Effect of initial moisture and temperature on the enzyme activity of pelleted high protein/fiber biomass

Elizabeth George[1], Shahram Emami[2], Lope G. Tabil[1]*, Leigh Campbell[3]

[1]*Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon, SK S7N 5A9, Canada*
[2]*Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada*
[3]*GNC Bioferm Inc., P.O Box, Bradwell, SK S0K 0P0, Canada.*
Email: lope.tabil@usask.ca
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High protein/fiber biomass, which is used as a feed enzyme to increase feed utilization efficiency of poultry rations, was pelletized. Feed enzymes reduce viscosity of gut contents and change nutrient absorption in animal digestive tract and as a result, improve the nutritional quality of feeds. However, the pelleting process may denature enzymes. Pelleting was optimized in terms of the initial moisture content of the biomass and the pelleting temperature. Preliminary pelleting tests were done in a 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% wb and pelletized at temperatures of 60.0, 77.5, and 95.0ºC during preliminary tests. Pellet durability and enzyme activity were measured to study the effect of each variable. To improve pellet durability, different binders were combined with the biomass and the feasibility of producing pellets in a pilot-scale pellet mill was tested. Although high pellet durability was obtained by pelleting the biomass in a pilot-scale pellet mill with steam conditioning at 14% moisture content, combined with 1.5% bentonite, and 5% fat, the enzyme activity of the pellets was low. However, pelleting the biomass without steam conditioning did not affect enzyme activity and can be a viable option for making durable pellet. **Keywords:** feed enzymes, heat-sensitive protein/fiber biomass, pelleting, binders.

De la biomasse à teneur élevée en protéine/fibre, utilisée comme enzyme dans l'alimentation des volailles afin d'augmenter l'efficacité de la prise alimentaire, a été granulée. Les enzymes alimentaires réduisent la viscosité du contenu du tube digestif et modifient l'absorption des nutriments améliorant ainsi la qualité nutritionnelle des aliments. Toutefois, le processus de granulation peut dénaturer les enzymes. Le granulage a été optimisé pour les paramètres suivants : teneur en eau initiale de la biomasse et température utilisée. Des essais préliminaires de granulage ont été faits avec un moulin à granulage unitaire et les résultats ont été validés en utilisant un moulin expérimental. Les essais préliminaires ont été réalisés avec des teneurs en eau initiales de la biomasse variant entre 14 et 22 % (base humide) et des températures de granulation de 60,0, 77,5, et 95,0°C. La solidité des granules et l'activité enzymatique ont été mesurées pour étudier l'effet de chacune des variables. Différents liants ont été ajoutés à la biomasse pour améliorer la solidité des granules, et la facilité de produire des granules avec ces mélanges a été testée avec le moulin expérimental. Des granules de grande solidité ont été obtenues lorsque le moulin expérimental a été utilisé avec la biomasse conditionnée à la vapeur à une teneur en eau de 14 %, à laquelle 1,5 % de bentonite et 5 % de gras ont été ajoutés. Malheureusement, l'activité enzymatique de ces granules était faible. Toutefois, le granulage de la biomasse sans conditionnement à la vapeur n'avait eu aucun effet sur l'activité enzymatique représentant, par conséquent, une option viable pour produire une granule solide. **Mots clés**: enzymes alimentaires, protéine/fibre de biomasse sensible à la chaleur, granulation, liants.

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 than in other animals (Choct and Kocher 2000). NSPs have chemical cross-linking and endogenous enzymes are needed to breakdown and digest them. The digestive system of poultry does not produce the exogenous enzymes required to break down and digest plant cell walls. NSPs are mostly soluble, increasing the viscosity in the intestine, leading to decreased feed passage rate and impaired digestion. 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. 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). 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 may increase 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) and poultry producers.

Despite the beneficial effects of feed enzymes, its use and application has certain issues. Ground or powdered biomass with enzymes have a low bulk density (40-200 kg m^{-3}) and handling this ingredient in in its natural fine/powdery form will lead to increased wastage and high transportation, storage, and handling costs. In order

to overcome these challenges, the bulk density of feed enzyme-containing biomass should be increased (600-800 kg m^{-3}) by pelleting to a denser product. Chemical, physico-chemical (Adapa 2011), and biological pre-treatments (Kashaninejad and Tabil 2011) can be done before densification of agricultural biomass to improve binding among particles. Pretreatments using chemicals and heat are not preferred because these pretreatments may denature the enzymes. The physico-chemical properties of biomass pellets are affected by various factors including moisture content of the biomass and pelleting temperature. Moisture content of the biomass during pelleting serves to bind particles together and may affect the enzyme activity of the biomass as high moisture coupled with heat can lessen its enzyme activity. High moisture contents and pelleting temperatures are essential for starch gelatinization and protein denaturation. 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 biomass 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 biomass with enzymes may also reduce its enzyme activity at high pelleting temperatures. Therefore, pelleting of enzyme-containing biomass should be conducted under a condition ensuring that enzymes are not denatured. This can be done by pelleting at the optimal combination of temperature and moisture.

Addition of commercial binders improves the physical quality of the feed pellets (Abdollahi et al. 2012). Pellet binders improve the bond among the biomass particles, significantly increasing the throughput of the pellet mill. Different binders such as lignosulfonates, bentonite, corn starch, mineral binders, and gums are available commercially. Certain binders are highly preferred as they increase the nutrient content of the feeds by increasing energy value and mineral content. 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 variables, i.e. pelleting temperature and moisture content of the feed material. In this study, different binders were included in the ingredient composition to study the effect of the variables 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 were: 1) to optimize the process of pelleting of high protein/fiber biomass using the single pelleting unit; 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 fungal biomass (obtained from GNC Bioferm Inc., Bradwell, SK) represented the end product of a fungal fermentation (*Aspergillus niger*) on a grain-based substrate source (barley, *Hordeum vulgare)*. The components of the biomass (Emami *et al.* 2013) were crude protein (29%, db), cellulose (17.9%, db), hemicellulose (20%, db), lignin (4.9%, db), crude fat (2.1%, db), total ash (7.8%, db), and moisture (8.5%, wb). The biomass was previously dried in an industrial forced-air dryer (inlet air 94°C) and ground using a hammer mill with 1.59 mm screen (4/64 inch). The material was mixed to ensure uniformity and stored in airtight plastic bags to ensure moisture constancy. Bentonite (Canadian Clay Products Inc., Wilcox, SK) and corn starch (Ingredion Canada Inc., Pointe-Claire, QC) used as binder in our previous study (Emami et al. 2013) were shown to have good binding quality and were used in this study. For pilot-scale pelleting, hydrogenated fat (called silver prills, Trident Feeds, UK) was used as lubricant.

Sample preparation

The initial moisture content of biomass that was divided into 4 groups of samples was determined to be 6.79% (wb) using AACC standard method 44-15A (AACC 2000). The moisture content of each group of biomass was adjusted to 14, 18, 20, and 22%, respectively, by adding appropriate mass of water and mixing with the biomass sample for uniform distribution. The samples were stored in airtight plastic bags at room temperature for 48 h to retain the moisture and mixed every 12 h to ensure moisture equilibration. Each moisture adjusted biomass sample was split and bentonite (1.5% wt) or corn starch (5.0% wt) was added to each. Both binders were in powder form and were mixed uniformly with the biomass.

Densification methods

Single pelleting The single pelleting unit (SPU) was 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 connected to the die were used to monitor the pelleting temperature (Tabil 1996).

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 (60.0, 77.5, and 95.0°C). For each run, a 5000 N force and 50 mm min^{-1} plunger crosshead speed was used to compress the sample by direct compression (Tabil 1996). The compression load was released after 60 s and then the pellet was ejected through the die hole (Kashaninejad et al. 2010). The mass and the dimensions of the pellet were measured. The pellets were stored in airtight plastic bags for 14 d before testing for durability and enzyme activity.

Pilot-scale pelleting A 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 mill) are the conditioning chamber and the pelleting mill assembly (Tumuluru 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, in which the samples are compressed into the die holes. The ring die rotates at 250 rpm, driven by a 1.5 kW motor. A 7.9 mm die diameter with length-to-diameter ($L\ D^{-1}$) ratio of 4.1 was used for this study.

Prior to pelleting, 5% hydrogenated fat was added uniformly to each biomass-binder combination to ensure pellets can be ejected through the die due to the lubrication effect of fat (Emami et al. 2013). To start processing in the CPM, 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 regimes: 1) without any heating source when the steam injection valve and the electric heating pads of the conditioning chamber wall were turned off; 2) by conditioning the biomass in the steam conditioner prior to passing through the pellet mill (steam injection valve and electric heating pads were turned on); and 3) by heating the biomass in the conditioning chamber when only electric heating pads were turned on (steam injection valve was turned off); the biomass sample was recirculated in the conditioning chamber until it attained a temperature of approximately 85°C because heating below 85°C reduced pellet durability and above 85°C resulted in plugging of the die.

Thermocouples placed in different parts of the CPM, monitored the temperature of the biomass and pellets. The ejected 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 airtight plastic bags for 14 d before measuring durability and enzyme activity. The power draw of the pellet mill was measured and recorded, and the specific energy consumption was calculated and expressed as kWh of energy consumed per tonne of pellets.

Evaluation methods

Pellet density The volume of a single pellet, as cylinder shape, was determined from length and diameter measured by a digital caliper (Shankar et al. 2007; Shankar et al. 2008). Density of the pellets was calculated by dividing the weight to the volume of each pellet.

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; Shrivastava et al. 1989; Sah et al. 1980). In this test, a single pellet was dropped from a height of 1.85 m on a metal plate. Due to impact, the pellet breaks. The ratio of the mass of the larger portion to the initial pellet mass was expressed as the percentage of the pellet durability.

Pellet durability (pilot-scale pelleting) Durability of pellets produced in CPM was measured using the tumbling test according to ASABE Standard S269 (ASABE 2011). Pellets (100 g) were placed in the tumbling test chamber and tumbled at 50 rpm for 10 min. The pellets were sieved using a 5.7 mm (US sieve size 3.5) round sieve to separate the fines from the coarse and broken pellets. The ratio of the mass of pellets retained on the sieve to the initial mass of pellets was expressed as a percentage of pellet durability.

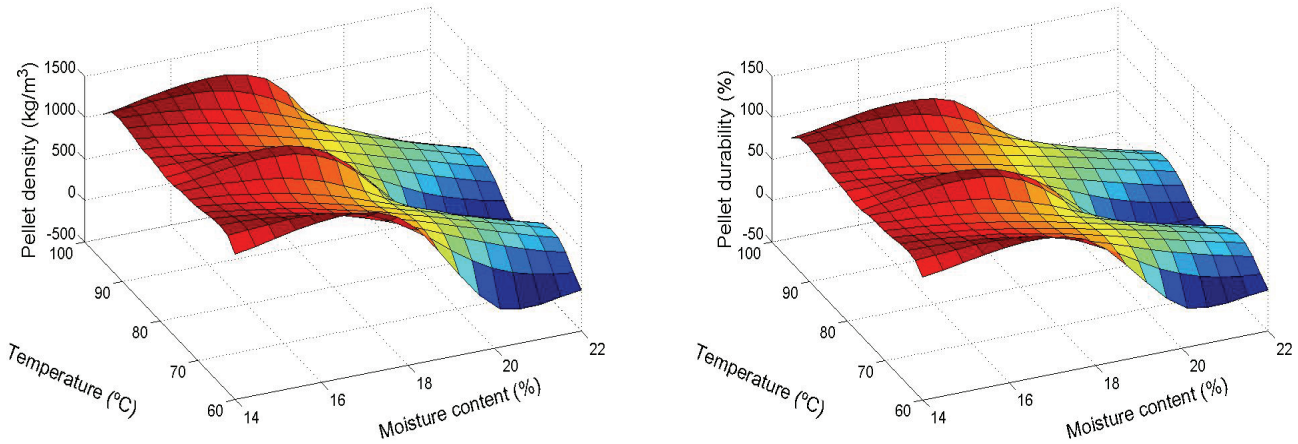
Bulk density Bulk density of the pellet made in the CPM was determined using a 0.5-L cylindrical container (SWA951, Superior Scale Co. Ltd., Winnipeg, MB). Empty container was weighed (W_1). The container was filled using a funnel, with its discharge opening located 55 mm above the top edge of the container. The funnel was removed from top of the container. The top of the container surface was leveled by rolling a cylindrical stainless steel bar across the container in two perpendicular directions. Subsequently, the container was weighed (W_2). The mass (W_2 - W_1) per unit volume presented the bulk density of the biomass in kg m^{-3}. The bulk density was determined in three replicates for each sample.

Enzyme activity A β-glucanase assay kit was used to determine the enzyme activity of the biomass pellet samples. Enzyme activity quantitation 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 (Emami et al. 2013). The reaction was initiated by weighing 30 mg of the sample and adding it to the reaction mixture. The reaction mixture comprised of 1 ml of 0.5% (w v^{-1}) 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, and 10 min. The reaction was stopped by adding 1.0 ml of 3,5-dinitrosalicylic acid solution (16 g NaOH, 300 g Na-K-tartrate, 10 g 3,5-dinitrosalicyclic acid in 1 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

Sample processing in SPU was conducted using a completely random experimental design with factorial treatment structure. There were three variable factors, the biomass moisture content (14, 18, 20, and 22%), binder type (control, 1.5 % bentonite, and 5.0% corn starch), and pelleting temperature (60.0, 77.5, and 95.0°C).

Analysis of variance (ANOVA) and comparison of means (Duncan's multiple range test at the 0.05 level) was performed using the Statistical Analysis System (Version 9.3, SAS Institute Inc., Cary, NC) and the GLM procedure was used to evaluate the effect of each variable and their interactions on the pellet quality.



Pellet Density **Pellet Durability**

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

RESULTS AND DISCUSSION

The biomass used in this study had high crude protein content (29%, db); it contained high levels of cellulose (17.9%) and hemicellulose (20%) (Emami et al. 2013). The protein levels were higher than the usual levels in other biomass because of the fungal material-containing enzyme.

Single pelleting

Pellet density, durability, and enzyme activity The highest pellet density (Table 1) was observed in pellet samples with corn starch, 14% moisture, and high pelleting temperatures (77.5 and 95.0°C). The interaction effect of biomass moisture content and pelleting temperature on the density and durability of pellets made in the SPU are shown in Fig. 1a and 1b, respectively. The effect of moisture content, pelleting temperature, and binder type on pellet density was significant ($P < 0.01$, Table 2). The density of pellets made with corn starch increased as temperature increased. This may be due to the combined effect of pelleting temperature and some water added leading to the 'natural binding' of the product which may be due to (partial) protein solubilisation and its inherent stickiness, increasing further the adhesive properties of the biomass particles. The pellet density of samples with binder was higher than the corresponding control sample (no binder) at lower moisture content (14%).

The effect of pelleting temperature and binder type on pellet durability was not significant ($P > 0.01$, Table 2) and all regimes had durability ranging from 96 to 100%. The highest durability (98-100%) was obtained in pellets made with bentonite. 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 durability of pellets. The statistical analysis for the pellet durability showed that only moisture content had significant effect on pellet durability ($P < 0.01$, Table 2); pellet durability increased as moisture content increased.

The enzyme activity of most of the biomass samples reduced after pelleting (Table 1). The lowest value for enzyme activity was observed in control feed enzyme pellets (no binder). The pellets made with moisture content of 22% at pelleting temperature of 60.0°C had the lowest enzyme activity (69.9%). With the inclusion of binders to the biomass samples, the enzyme activity was between 85 and 99%. In the SPU, the residence time of the samples affected enzyme activity. Since the residence time of the biomass samples in the pelleter die was only about 105 s per run, the biomass samples may not have attained the set-point temperature (the temperature of ejected biomass pellets was not determined). The statistical analysis of the samples shows that the effect of temperature on the enzyme activity of the samples is insignificant ($P > 0.01$, Table 2).

In general, pellets with bentonite had higher pellet density and durability values. Pellets were not formed at high moisture contents (20 and 22%) because the biomass was too wet to form pellets that stay together. The pelleting regime containing corn starch or bentonite at a moisture content of 14% and pelleting temperature of 77.5°C produced pellets with good physical quality. Bentonite has the lowest cost (approx. CAD\$97 t^{-1}) in comparison to corn starch (approx. CAD\$500-700 t^{-1}). Therefore, bentonite was selected for pilot-scale pelleting to reduce production costs.

From the results of single pelleting, the pelleting regime with 14% moisture content and 1.5% bentonite was selected as the optimal combinations for pelleting biomass used as feed enzymes. In order to validate the results of single pelleting, the same combinations were used in the CPM.

Pilot-scale pelleting

Pilot-scale pelleting of the biomass samples mixed with 1.5% bentonite was done to confirm the results of single pelleting tests. Prior to pelleting in the CPM, hydrogenated fat was added to the biomass samples to

Table 1. Pellet 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	Pellet density	Pellet durability	Enzyme activity‡
Blank	14	60.0	1229 (14)^{ef}†	97 (3)^{ab}	92.9^{abc}
		77.5	1243 (19)^{cd}	97 (3)^{ab}	87.1^{abcd}
		95.0	1267 (19)^{b}	100 (0)^{a}	80.2^{cd}
	18	60.0	1222 (12)^{f}	99 (0)^{a}	91.3^{abc}
		77.5	1226 (20)^{ef}	100 (0)^{a}	98.7^{ab}
		95.0	1220 (9)^{f}	96 (11)^{ab}	85.6^{abcd}
	20	60.0	0^{g}	0^{c}	94.0^{abc}
		77.5	0^{g}	0^{c}	90.0^{abc}
		95.0	0^{g}	0^{c}	80.2^{cd}
	22	60.0	0^{g}	0^{c}	69.9^{d}
		77.5	0^{g}	0^{c}	89.3^{abc}
		95.0	0^{g}	0^{c}	83.1^{abcd}
Bentonite (1.5%)	14	60.0	1249 (17)^{c}	100 (0)^{a}	99.1^{ab}
		77.5	1266 (14)^{b}	99 (3)^{ab}	88.4^{abc}
		95.0	1272 (9)^{b}	98 (2)^{ab}	91.7^{abc}
	18	60.0	1250 (19)^{c}	100 (0)^{a}	89.2^{abc}
		77.5	1248 (15)^{c}	100 (0)^{a}	100.0^{a}
		95.0	1239 (18)^{cde}	100 (0)^{a}	85.8^{abcd}
	20	60.0	0^{g}	0^{c}	99.8^{a}
		77.5	0^{g}	0^{c}	90.3^{abc}
		95.0	0^{g}	0^{c}	86.9^{abcd}
	22	60.0	0^{g}	0^{c}	88.0^{abc}
		77.5	0^{g}	0^{c}	80.1^{cd}
		95.0	0^{g}	0^{c}	88.8^{abc}
Corn Starch (5.0%)	14	60.0	1270 (15)^{b}	96 (2)^{ab}	94.7^{abc}
		77.5	1293 (14)^{a}	99 (1)^{a}	94.5^{abc}
		95.0	1291 (16)^{a}	100 (0)^{a}	84.8^{abcd}
	18	60.0	1229 (12)^{ef}	95 (11)^{b}	87.0^{abcd}
		77.5	1230 (13)^{ef}	99 (1)^{a}	94.7^{abc}
		95.0	1233 (20)^{def}	100 (0)^{a}	91.6^{abc}
	20	60.0	0^{g}	0^{c}	95.5^{abc}
		77.5	0^{g}	0^{c}	94.3^{abc}
		95.0	0^{g}	0^{c}	87.3^{abcd}
	22	60.0	0^{g}	0^{c}	81.4^{bcd}
		77.5	0^{g}	0^{c}	95.5^{abc}
		95.0	0^{g}	0^{c}	85.2^{abcd}

† Mean values with at least one common letter are not significantly different at P = 0.05; ‡ 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.

prevent plugging of the CPM, since hydrogenated fats have the advantage of being waxy as opposed to oily or greasy (below 50°C). Hydrogenated fat is commercially available for approximately CAD$2400-2650 t^{-1} and its inclusion increases the final product cost. However, hydrogenated fat inclusion is necessary to prevent plugging of the CPM die and reduce the dustiness of biomass pellets. Two levels of hydrogenated fat, 5 and 10%, were selected in this part of the study. When 5% fat was added, biomass samples were pretreated in three ways, based on heating methods discussed previously. Steam conditioning increased the moisture content and the temperature of the biomass. This led to changes in the biomass ingredients such as protein, lignin, cellulose, simple sugars, and hemicellulose, which in turn increased particle binding, leading to higher durability values of pellets (Wood 1987). In the CPM, pellets are formed by layer-by-layer deposition of particles in the die hole by the roller in the ring die assembly (George 2012). Since the melting point of the hydrogenated fat ranges from 50 to

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	< 0.01	873428.3	< 0.01	640.2	0.02
T	2	2453.1	< 0.01	24.7	0.21	536.6	0.13
B	2	9032.5	< 0.01	24.3	0.22	256.1	0.02
M × T	6	9472.8	< 0.01	43.2	0.49	976.4	0.83
M × B	6	20853.8	< 0.01	39.3	0.55	166.8	0.02
T × B	4	506.5	0.40	78.5	0.04	273.6	0.35
M × T × B	12	2084.7	0.16	182.6	0.03	651.1	0.55
Total	359	140458170.1	---	876395.1	---	6761.0	---

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

55°C, the temperature in the conditioning chamber was sufficient to melt the fat and improve lubricity and throughput rate of the pellet mill. The discharge temperatures of the pellets as they exited the pellet die of the CPM are given in Table 3. The pellets made after heat conditioning by electric heating pads were discharged at 88.8°C, and pellets made after steam conditioning were 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. Upon increase of the added fat in the sample from 5 to 10%, the specific energy consumption decreased for direct pelleting and the throughput rate increased (Table 3). This could be due to the lubricity effect of hydrogenated fat leading to the reduction of friction between the biomass particles and the die. In the case of steam conditioning prior to pelleting, the total specific energy consumption was higher due to added energy requirement for steam production. The highest energy consumption was in case of heat conditioning due to the continuous running of the conditioning chamber during recirculation of biomass to reach 85ºC. The results also showed that specific energy consumption and throughput rate of the CPM were inversely proportional which was similarly reported by Tumuluru et al. (2011) and George (2012).

Pellet density, durability, and bulk density Table 4 shows the pellet durability, bulk density, and pellet density of the biomass made in the CPM at added fat of 5 and 10% and three heating methods. The pellets made with steam conditioning and using 5% added fat had the highest durability (91.1%) and pellets made after heat conditioning had the highest bulk density and pellet density (781.1 and 1288.39 kg m^{-3}, respectively). Biomass which was directly pelleted (no heating) had high durability (85.0%) 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). Heat conditioned pellets had the lowest durability value (79.7%). Increasing the added fat of biomass to 10% in regimes without heat treatment (direct pelleting) and with steam conditioning resulted in pellets with slightly lower durability, pellet density, and bulk density. Similar to the results for 5% added fat, the pellets made after steam conditioning with 10% added fat had high durability (89.0%). Pellets with 5% added fat had higher durability and bulk density than those made with 10% added fat. In addition, the cost of pellet production with 5% added fat is lower than 10%. Therefore, 5% added is suggested for industrial scale production.

Enzyme activity Enzyme activity of the pellets made in the CPM is given in Table 4. Application of steam in the CPM resulted in direct steam contact that caused enzyme activity loss, presumably due to the high temperatures at the point of contact/condensation. Enzyme activity of steam conditioned biomass pellets reduced drastically (17.7 and 16.2%). Cowan (1993) reported that enzyme

Table 3. Discharge temperature, throughput rate, and specific energy consumption of pellets made in the pilot-scale pellet mill from biomass with moisture content of 14% and 1.5% bentonite.

Fat content (%)	Heating method	Discharge temperature (°C)	Throughput rate (kg h^{-1})	Specific energy consumption (kWh t^{-1})	
				Net	Total
5	Steam conditioning	68.6	16.51	20.36	43.13
5	Direct pelleting (no heating)	61.9	15.22	36.16	60.81
5	Heat conditioning	88.8	9.02	69.52	111.24
10	Steam conditioning	69.3	8.59	37.16	80.91
10	Direct pelleting (no heating)	68.4	23.85	14.06	29.81

Table 4. Durability, bulk density, pellet density, and enzyme activity of pellets made by pilot-scale pellet mill from biomass with moisture content of 14% and 1.5% bentonite.

Added fat (%)	Heating method	Durability (%)	Bulk density (kg m^{-3})	Pellet density (kg m^{-3})	Enzyme activity (%)
5	Steam conditioning	91.1[a†]	773.6[b]	1213.8[b]	17.7[c]
5	Direct pelleting (no heating)	85.0[c]	747.9[c]	1215.7[b]	100.0[a]
5	Heat conditioning	79.7[d]	786.1[a]	1288.4[a]	90.5[b]
10	Steam conditioning	89.0[b]	674.7[d]	1142.6[c]	16.2[c]
10	Direct pelleting (no heating)	80.6[d]	665.7[e]	1144.1[c]	91.4[b]

[†]Mean values with at least one common letter in the column are not significantly different at P = 0.05.

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 heating, the samples produced by direct pelleting had the highest enzyme activity values (100.0%) although the durability of the resulting pellets in these treatments was lower than pellets subjected to steam conditioning. In the CPM, the biomass feed generally travels through to the length of the conditioning chamber for 20 to 25 s and in the pellet die for 5 to 10 s. With exposure to steam, the biomass increased its temperature in a long enough residence time, resulting in a reduction of the enzyme activity of steam-conditioned pellets. When neither heat by steam nor heating pads on the conditioner walls was applied to the biomass, 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 heat conditioning (dry heat) was employed. Even though the application of steam to the biomass produced durable pellets, it caused a marked reduction in the enzyme activity. In comparison, the enzyme activity of the pellets made in the SPU and the CPM showed that the results for direct pelleting in the CPM were almost similar to the results from SPU (with 1.5% bentonite). The slight difference in the values was related 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 provide a complete picture of enzyme activity because the replication of the steam conditioning in the single pelleting unit for a very small amount of sample was difficult.

CONCLUSION

The pellets formed had high durability, and pellet and bulk density, which will potentially reduce wastage, dustiness, and handling, transportation, and storage costs. 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 95.0ºC) did not adversely affect the enzyme activity, which was highly preferable. Even though fat inclusion increases the production costs, it is needed for lubrication, to increase the throughput rate of the pellet mill, reduce energy consumption in each run, and reduce the dustiness of biomass pellets. Among the heating methods used, steam conditioning resulted in pellets with the highest durability but the lowest enzyme activity. In terms of retaining the enzyme activity in biomass pellets, direct pelleting of samples without heating was preferred. Finally, since the change in added fat from 5 to 10% did not increase the values for durability, a 5% added fat is preferred to reduce production cost and to obtain pellets with better physical quality.

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REFERENCES

AACC. 2000. Approved methods of the AACC. Method 44-15A. Moisture - Air-oven method. St. Paul, MN: American Association of Cereal Chemists (AACC International).

Abdollahi, M.R., V. Ravindran, T.J. Wester, G. Ravindran and D.V. Thomas. 2012. Effect of improved pellet quality from the addition of a pellet binder and/or moisture to a wheat-based diet conditioned at two different temperatures on performance, apparent metabolisable energy and ileal digestibility of starch and nitrogen in broilers. *Animal Feed Science and Technology* 175: 150–157. http://dx.doi.org/10.1016/j.anifeedsci.2012.05.001

Adapa, P.K., L.G. Tabil, G.J. Schoenau, B. Crerar and S. Sokhansanj. 2002. Compression characteristics of fractionated alfalfa grinds. *Powder Handling and Processing* 14(4): 252-259.

Adapa, P.K., L.G. Tabil and G.J. Schoenau. 2009. Compaction characteristics of barley, canola, oat, and wheat straw. *Biosystems Engineering* 104(3): 335-344. http://dx.doi.org/10.1016/j.biosystemseng.2009.06.022

Adapa, P.K. 2011. Densification of selected agricultural crop residues as feedstock for the biofuel industry. Unpublished Ph.D. thesis. Saskatoon, SK: Department of Chemical and Biological Engineering, University of Saskatchewan.

Al-Widyan, M.I. and H.F. Al-Jalil. 2001. Stress-density relationship and energy requirement of compressed only cake. *Applied Engineering in Agriculture* 17(6): 749-753.

ASABE. 2011. ASABE S269 - Densified products for bulk handling - definitions and method. St. Joseph, MI: American Society of Agricultural and Biological Engineers.

Campbell, G. L. and M.R. Bedford. 1992. Enzyme applications for monogastric feeds: A review. *Canadian Journal of Animal Science* 72: 449-466. http://dx.doi.org/10.4141/cjas92-058

Choct, M. and A. Kocher. 2000. Non-starch carbohydrates: digestion and its secondary effects in monogastrics. *Proceedings of the Nutritional Society of Australia* 24: 31–38.

Coffey, R.D., G.R. Parker and K.V. Laurent. 1995. Feeding and managing the weanling pig. University of Kentucky Online Publications ASC-149: 1-8.

Cowan, W.D. 1993. The stability of enzymes in animal feeds. Feed International 22–26.

Emami, S., L.G. Tabil, L. Campbell, E. George and P. Adapa. 2013. Densification of heat sensitive protein/fibre biomass. *Canadian Biosystems Engineering* 55: 3.19-3.28.

George, E. 2012. Investigation on the processing of wheat bran and condensed distillers solubles as animal feed. Unpublished M.Sc. thesis. Saskatoon, SK: Department of Chemical and Biological Engineering, University of Saskatchewan.

Kashaninejad, M., B. Kashaninejad and L.G. Tabil. 2010. Enhancing compaction characteristics of barley straw by microwave/chemical pretreatment. Paper No. CSBE100523. XVIIth World Congress of the International Commission of Agricultural and Biosystems Engineering (CIGR). Quebec City, Quebec: Canadian Society for Bioengineering. June 13-17.

Kashaninejad, M. and L.G. Tabil. 2011. Effect of microwave-chemical pre-treatment on compression characteristics of biomass grinds. *Biosystems Engineering* 108(1): 36-45. http://dx.doi.org/10.1016/j.biosystemseng.2010.10.008

Khan, S.H., R. Sardar and B. Siddique. 2006. Influence of enzymes on performance of broilers fed sunflower-corn based diets. *Pakistan Veterinary Journal* 26(3): 109-114.

Khankari, K.K., M. Shrivastava and R.V. Morey. 1989. Densification characteristics of rice hulls. ASAE Paper No. 89-6093. St. Joseph, MI: American Society of Agricultural and Biological Engineers.

Marquardt, R.R., D. Boros, W. Guenter and G. Crow. 1994. The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a Trichoderma reesei enzyme preparation. *Animal Feed Science and Technology* 45: 363–378. http://dx.doi.org/10.1016/0377-8401(94)90038-8

Sah, P., B. Singh and U. Agrawal. 1980. Compaction behavior of straw. *Journal of Agricultural Engineering-India* 18(1): 89-96.

Samarasinghe, K., Messikommer, R. and C. Wenk. 2000. Activity of supplemental enzymes and their effect on nutrient utilization and growth performance of growing chickens as affected by pelleting temperature. Archive Fur Tierernahrung 53(1): 45-58. http://dx.doi.org/10.1080/17450390009381937

Shankar, T.J., K. Xingya, S. Sokhansanj, C.J. Lim, X. Bi and S. Melin. 2007. Studies on off- gassing during storage of wood pellets. ASABE Paper No. 071022. St. Joseph, MI: American Society of Agricultural and Biological Engineers.

Shankar, T.J., K. Xingya, S. Sokhansanj, C.J. Lim, X. Bi and S. Melin. 2008. Effect of storage temperature on off- gassing and physical properties of wood pellets. ASABE Paper No. 084248. St. Joseph, MI: American Society of Agricultural and Biological Engineers.

Shrivastava, M., P. Shrivastava and K.K. Khankari. 1989. Densification characteristics of rice husk under cold and hot compression. In Agricultural Engineering: Proceedings of the 11th International Congress on Agricultural Engineering, eds. V.A. Dodd and P. M. Grace. Rotterdam: A.A. Balkema Publishers.

Silversides, F.G. and M.R. Bedford. 1999. Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. *Poultry Science* 78:1184-1190. http://dx.doi.org/10.1093/ps/78.8.1184

Slominski, B.A., T. Davie, M.C. Nyachoti and O. Jones. 2007. Heat stability of endogenous and microbial phytase during feed pelleting. *Livestock Science* 109: 244–246. http://dx.doi.org/10.1016/j.livsci.2007.01.124

Tabil, L. G. 1996. Binding and pelleting characteristics of alfalfa. Unpublished Ph.D. thesis. Saskatoon, SK: Department of Agricultural and Bioresource Engineering, University of Saskatchewan.

Tumuluru, J.K., L. Tabil, A. Opoku, M.R. Mosqueda and O. Fadeyi. 2010. Effect of process variables on the quality characteristics of pelleted wheat distillers dried grains with solubles. *Biosystems Engineering* 105(4): 466-475. http://dx.doi.org/10.1016/j.biosystemseng.2010.01.005

Tumuluru, J.K., C.T. Wright, J.R. Hess and K.L. Kenney. 2011. A review of biomass densification systems to develop uniform feedstock commodities for bioenergy application. *Biofuels, Bioproducts and Biorefining* 5(6): 682- 707.

Wood, J.F. 1987. The functional properties of feed raw materials and their effects on the production and quality of feed pellets. *Animal Feed Science and Technology* 18(1): 1-17. http://dx.doi.org/10.1016/0377-8401(87)90025-3