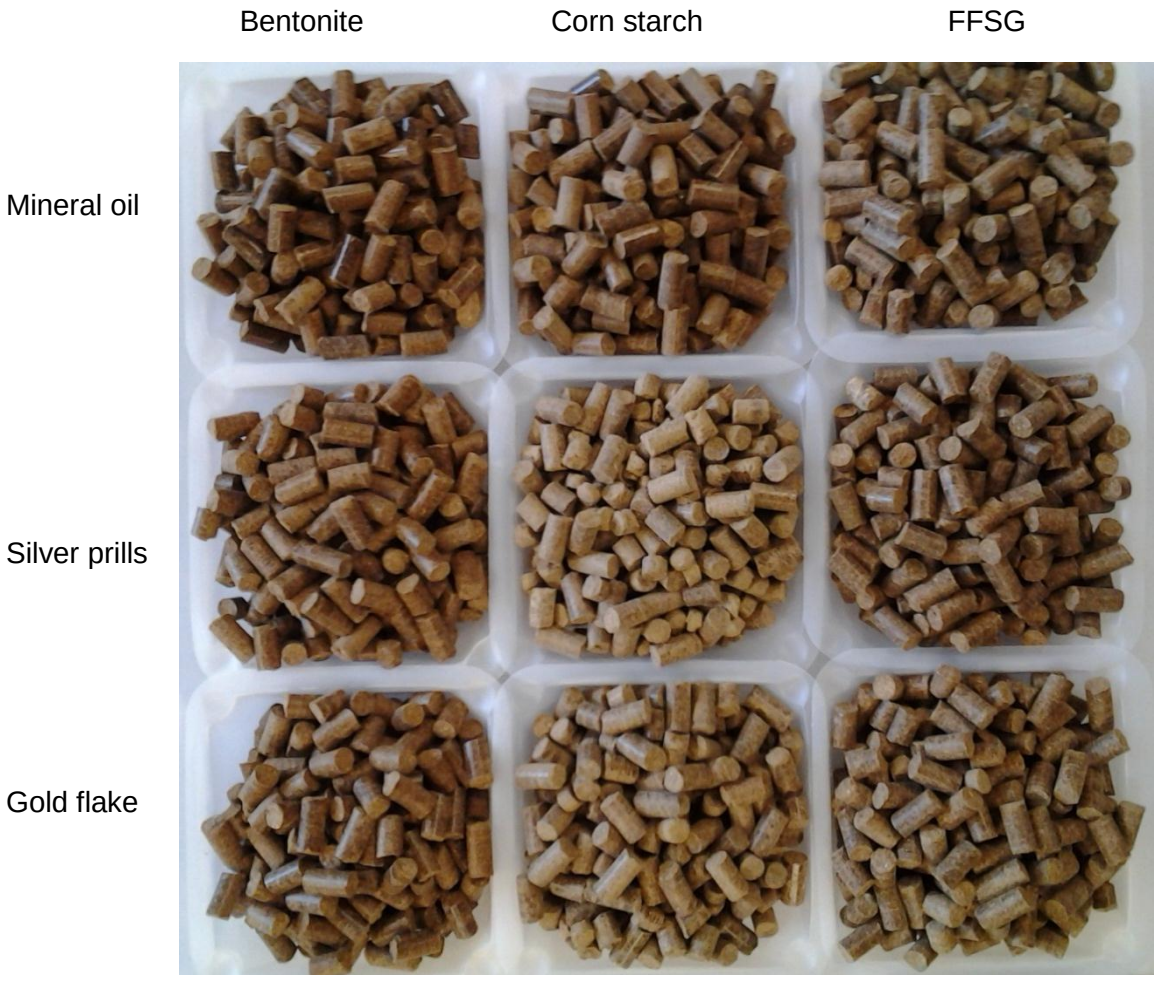

Figure 2. Photograph of pellets manufactured using bentonite, corn starch, and FFSG as binder and mineral oil, golden flake, and silver prills as lubricant in pilot-scale pellet mill.

Pellet density was significantly affected by 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. 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). The highest average of durability was observed in pellets with FFSG (94.9%) followed by bentonite and corn starch. Similar to the result of bulk density, the highest durability was obtained when golden flake was employed as lubricant. Although pellets made 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 lubricant resulted in higher durability compared to silver prills and mineral oil 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 reduction in enzyme activity of pellets was negligible. The enzyme activity in majority of treatments ranged from 96 to 100%, except in pellets made from bentonite and silver prills which had enzyme activity of 89.5%. The enzyme activity in even the latter treatment is acceptable in feed industry.

Pellet mill throughput of all treatments lubricated by mineral oil was 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 total specific energy (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.

Table 8 presents the moisture content and ash content of biomass and pellets manufactured by the pilot-scale pellet mill. Moisture content of different treatments were different depending on the binder type and lubricant used to be manufactured. Adding binders did not increase the ash content markedly, even in the bentonite treatment. This may be due 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 for microwave pretreatment of biomass.

Table 4. Temperature of pellets discharged from CPM die

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

Table 5. Effect of binder (B) and lubricant (L) type on bulk density and durability of pellets made by CPM

Source of variation	DF	Bulk density		Durability	
		SS	P-value	SS	P-value
B	2	13785.92	<0.01	712.98	<0.01
L	2	388038	<0.01	1906.77	<0.01
B × L	4	5251.84	<0.01	1329.51	<0.01
Residuals	18	2992.57	---	14.51	---
Total	26	25910.71	---	3963.77	---

Table 6. Bulk density, pellet density, durability, moisture and ash contents of pellets made by CPM using different binders at lubricants (mean ± standard deviation)

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
	Golden flake	800+9^{bc}	1229±48^{b}	95.8+1.6^{b}	9.44	352	100.0
	Silver prills	760+7de	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
	Golden flake	804+12^{bc}	1238±62^{ab}	91.7+0.8^{cd}	12.35	259	100.0
	Silver prills	764+1de	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
	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

[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.

Table 7. Effect of binder (B) and lubricant (L) type on pellet density of pellets made by CPM

Source of variation	DF	Pellet density	
		SS	P-value
B	2	68600.56	<0.01
L	2	65946.29	<0.01
B × L	4	72349.78	<0.01
Residuals	81	208954.00	---
Total	89	415850.62	---

Table 8. Moisture and ash contents of pellets made by CPM using different binders at lubricants (n = 2, mean ± standard deviation)

Binder	Lubricant	Moisture content (% wb)	Ash (% wb)
Feed enzyme	---	8.5±0.1	7.8±0.0
Bentonite	Mineral oil	10.9±0.1^{a}	8.3±0.0^{d}
	Golden flake	8.4+0.3^{cd}	7.6+0.0^{e}
	Silver prills	7.3+0.1^{e}	8.9+0.0^{c}
Corn starch	Mineral oil	10.0+0.0^{b}	7.4+0.2^{f}
	Golden flake	8.7+0.2^{c}	9.1+0.1^{b}
	Silver prills	6.2+0.1^{g}	7.6+0.0^{e}
FFSG	Mineral oil	6.7+0.3^{f}	9.3+0.1^{a}
	Golden flake	8.1+0.0^{d}	7.3±0.1^{f}
	Silver prills	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

CONCLUSION The effect of different binders and pelleting temperature on density, durability, and enzyme activity of pellets made in SP from feed enzyme-containing biomass was successfully investigated. Among all the tested binders, bentonite, corn starch, and FFSG resulted in higher pellet density and durability with very minor enzyme activity loss at higher pelleting temperatures. These three binders in combination with three different oils (mineral oil, hydrogenated golden flake, and hydrogenated silver prills) as lubricant were mixed with biomass and was pelleted using the pilot-scale pellet mill. Pellets manufactured in the pilot-scale pellet mill had high bulk density, pellet density and durability. Biomass sample mixed with FFSG and golden flake resulted in the most durable pellets with low moisture content (7.3% wb) and high enzyme activity (100.0%).

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