The Canadian Society for Bioengineering
The Canadian society for engineering in agricultural, food, environmental, and biological systems.

La Société Canadienne de Génie Agroalimentaire et de Bioingénierie
La société canadienne de génie agroalimentaire, de la bioingénierie et de l'environnement

CSBE | SCGAB

Paper No. CSBE13-024

Effect of Initial Moisture and Temperature on the Enzyme Activity of Pelleted High Protein/Fiber Biomass

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Written for presentation at the
CSBE/SCGAB 2013 Annual Conference
University of Saskatchewan, Saskatoon, Saskatchewan
7-10 July 2013

ABSTRACT High protein/fiber biomass which is used as a feed enzyme to increase feed utilization efficiency of poultry rations was pelleted. Feed enzymes cause viscosity reduction and changes in nutrient absorption, improving the nutritional quality of feeds. However, the pelleting process may cause enzyme denaturation. Optimization studies were conducted by varying the initial moisture content of the biomass and the pelleting temperatures. Tests were done in the single-pelleting unit and the results were validated using the pilot-scale pellet mill. The initial moisture content levels of biomass was varied from 14 to 22% w.b. and pelletized at temperatures of 60, 77.5 and 95ºC and pellet durability and enzyme activity were measured. To improve pellet durability, different binders were combined with the feed ingredients and the feasibility of producing pellets in a pilot-scale pellet mill was tested. The study showed that although high pellet durability was obtained from the biomass by pelleting with steam conditioning at 14% MC, combined with

1.5% bentonite, and 5% fat, the enzyme activity of the pellets were low. Thus, pelleting the biomass without steam conditioning can be a viable option provided that pellet durability is high.

Keywords: Feed enzymes, heat-sensitive protein/fiber biomass, pelleting, binders

INTRODUCTION The animal feed industry is highly dependent on cereal grains as animal feed ingredient. Cereal-based (wheat, barley, oats, etc.) feeds are high in non-starch polysaccharides (NSP). However, the digestibility of NSP in poultry is lower (Choct and Kocher, 2000). NSPs have chemical cross linking and endogenous enzymes are needed to digest them. The digestive system of poultry does not produce the endogenous enzymes needed to breakdown and digest plant cell walls. Therefore, feed enzymes are used to hydrolyze NSPs to improve feed digestibility, which in turn improves the growth and feed conversion efficiency of the poultry. NSPs are mostly soluble, increasing the viscosity in the intestine, leading to decrease in the feed passage rate and digestion impairment. According to Campbell and Bedford (1992), enzyme supplementation leads to decrease in the viscosity of the gut contents. As a result, the animal growth rate increases and the ratio of feed to weight gain increases (Marquardt et al. 1994; Silversides and Bedford, 1999). Due to this, the poultry feed industry has demonstrated an increased utilization of biomass-based feed enzymes as feed supplements. Using enzymes, animal diets with lower nutrient value can be formulated from readily available ingredients, such that the enzymes might uplift the nutrient content of the final product to the required value. Overall, the cost of the feeds is reduced, benefitting feed manufacturers (Khan et al., 2006). Therefore, the aforementioned reasons support the use of feed enzymes as poultry feed supplements.

However, the application of biomass-based feed enzymes has certain issues. Granulated or powdered enzymes have a low bulk density (40-200 kg/m^3). Using feed ingredients (including enzymes) in their natural fine/powdery form will lead to increased wastage, and high transportation, storage, and handling costs. In order to overcome these challenges, the feed enzyme-containing biomass can be densified to the pellet-form, increasing the bulk density (600-800 kg/m^3) of the final product. Chemical, physico-chemical (Adapa 2011), and biological pre-treatments (Kashaninejad and Tabil, 2011) to improve binding among particles can be done before the densification of agricultural biomass. Nonetheless, heat pretreatments using chemicals are not preferred because they may denature the enzyme. Under these circumstances, pelleting is a potential method of densification which can be used. Pelleting is affected by various factors, among them being moisture content and pelleting temperature, two of the most important. Moisture content of feed particles during pelleting serves as the conduit for the transfer of the heat into the feed particles. High pelleting temperatures are essential for complete starch gelatinization. During gelatinization, the starch molecules adhere to each other and to other particles, increasing the pellet durability. During pelleting, the feed ingredients are modified, such that starch molecules are gelatinized, sugars are recrystallized, and protein molecules are denatured. These changes improve the binding characteristics of the feed pellets. However, low temperature and moisture during pelleting can affect pellet quality adversely, negating the benefits of pelleting. On the other hand, according to Slominski et al. (2007), higher moisture content of the enzymes may also reduce the enzyme activity at high pelleting temperatures. Therefore, pelleting of enzyme-containing biomass should be done ensuring that enzymes are not denatured. This can be done by pelleting at low temperature and moisture conditions. When binders are included in the feed ingredients, this issue may be resolved.

Addition of commercial binders improves the physical quality of the feed pellets (Abdohalli et al., 2012). Pellet binders improve the bond among the feed particles, significantly increasing the throughput of the pellet mill, whilst reducing the power consumption. Different binders such as lignosulfonates, bentonite, corn starch, mineral binders, and gums are available commercially. The inclusion rate of the binders should be controlled to ensure that they disperse uniformly throughout the feed. Certain binders increase the nutrient content of the feeds by acting as the source of minerals, therefore, they are highly preferred. In addition to binders in commercial pelleting process, fats are also included in the feed ingredients for lubrication and to reduce the generation of dust from fines generated by the pellets. Fats lubricate the pellet die (Coffey et al., 1995), increasing the output and reducing the energy consumption of the pellet mill.

A study should be conducted to optimize the pelleting process of high protein/fiber biomass containing enzymes, focusing on two main factors, i.e. pelleting temperature and moisture content of the feed. In this study, different binders were included in the ingredient composition to study the effect on the physical properties of the pellets formed and the enzyme activity of the high protein/fiber biomass. Therefore, the two main objectives of this study are as follows. 1) to optimize the pelleting processing to improve the physical characteristics of the high protein/fiber biomass; and 2) to determine the combination of feed ingredients and processing conditions which maintain the enzyme activity of the biomass-based feed enzyme.

MATERIALS AND METHODS

Materials The grain-based fungal biomass containing enzyme used in this study was obtained from GNC Bioferm Inc., Bradwell, SK. The biomass was barley-based and it was developed by passing it through a bed of reactors, followed by inoculation of a certain strain of mold. It was developed as an enzyme supplementation for poultry feed. The biomass was stored in air-tight containers to prevent moisture loss. The two binders used in the study were bentonite and corn starch because both the binders have good binding quality. Bentonite was supplied by Canadian Clay Products Inc., Wilcox, SK and corn starch was obtained from Ingredion Canada Inc., Pointe-Claire, QC. For pilot-scale pelleting, hydrogenated fat was used for lubricant. The hydrogenated fat, i.e. silver prills was supplied by Trident Feeds, UK.

Sample Preparation The biomass samples were divided in to four groups. The initial moisture content of each group of samples was determined to be 6.79% (w.b.) using AACC standard 44-15A (AACC, 1995). The moisture content of each group was adjusted to 14, 18, 20, and 22% respectively by adding water and mixing for uniform distribution. For adjusting the water levels, the required amount of water was calculated by mass balance between the original moisture content of the ground sample and the desired mash moisture content. The samples were stored in double Ziploc bags at room temperature to retain the moisture content and mixed every 12 h to ensure moisture equilibration. To each sample, the two binders were added as follows: 1.5% bentonite (by mass) and 5% corn starch. Both the binders were in powdered form and were mixed uniformly to ensure that they bind with the particles thoroughly.

Densification Methods

Single pelleting - The single pelleter unit (SPU) used in this study comprised of a plunger-die assembly (Adapa et al. 2002). The main components of the pelleter were: a cylindrical die (125 mm internal length and 6.35 mm diameter), a plunger, and a crosshead (Kashaninejad and Tabil 2011). Thermocouples were used to monitor the pelleting temperature (Tabil 1996). After pelleting, the pellets were ejected through the hole of the stainless steel die.

Approximately 0.55-0.65 g of the moisture adjusted biomass-binder sample was loaded into the cylindrical die for each run. The samples were pelleted under varying temperatures (60, 77.5, and

95˚C). For each run, a 5000 N force and 50 mm/min plunger crosshead speed was used to compress the samples by direct compression (Tabil 1996). After the pellets ejected through the die hole (Kashaninejad et al. 2010), the mass and the dimensions of the pellets were measured. The pellets were stored in Ziploc bags for 14 d before they were tested for durability and enzyme activity.

Pilot-scale pelleting - The pilot-scale pellet mill (Laboratory Model CL-5, California Pellet Mill Co., Crawfordsville, IN) was used to produce biomass pellets. The two main components of the pilot-scale mill (CPM) are a conditioning chamber and the pelleting assembly. The steam conditions the samples in the conditioning chamber (Tumurulu et al. 2010). The chamber is 830 mm in length and 102.7 mm in diameter. The pellet mill has a rotating ring die assembly (Tabil 1996; Adapa et al. 2004), in which the samples are compressed into the die holes. The roller mill runs at 250 rpm, driven by a 1.5 kW motor (Adapa et al. 2010). A 7.9 mm die diameter with length-to-diameter (L/D) ratio of 4.1 was used for this study.

Prior to pelleting, to each biomass-binder combination, 5% hydrogenated fat was mixed uniformly to ensure lubrication during the pelleting process. Prior to running the biomass samples through the mill, ground wheat with about 1-2% added oil was run through the mill to clean out the interiors and avoid clogging (Tabil 1996). The biomass samples were pelleted in three conditions in the pilot-scale pellet mill: 1) the sample was pelleted without heating (steam and the electric heating pads of the conditioning chamber wall were OFF); 2) the biomass sample was pelleted with steam conditioning (loaded into the CPM and passed through the conditioner, where superheated steam conditioned the samples); 3) the samples was pelleted by first running the mash continuously through the conditioning chamber to reach the desired temperature (85ºC) without steam conditioning (but the electric heating pads of the conditioning chamber wall were ON).

Thermocouples placed in different parts of the pilot-scale pellet mill, monitored the temperature of the mash and pellets. The throughput of the pellets from the pellet mill was determined by collecting the amount of pellets from each run. The pellets were spread for cooling to room temperature for approximately 24 h. The mass, length, and diameter of the cooled pellets were measured and the pellets were stored in Ziploc bags for 14 d before they were tested for durability and enzyme activity. The specific energy consumption was calculated and expressed as kWh of energy consumed per tonne of pellets.

Evaluation Methods

Pellet density - The density of the pellets was calculated by measuring the weight, length, and diameter of the pellets. A digital caliper was used to measure the length and diameter, whereas, an electronic balance with a 0.01 g precision measured the weight of the pellets. Pellet density was expressed as the mass and volume ratio (Shankar et al. 2007; Shankar et al. 2008).

Pellet durability (single pelleting) - Durability of the biomass pellets 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). In this test, a single pellet was dropped from a height of 1.85 m on a metal plate. Due to impact, the pellets break. The weight of the larger portion of the pellet retained was measured and expressed as the percentage of the initial weight of the pellet yielding the pellet durability.

Pellet durability (pilot-scale pelleting) - For the pilot-scale pellet samples, the durability of pellets was measured using the tumbling test, as given in ASABE Standard S269 (ASABE, 2011). For this test, 100 g pellets were placed in the tumbling test chamber and tumbled for 10 min at 50 rpm. The pellets were sieved using a 5.7 mm (US sieve size 3 1/2) round sieve to separate the fines from the coarse and broken pellets. To determine durability, the pellets retaining in the sieve were weighed and expressed as a percentage of the initial weight of the pellets.

Bulk density - The bulk density apparatus (SWA951, Superior Scale Co. Ltd., Winnipeg, MB) was used for measuring the bulk density of the pellet samples from CPM. The apparatus comprised of a funnel and a 0.5 L steel cylinder (Kashaninejad et al. 2010). The weight of the empty cylinder was recorded, following which, the pellet samples were dropped continuously in to the cylinder, filling it. The samples were leveled with a steel rod and weighed (Adapa et al. 2009). The mass by volume ratio for the pellet samples was calculated which represented the bulk density of the samples (Adapa et al. 2010). Three replicates of the bulk density test were performed for each sample.

Enzyme activity - The β-glucanase assay was used to determine the enzyme activity of the biomass pellet samples. The activity of β-glucanase was determined to be 276, which is used as the reference standard value. Using the assay, the reducing sugars in the samples were determined for enzyme quantification. The reaction was initiated by weighing 30mg of the sample and adding it to the reaction mixture. The reaction mixture comprised of 1 ml 0.5% (w/v) lichenan (Icelandic moss, Sigma-Aldrich Canada Co., Oakville, ON) in 8 ml of 0.1 M sodium-acetate buffer, pH 4.0. The sample-reaction mixture was incubated at 30ºC for 0, 5, or 10 min. The reaction was stopped by the addition of 1.0 ml of 3,5-dinitrosalicylic acid solution (16 g NaOH, 300 g Na-K-tartrate, 10 g 3,5-dinitrosalicyclic acid in one l deionized water), followed by cooling the samples using 8 ml deionized water. The amount of reducing sugars was measured by determining the optical density (OD) of the samples at 540 nm. The OD readings were expressed as a percentage of the reference enzyme standard.

Statistical analysis

The statistical analysis was done using the Statistical Analysis System (Version 9.3, SAS Institute Inc., Cary, NC). The study aimed at determining the temperature (60, 77.5 or 95°C) and moisture content (14, 18, 20 or 22%) threshold for enzyme activity in the biomass-based feed enzyme. Statistical analysis was used to determine whether the absence or presence of binder (bentonite or corn starch), heating methods used, and inclusion of fat improved the physical properties of the pellets. The GLM procedure and analysis of variance (ANOVA) was used to evaluate the effect of each variable and their interactions on the pellet quality.

RESULTS AND DISCUSSION

The aim of this study was to develop enzyme supplements for poultry feeds. The biomass used in this study had almost 20% protein and it contained high levels of lignin, cellulose and hemicellulose (almost 20%). The protein levels were higher than the usual levels in other biomass, which could be due to the presence of enzyme.

Single Pelleting

Pellet density, durability, enzyme activity - The three factors which were varied in this study were the binders used, the initial moisture content of the biomass samples, and the pelleting temperatures. Table 1 shows the results for pellet density, durability, and enzyme activity for the pellet samples with different combinations of binders, moisture levels, and pelleting temperatures made in the single pelleting unit (SPU). The highest pellet density was observed in pellet samples with corn starch, 14% MC, and higher pelleting temperatures (77.5 and 95°C). For better understanding of the combined effect of moisture and temperature levels on the pellet density and durability of pellets made in the SPU, response surface plots were produced, as shown in Figure 1 (a, b). Corn starch is used as a binding and thickening agent, which increases particle binding quality. Higher temperatures lead to changes in the lignin, cellulose, hemicellulose, and sugars present in the biomass, increasing their adhesive properties further. The effect of moisture levels, pelleting temperatures, binders, and their interactions on pellet density were significant ($P < 0.01$, Table 2). In comparison to control samples (no binder), the pellet density values were higher for lower moisture levels (14%) for all binder combinations.

Pellet durability is the measure of the ability of the pellet to withstand handling and transportation prior to consumption. In this study, the pellet durability values for all the combinations of binders and pelleting temperatures were almost similar. The highest durability was observed for pellets with bentonite (98-100%) as the binder. Bentonite has a high water absorption capacity and is commonly used for feed pelleting (Tabil, 1996) to reduce the production of fines and to increase the binding quality of pellets. The statistical analysis for the pellet durability results showed that only moisture levels had significant effect on pellet durability ($P < 0.01$, Table 2).

The enzyme activity of most of the biomass samples reduced (but not statistically significant compared to blank samples) after pelleting. The lowest value for enzyme activity was observed for feed enzyme pellets made without the usage of binders. The pellets made with 22% MC and 60°C had the lowest enzyme activity (69.2%). With the inclusion of binders to the biomass samples, the enzyme activity was between 85 and 99%. Binders might play an important role in the prevention of enzyme denaturation. Further studies are required to establish the effect of binders on enzyme activity. Statistical analysis showed that moisture content, binder and their interaction had slightly significant effect on enzyme activity (P value < 0.05). In the single pelleting unit, the residence time of the samples affects enzyme activity. Since the residence time of the biomass samples in the pelleter die is only about 105 s per run, the biomass samples are not exposed to the set-point temperature for too long. Due to this, temperature may not affect the enzyme activity of the biomass samples. The statistical analysis of the samples also shows that the effect of temperature on the enzyme activity of the samples is insignificant (Table 2).

In general, pellets with bentonite had higher pellet density and durability values. It was also observed that at high moisture content levels (20 and 22%), pellets were not formed.

The combination of corn starch/bentonite and high pelleting temperatures along with optimum moisture levels leads to development of pellets with good physical quality. Bentonite has the lowest cost (approx. CAD$ 97/t) in comparison to corn starch (approx. CAD$ 500-700/t). Therefore, bentonite is selected for pilot-scale pelleting to reduce production costs.

From the results of single pelleting, 14% initial moisture content and 1.5% bentonite were selected as optimal combinations of pelleting biomass used as feed enzymes. In order to validate the results of single pelleting, the same combinations were used in the pilot-scale pellet mill (CPM).

Pilot-Scale Pelleting

Pilot-scale pelleting of the biomass samples combined with bentonite was done to confirm the results of single pelleting tests. Prior to pelleting in the CPM, fat (lubricant) was added to the biomass samples to prevent plugging of the CPM. Hydrogenated fat is commercially available for approximately CAD$ 2400-2650/t and its inclusion affects the production economics. However, fat inclusion is necessary to prevent plugging of the CPM and reduce the dustiness of biomass pellets. Two levels of lubrication, 5 and 10%, were selected to study the effect of varying the fat levels on the physical quality of the pellets. When 5% fat was added, pellets were made in three groups, based on heating methods used as pretreatments. In the first group, steam conditioning was given prior to pelleting. Steam conditioning increased the moisture content and the temperature of the pellets. This led to changes in the biomass ingredients such as lignin, cellulose, simple sugars, and hemicellulose, which in turn increases particle binding, leading to higher durability values (Wood, 1987). The second group of pellets was made by directly pelleting the samples in the CPM without any heat treatment. In the CPM, pellets are formed by layer-by-layer deposition in the die hole (George, 2012). For the third group, pellets were made after conditioning the pellets to reach 85°C. For this, the samples were recirculated in the conditioning chamber until the desired temperature was reached. Since the melting point of the hydrogenated fat ranges from 50°C to 55°C, the temperature in the conditioning chamber was sufficient to melt the fat, improving lubrication and throughput rate of the pellet mill. The discharge temperatures of the pellets as they exited the pellet die are given in Table 3. The pellets made after conditioning (continuous heating of the mash) discharged at 88.8°C, and pellets made after steam conditioning discharged at temperatures from 68.6 to 69.3°C. The discharge temperature of pellets made directly, without any heat treatment was close to temperatures observed for steam conditioned samples. Table 3 also shows the results for the specific energy consumption and throughput rate of the CPM for the different biomass samples. Upon increase of the fat content in the sample from 5 to 10%, the energy consumption decreased for direct pelleting (no conditioning) and the throughput rate increased. This could be due to increased lubrication effect of the fat. In the case of steam pelleting, the total energy consumption was higher due to added energy requirement for steam production. The highest energy consumption was in case of conditioning due to the continuous running of the mill during recirculation of biomass to reach 85ºC. The results also showed that specific energy consumption and throughput rate of the CPM are inversely proportional, as observed by Tumurulu et al. (2011) and George (2012).

Pellet density, durability, bulk density - Table 4 shows the results for pellet durability, bulk density, and pellet density of the samples made in the pilot-scale pellet mill by varying the levels of fat and the heating methods. The pellets made after steam conditioning, using 5% fat had highest durability and bulk density values (91.08% and 786.11 kg/m^3 respectively) and pellets made after conditioning had the highest pellet density value (1288.39 kg/m^3). Pellets made directly had high durability (85.01%), but low bulk density (747.94 kg/m^3). High durability and bulk density values are an indication of lower handling and transportation costs, making the production of feed pellets more economical (Adapa et al., 2009). Conditioned pellets had lowest durability value (79.66%). When the fat levels were increased to 10% and pellets were made directly and after steam conditioning, the durability, pellet density, and bulk density values were slightly lower. Similar to the results for 5% fat, the pellets made after steam conditioning had higher durability and bulk density values (88.96% and 674.67 kg/m^3 respectively). When the durability results for 5 and 10% fat were compared, there was no significant difference. Therefore, 5% fat level is suggested for lowering production costs.

Enzyme activity – The results for enzyme activity of the pilot-scale pellet mill biomass samples are given in Table 4. Application of steam in the pilot-scale pellet mill may have denatured the enzymes and reduced the enzyme activity drastically. Cowan (1993) had reported that enzyme denaturation occurs at high pelleting temperatures in the presence of steam, reducing the enzyme activity to almost 30% of the starting level. Another study conducted by Samarasinghe et al. (2000) showed that when pelleting temperatures reached 90ºC, the activity of cellulose enzyme, supplemented in chicken feed, reduced by 73%. Since direct pelleting did not have any heat treatments, the samples produced by direct pelleting had the highest enzyme activity values although the durability of the resulting pellets in these treatments was lower than pellets treated by steam. Generally, in the pilot-scale pellet mill, the mash travels the conditioning chamber in 20 to 25 s and 5 to 10 s in the pellet die. With exposure to steam conditioning (increased temperature and long enough residence time), a reduction in the enzyme activity of steam conditioned pellets was observed. When neither heat by steam nor heating pads on the conditioner walls was applied to the biomass mash, the enzyme activity of the pellets had only a small reduction compared to the standard. Table 4 shows that the enzyme activity of the pellets was 90.5% when dry heat was employed. Even though the application of steam to the biomass mash produced durable pellets, it caused a marked reduction in the enzyme activity and hence is not recommended. Comparison the enzyme activity of the pellets made in the single pelleting unit and the pilot-scale pellet mill, it was seen that the results for direct pelleting in the CPM were almost similar to the results from single pelleting (with 1.5% bentonite). The slight difference in the values could be due to the presence of fat, which reduces the exposure time to heat by increasing the throughput rate of the pellets. The single pelleting results did not give us a complete picture as to enzyme activity of biomass, thus the need for pilot-scale pelleting. This is because it is difficult to replicate steam conditioning in the single pelleting unit for a very small amount of sample.

CONCLUSION

The study shows that pelleting of high protein/fiber biomass is beneficial. The pellets formed have high durability, and pellet and bulk density, which will potentially reduce wastage, dustiness, and handling, transportation, and storage costs. Both the SPU and CPM results show that various processing variables and ingredient composition affect the quality of the pellet formed. Among these, moisture content and pelleting temperatures are very important. Pelleting the biomass at 14 and 18% moisture levels, with the inclusion of binders improved the physical quality of the pellets. The moisture content levels used and the high pelleting temperatures (77.5 and 95ºC) did not adversely affect the enzyme activity, which was highly preferable. Since pellets could not be formed at very high moisture levels (20 and 22%), they were not selected for CPM studies. When pelleting was done in the CPM, fat was included in the sample composition to prevent machine plugging. Even though fat inclusion increases the production costs, it is needed for lubrication, to increase the throughput rate of the pellet mill, reduce the energy consumption in each run, and also to reduce the dustiness of biomass pellets. Among the heating methods, steam conditioning gave highest value of durability and lowest values for enzyme activity. Steam conditioning increases the heat and moisture levels of the samples, thereby improving their binding ability, but at the same time, exposure to steam even for a short duration leads to enzyme denaturation. Therefore, to prevent reduction in enzyme activity, direct pelleting of samples is preferred. Finally, since the change in fat levels from 5 to 10% did not increase the values for durability, 5% fat level is preferred to control production cost and to obtain pellets with better physical quality.

Acknowledgement. The authors would like to acknowledge the financial contribution from the Mitacs Accelerate Program and GNC Bioferm Inc. We are very grateful to the GNC Bioferm Inc. for providing the material for the project. The technical assistance of Louis Roth (Department of Chemical and Biologiocal Engineering, University of Saskatchewan) and Lily Ketabi to the project is appreciated.

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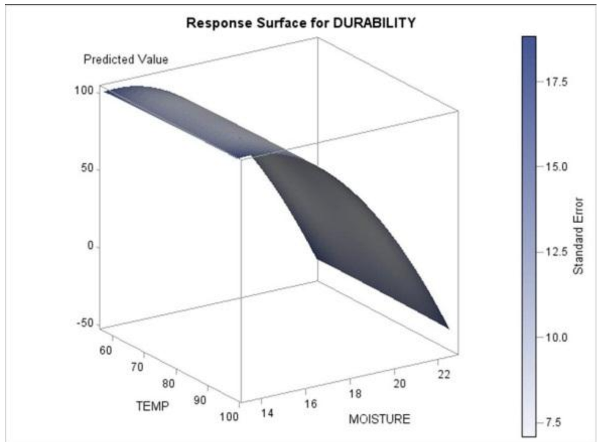
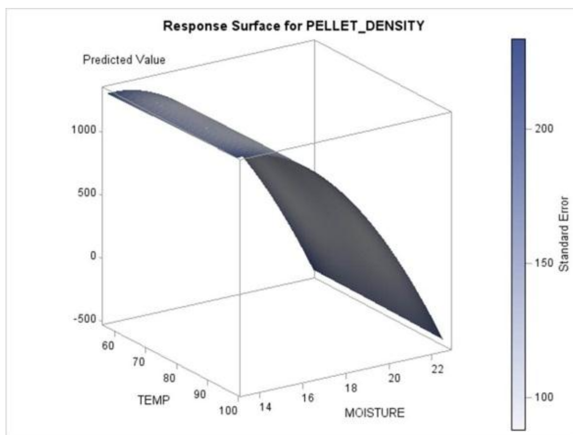


a) Durability



b) Pellet density

Figure 1. Effect of moisture and temperature levels on the durability and density of biomass pellets from single pelleting unit.

Table 1. Density, durability, and enzyme activity of pellets made by the single pelleting unit using different binders at different moisture levels and temperatures (n = 10; mean (standard deviation)).

Binder	Initial moisture (%)	Temperature (°C)	Pellet density (kg/m^3)	Pellet durability (%)	Enzyme activity$^{(2)}$ (%)
Blank	14	60	1229 (14)$^{(1)ef}$	97 (3)^{ab}	92.9^{abc}
		77.5	1243 (19)^{cd}	97 (3)^{ab}	87.1^{abcd}
		95	1267 (19)^{b}	100 (0)^{a}	80.2^{cd}
	18	60	1222 (12)^{f}	99 (0)^{a}	91.3^{abc}
		77.5	1226 (20)^{ef}	100 (0)^{a}	98.7^{ab}
		95	1220 (9)^{f}	96 (11)^{ab}	85.6^{abcd}
	20	60	0^{g}	0^{c}	94.0^{abc}
		77.5	0^{g}	0^{c}	90.0^{abc}
		95	0^{g}	0^{c}	80.2^{cd}
	22	60	0^{g}	0^{c}	69.9^{d}
		77.5	0^{g}	0^{c}	89.3^{abc}
		95	0^{g}	0^{c}	83.1^{abcd}
Bentonite (1.5%)	14	60	1249 (17)^{c}	100 (0)^{a}	99.1^{ab}
		77.5	1266 (14)^{b}	99 (3)^{ab}	88.4^{abc}
		95	1272 (9)^{b}	98 (2)^{ab}	91.7^{abc}
	18	60	1250 (19)^{c}	100 (0)^{a}	89.2^{abc}
		77.5	1248 (15)^{c}	100 (0)^{a}	100.0^{a}
		95	1239 (18)^{cde}	100 (0)^{a}	85.8^{abcd}
	20	60	0^{g}	0^{c}	99.8^{a}
		77.5	0^{g}	0^{c}	90.3^{abc}
		95	0^{g}	0^{c}	86.9^{abcd}
	22	60	0^{g}	0^{c}	88.0^{abc}
		77.5	0^{g}	0^{c}	80.1^{cd}
		95	0^{g}	0^{c}	88.8^{abc}
Corn Starch (5%)	14	60	1270 (15)^{b}	96 (2)^{ab}	94.7^{abc}
		77.5	1293 (14)^{a}	99 (1)^{a}	94.5^{abc}
		95	1291 (16)^{a}	100 (0)^{a}	84.8^{abcd}
	18	60	1229 (12)^{ef}	95 (11)^{b}	87.0^{abcd}
		77.5	1230 (13)^{ef}	99 (1)^{a}	94.7^{abc}
		95	1233 (20)^{def}	100 (0)^{a}	91.6^{abc}
	20	60	0^{g}	0^{c}	95.5^{abc}
		77.5	0^{g}	0^{c}	94.3^{abc}
		95	0^{g}	0^{c}	87.3^{abcd}
	22	60	0^{g}	0^{c}	81.4^{bcd}
		77.5	0^{g}	0^{c}	95.5^{abc}
		95	0^{g}	0^{c}	85.2^{abcd}

(1) Mean values with at least one common letter are not significantly different at P = 0.05, (2) The enzyme activity percentage was calculated relative to the biomass before adding any binder or pelletizing. Entries with ‘0’ for pellet density and durability indicate no pellets produced.

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

Source of variation	DF	Pellet density		Pellet durability		Enzyme activity	
		SS	P-value	SS	P-value	SS	P-value
M	3	140373680.2	<.0001	873428.2545	<.0001	640.2118	0.0204
T	2	2453.1	<.0001	24.7043	0.2128	536.6422	0.1291
B	2	9032.5	<.0001	24.2761	0.2186	256.1363	0.0162
M x T	6	9472.8	<.0001	43.2315	0.4901	976.4236	0.8337
M x B	6	20853.8	<.0001	39.2788	0.5520	166.7512	0.0228
T x B	4	506.5	0.3952	78.4617	0.0447	273.5778	0.3496
M x T x B	12	2084.7	0.1621	182.6049	0.0319	651.0924	0.5517
Total	359	140458170.1	---	876395.1268	---	6761.0290	---

*DF= degrees of freedom, **SS= Sum of squares, ***P= probability

Table 3. Specific energy consumption and throughput rate, and discharge temperature of the biomass (FE) pellets made in the pilot-scale pellet mill.

Sample	Specific energy consumption (kWh/t)		Throughput rate (kg/h)	Discharge temp. (°C)
	Net	Total		
FE (14%MC), 1.5%Bentonite, 5%Fat- Steam conditioning	20.36	43.13	16.51	68.6
FE (14%MC), 1.5%Bentonite, 5%Fat- Direct pelleting	36.16	60.81	15.22	61.9
FE (14%MC), 1.5%Bentonite, 5%Fat- Conditioning	69.52	111.24	9.02	88.8
FE (14%MC), 1.5%Bentonite, 10%Fat- Steam conditioning	37.16	80.91	8.59	69.3
FE (14%MC), 1.5%Bentonite, 10%Fat- Direct pelleting	14.06	29.81	23.85	68.4

Table 4. Durability, bulk density, pellet density, and enzyme activity of biomass (FE) pellets made by pilot-scale pellet mill.

Sample	%Fat	Heating method	Durability (%)	Bulk density (kg/m3)	Pellet density (kg/m3)	Enzyme activity (%)
FE(14%MC*)1.5%Bentonite	5	Steam conditioning	91.08a	773.64b	1213.83b	17.7c
FE(14%MC),1.5%Bentonite	5	Direct pelleting	85.01c	747.94c	1215.66b	100.0a
FE(14%MC),1.5%Bentonite	5	Conditioning	79.66d	786.11a	1288.39a	90.5b
FE(14%MC), 1.5%Bentonite	10	Steam conditioning	88.96b	674.67d	1142.62c	16.2c
FE(14%MC), 1.5%Bentonite	10	Direct	80.58d	665.65e	1144.14c	91.4b

pelleting

*MC=moisture content