Research Article

Optimization of Extrusion Process for Pentosanase-Supplemented Swine Feed: Evaluation of Physical Properties and Enzyme Stability

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Abstract
Extrusion of pentosanase supplemented swine feed to improve digestibility was optimized and the extrudates' physical properties, and enzyme stability were investigated. The effects of feed moisture content, die end temperature, and screw speed on product responses, including expansion ratio, bulk density, and hardness were evaluated using the response surface methodology. Following conditions yielded the swine feed with physical properties comparable to the commercial products, feed moisture content at 18–21% (w/w), die end temperature of 95–120 °C, and screw speed at 100–150 rpm. Crude pentosanase was added to the ingredients at 0.5 g/kg and extruded with 2 levels of die end temperature at 95 and 110 °C. Residual activity of pentosanase in extruded swine feed indicated that an increase in die end temperature reduced the activity by 34–35%. Higher activity and stability of pentosanase were observed at pH 3.0 compared to pH 6.8. A significant decrease in the enzyme activity was observed during a 4 week storage period at room temperature. Optimal conditions for the extrusion of pentosanase-supplemented swine feed were obtained. However, the enzyme stabilization in extruded swine feed during the long storage requires further study.

Keywords: Swine feed, Extrusion, Pentosanase, Expansion ratio, Enzyme stability, Xylanase, In vitro study

1 Introduction

Over a billion tons of feed per year are produced globally, a large part of which is fed to livestock. A conventional method for producing corn and soybean meal-based swine feed is usually to provide in a mash form; further processing other than grinding and mixing is not utilized in most cases [1]. Most ingredients are ground to reduce the particle size and increase digestibility [2]. Heat processing is sometimes applied to the feed ingredients to reduce antinutritional factors, such as trypsin inhibitor in soybean and may affect the energy and nutrient digestibility [3]. Other processing techniques that may be used for swine feed include expander processing [4], [5], pelleting [6], [7], and extrusion [4], [8]. Before pelleting, steam conditioning might be used prior to pelleting to obtain a durable feed pellet [9]. In association with pelleting, extrusion is often used in the feed industry [1]; with 95% of pet foods in the United States using extrusion, this is one of the leading technologies for pet food production [10].

Extrusion processing combines many unit operations, such as mixing, heating, and forming the feed material through a barrel by using an extruder [11]. Extrusion is a continuous process with high production capacity, versatility, and low cost per product unit, and utilized for many food products, such as snacks, pasta, noodles, and instant rice, including for value addition of agricultural wastes and by-products [12]–[18]. Extrusion variables, such as feed moisture content, barrel temperature, and screw speed significantly affected the physical and nutritional properties of the extruded products [14], [18]. Extrusion cooking may alter nutritional ingredients, such as dietary fiber, protein, amino acid profile, and other nutrients, both positively or negatively [19]. The process could convert insoluble fibers in the nutrition rich by-products such as orange pulps, defatted soybean meal, germinated brown rice meal, and mango peel fiber [14] into soluble fiber and a good balance of both types of fibers. The extrusion of cereal grains can the increase energy and nutrient digestibility of the raw materials, possibly resulting in a higher feed conversion rate and growth performance in pigs [6]. Rojas *et al.* [20] experimented to determine the effects of pelleting, extrusion, and extrusion plus pelleting on energy and nutrient digestibility in diets containing different levels of fiber-fed to growing pigs. Results indicated that pelleting alone did not increase the digestible or metabolizable energy of the high-fiber diets, while extrusion and the combination of extrusion and pelleting did.

Thailand is an agricultural-based country with plenty of agricultural produces and food processing by-products such as rice bran, cassava, sugarcane, and defatted soy meal [21]. Some primary ingredients used in feed production in Thailand are corn, broken rice, cassava chip, palm oil, fish oil, soybean meal, fish meal, and rice bran. The main hemicellulosic polysaccharide in cereals and their co-products is xylan, 60–70% composed of arabinoxylan [22]. The second most abundant compounds in the cereal used in swine feed are mannan [23]. The digestive systems of non-ruminant animals such as pigs and poultry do not have the enzymes to digest hemicellulose in feed [21]. The inclusion of pentosanase to hydrolyze arabinoxylan in the feed can increase the energy values by increasing the digestibility of nutrients [24]. Pentosanase enzyme can be derived from a selected strain of fungal species [25], which is developed to break down the xylan, arabinan, and mannan in cereal grains. Mannanase and cellulase were produced from three agricultural wastes by Bacillus subtilis P2-5 strain [26]. Supplementation of exogenous enzymes can be helpful in depolymerizing non-starch polysaccharides into shorter chains, increasing the entrapped nutrients, digestibility, and fermentability of nutrients. Chen *et al.* [27] reported that the supplemental xylanase could reduce the digestate viscosity, improve the nutrient release, and exhibit health benefits in nursery pigs fed with corn distillers' dried grains.

To the best of our knowledge, very little information on the extrusion of swine feed in Thailand has been published [28]. There is also a lack of information about the extrusion of swine feed with pentosanase supplementation. Therefore, the objectives of this research were to study the effect of extrusion on the production of pentosanase-supplemented swine feed. The effects of the extrusion parameters, including feed moisture content, die end temperature, screw speed, on physical properties of the extruded swine feed were investigated using response surface methodology (RSM). Crude pentosanase enzyme was added to the feed mix before extrusion, and their enzyme activity and stability during the storage were evaluated. The pieces information obtained will be beneficial for the swine feed industry.

2 Materials and Methods

2.1 *Raw materials for the swine feed*

The feed ingredients were obtained from various sources which were manufacturers in Thailand: broken rice from Ake Rice Mill Co., Ltd. (Thailand), ground corn from Unigro International Co., Ltd., rice bran from Charoen Phon Rice Mill Co. Ltd., soybean meal (44% protein content) from Thai Vegetable

Oil Public Co., Ltd., fishmeal (55% protein content) from Sun Feed Co., Ltd., dicalcium phosphate (P18) from Connell Bros. Co. (Thailand), Ltd., sodium chloride from Saha Patthanapibul Public Co., Ltd., premix from Agintel Co., Ltd. D-xylose and oat spelt xylan were purchased from Sigma-Aldrich Co. (St. Louis, Mo., USA). Crude pentosanase powder was kindly provided by Dr. Pongsuda Poathanya, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA), Thailand.

2.2 *Effects of extrusion variables: response surface experimental design*

The swine feed formulationfor finishing pigs (60–100 kg weight) was adapted from the swine nutrition guide [29], as shown in Table 1. All ingredients were mixed in a Kenwood mixer (KM230, England) with speed level 2 for 10 min and kept at room temperature for 2 h to equilibrate their moisture content. The laboratory single-screw extruder (Brabender 19/20, DGE 330, PL 2200, Germany) with the barrel bore diameter (D) 19.1 mm, barrel length (L) of 20D, and a screw compression ratio of 4:1 was used to produce the swine feed. There were three independent temperature zones along the barrel, Zone 1 (entering zone), Zone 2 (kneading zone), and Zone 3 at the dead end before the exit. The diameter of the circular die was 3 mm.

The extrusion was carried out with the barrel temperature of Zone 1 and 2 controlled at 50 and 70 °C, respectively. The effects of three independent extrusion variables including feed moisture content (X_1) 20–35% (w/w), die end temperature (X_2) 70–120 °C, and screw speed (X_3) 100–200 rpm on physical properties of the extrudates, were studied using the central composite design. A total of 17 extrusion runs were carried out, as presented in Table 2. All variables were studied at five different levels with α = 1.68 (–α, –1, 0, 1, +α, α = 1.68) according to Box and Wilson [30]. The extrudate was dried in a tray dryer (Binder, Germany) at 50 °C for 3 h, and stored at room temperature (30 ± 2 °C) in polypropylene zip-lock bags until further analysis for physicochemical properties of the extrudate including color (L*, a*, b*), diameter, expansion ratio, hardness, bulk density, and water activity (aw). All experiments were carried out with at least two replications.

Table 1: Formulation for the extrusion of swine feed

Raw Materials	% (w/w)
Broken rice	42.80
Ground corn	30.00
Rice bran	10.00
Soybean meal (44% CP)	9.30
Fish meal (55% CP)	5.50
Dicalcium Phosphate (P18)	1.80
Sodium chloride	0.35
Premix minerals	0.25

Table 2: Experimental design for the 3-factor orthogonal central composite design (CCD) with a single center point

Condition	X_1, Feed Moisture	X_2, Zone 3 Temperature	X_3, Screw Speed
1	–1 (23)	–1 (80)	–1 (70)
2	–1 (23)	–1 (80)	1 (130)
3	–1 (23)	1 (110)	–1.68 (70)
4	–1 (23)	1 (110)	1 (130)
5	1 (32)	–1 (80)	–1 (70)
6	1 (32)	–1 (80)	1 (130)
7	1 (32)	1 (110)	–1 (70)
8	1 (32)	1 (110)	1 (130)
9	–1.68 (20)	0 (95)	0 (100)
10	1.68 (35)	0 (95)	0 (100)
11	0 (28)	–1.68 (70)	0 (100)
12	0 (28)	1.68 (120)	0 (100)
13	0 (28)	0 (95)	–1.68 (50)
14	0 (28)	0 (95)	1.68 (50)
15	0 (28)	0 (95)	0 (100)
16	0 (28)	0 (95)	0 (100)
17	0 (28)	0 (95)	0 (100)

Response surface plot and statistical analysis of the experimental data were done by response surface methodology [31] using Statica version 14.0 (TIBCO, USA). The optimal point of the responses was predicted by the second-order polynomial model expressed as following Equation (1).

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \ldots + \varepsilon \quad (1)$$

where y indicated the response variable, β_0 represented the intercept while β_1, β_2 and β_3 were linear terms with β_{12}, β_{13}, and β_{23} as interaction terms and β_{11}, β_{22}, and β_{33} as quadratic terms. X_1, X_2, and X_3 represented independent variables coded for moisture content, die end temperature, and screw speed, respectively.

2.3 *Supplementation of pentosanase and enzyme activity*

Crude pentosanase was added to the feed ingredients at 0.5 g/kg. The extrusion was carried out with optimal conditions based on Section 2.2; feed moisture content at 20% (w/w), screw speed at 130 rpm, and 2 levels of die end temperature at 95 and 110 °C. The extruded feeds were dried at 50 °C for 3 h before determining their physical properties. The dried samples were preserved in polypropylene zip-lock bags and stored at room temperature (30 ± 2 °C) for 4 weeks. Pentosanase was extracted from the swine feed and determined for the residual activity at pH 3.0, and 6.8 using oat spelt xylan as the substrate every week.

2.4 *Analytical methods*

2.4.1 Chemical composition

The raw materials' protein, lipid, and crude fiber content of were analysed by AOAC [32] methods 12.1.07 and 920.39C, and 978.10, respectively. The ash and moisture content were determined by AACC methods 8-01 and 44-19 [33].

2.4.2 Physical properties

A Vernier caliper and data randomly measured the diameter of twenty extrudates were averaged. were averaged. The expansion ratio of the feed samples was calculated by following Equation (2).

$$\text{Expansion ratio} = \text{Extrudate diameter}/\text{Die diameter} \quad (2)$$

Ten pieces of the feed samples were randomly selected, to determine the bulk density, and their total weights were recorded. The samples' volume was determined by substituting with sesame seeds in a 100 mL cylinder, and bulk density (g/cm^3) was then calculated [34].

The hardness of the samples was analyzed using a Texture Analyzer (Stable Micro System Co., Ltd., UK). Ten samples were used for the measurement using a cylinder aluminum P_{50} (50 mm) probe at 5.0 and 10.0 mm/s as a pre-test and a post-test speed, respectively. The hardness was presented as the highest force to break the samples, and the data were averaged.

The color of the swine feed based on the L*, a*, and b* color scale was measured using a Hunter Lab (Color Quest XE, USA) in which L* represents the lightness, a* (red/green), and b* (yellow/blue) coordinates. Water activity was measured using an Aqua Lab/CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA, USA) at 25 °C, following the method described in the manual.

2.4.3 Crude enzyme extraction and assay

Pentosanase was extracted from 1.0 g of swine feed extrudates by adding 10 mL of distilled water, shaken for 2 min, and centrifuged at 10,000 rpm, 4 °C for 10 min. The supernatants were separated and used as crude enzyme [35] to determine their activity on oat spelt xylan (1% w/v) as substrate at two different pHs, 3.0 and 6.8, using citrate phosphate buffer (CPB).

The xylanase assay was determined by mixing 200 µL crude enzyme and 1.80 mL of D-xylan solution and incubated at 39.5 °C for 5 min. Then 3 mL of dinitrosalicylic acid (DNS) was added to the reaction mixture and heated in boiling water for 15 min before cooling in the ice bath and centrifuged at 2000 rpm for 10 min. The supernatant was measured for its absorbance at 540 nm using a spectrophotometer [36]. The enzyme assay was carried out both at pH 3.0, and 6.8.

2.5 *Data analysis*

Statistical analyses were performed using SPSS 11.5 program (SPSS Inc, Chicago, IIinois, US). Duncan's multiple range test determined the differences between data at 95% confidence level ($p < 0.05$).

3 Results and Discussion

3.1 *Composition of raw materials*

Chemical compositions of the raw materials used for the swine feed extrusion are presented in Table 3. The fish meal contained the highest protein content (54.23%), followed by soybean meal (39.82%), rice bran (12.56%), ground corn (7.81%), and broken rice (7.05). The protein content in broken rice and soy meals was close to those reported earlier [13], [14]. The protein requirement of pigs varies according to the stage of development. Fishmeal, soybean meal, and broken rice composed of protein content at 55.0%, 42.0%, and 8.0%, respectively, which were recommended for the growth and development of finishing pigs [29]. However, the other sources of raw materials including rice bran and ground corn, could supplement the protein in feed. Rice bran contained high fat (15.36%), ash (7.98%), and fiber content (11.25%). The non-starch polysaccharides in the fiber of each ingredient are composed mainly of cellulose, hemicellulose, and lignin, which could cause a problem in the digestive system of monogastric animals, especially poultry and pigs [21]. The higher temperatures during the feed processing can destroy the nutrition inhibitors, increase the digestibility of protein, might lower the heat-sensitive nutrition such as vitamins and minerals at the same time [37].

Table 3: Chemical compositions of raw materials for swine feed (% w/w)

	MC (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)
BR	9.51 ± 0.11	7.05 ± 0.21	2.55 ± 0.28	0.04 ± 0.02	1.03 ± 0.03
GC	6.09 ± 0.35	7.81 ± 0.04	5.42 ± 0.88	1.45 ± 0.14	2.46 ± 0.16
RB	7.49 ± 0.01	12.56 ± 0.10	15.36 ± 0.26	7.98 ± 0.29	11.25 ± 0.05
SM	9.27 ± 0.08	39.82 ± 0.08	2.88 ± 0.23	5.48 ± 0.13	6.80 ± 0.08
FM	6.68 ± 0.28	54.23 ± 0.08	10.02 ± 0.15	32.16 ± 1.84	0.61 ± 0.06

Note: BR (broken rice), GC (ground corn), RB (rice bran), SM (soy bean meal), FM (fish meal)
Values are mean ± SD from triplicate determinations.

3.2 *Effects of extrusion variables on physical properties of swine feed extrudates*

Table 4 summarizes the physical properties of the swine feeds produced from 17 extrusion runs with 3 independent variables, feed moisture content (X_1, 20–35% w/w), die end temperature (X_2, 70–120 °C), and screw speed (X_3, 100–200 rpm). The visual appearance of the extruded feeds from each extrusion run is shown in Figure 1. All variables significantly affected the expansion ratio and feed hardness. The major components of the feed ingredients, broken rice (42.80% w/w) and ground corn (30% w/w), consisted mainly of starches. Extrusion is a thermomechanical process to break the bonds in starch and transform them

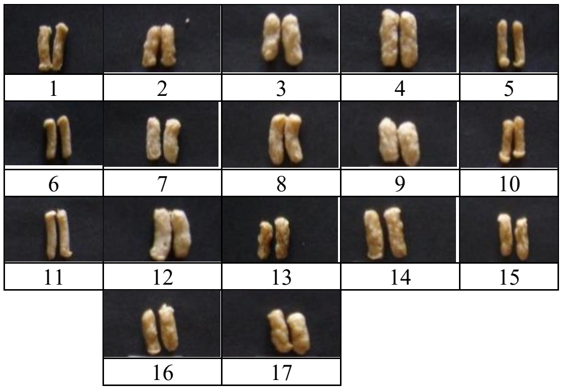
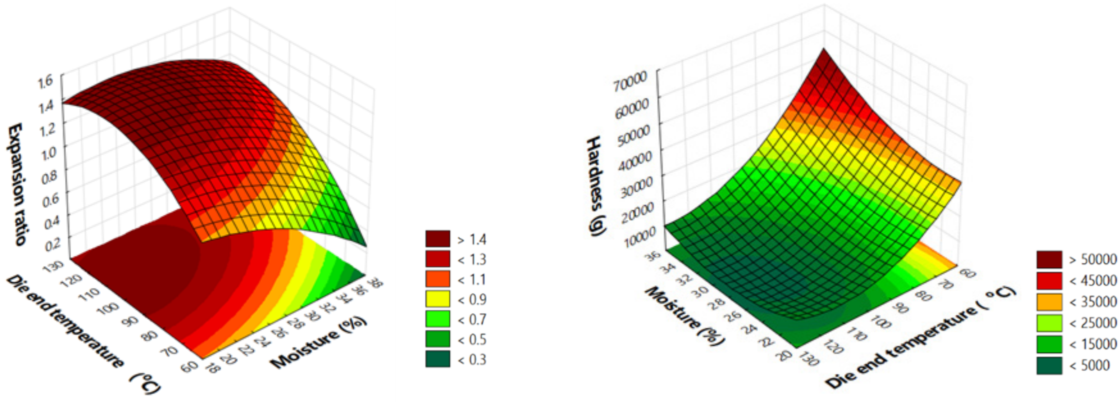


Figure 1: The appearance of swine feeds prepared from 17 extrusion runs.

into the viscoelastic dough by melting, breaking down, and gelatinization processes. Broken rice and corn contain amylose and amylopectin (α-glucan polymer molecules) gelatinized during heating at higher moistures [38]. An increase of feed moisture content tended to yield extrudates with a lower expansion ratios, while higher die end temperatures resulted in higher expansion ratios. The extrudate expansion depends on the starch gelatinization due to the interactions among water content, pressure, shear stresses and strains during the extrusion, and transformation of starch molecular during the evaporation process [39], [40]. The resistance to flow increases and results in a melt with higher viscosity under the low moisture content, while high moisture content can affect the amylopectin macromolecular structure, reduce the melt elasticity, and thus result in products with less expansion [41]. The higher expansion ratio of the extruded swine feeds indicated a lower bulk density and hardness. The higher die end temperature could increase the degree of gelatinization, the extent of superheated steam and the vapor pressure of the moisture, and thus a degree of puffing [14], [42].

The preferred appearance of these extrusion runs should be considered together with their physical properties, which were discussed in the next section (3.2).

An increase of screw speed presented a similar trend with the die end temperature, tended to increase the expansion ratio but less. Similar models for the effects of feed moisture content and die end temperature on expansion ratio were observed at all screw speeds examined. An increase in the expansion ratio resulted



(a) (a)

Figure 2: Response surfaces for the effects of feed moisture content and die end temperature on expansion ratio (a), and hardness (b) of the extruded swine feeds.

Table 4: Physical properties of swine feed obtained from 17 extrusion runs

Run	Feed MC (%)	Zone 3 Temp. (°C)	Screw Speed (rpm)	Physical Properties of Extruded Swine Feed							
				Diameter (mm)	Exp. Ratio	Bulk Density (g/cm³)	Hardness (g)	L*	a*	b*	MC (%)
1	23.04	80.12	70.24	3.50	1.17	0.66	23,343	45.02	5.97	21.67	5.95
2	23.04	80.12	129.76	3.71	1.24	0.60	14,476	45.97	5.69	20.95	4.90
3	23.04	109.88	70.24	4.09	1.36	0.35	5,627	50.60	5.12	21.82	4.05
4	23.04	109.88	129.76	4.51	1.50	0.38	7,003	51.30	4.98	21.55	5.20
5	31.96	80.12	70.24	3.00	1.00	0.66	23,770	43.88	5.85	21.20	6.25
6	31.96	80.12	129.76	3.09	1.03	0.62	25,144	46.43	5.32	20.33	4.75
7	31.96	109.88	70.24	3.90	1.30	0.37	5,711	48.13	4.92	19.44	5.75
8	31.96	109.88	129.76	4.13	1.38	0.36	8,852	46.93	4.92	19.11	5.90
9	20.00	95.00	100.00	4.75	1.58	0.43	6,888	52.12	4.68	21.59	6.95
10	35.00	95.00	100.00	3.13	1.04	0.64	18,575	41.44	6.68	21.11	4.10
11	27.50	70.00	100.00	3.08	1.03	0.59	25,031	45.97	4.86	18.07	6.05
12	27.50	120.00	100.00	4.35	1.45	0.32	3,464	52.06	4.48	19.76	4.50
13	27.50	95.00	50.00	3.60	1.20	0.51	8,810	45.14	5.42	20.09	7.00
14	27.50	95.00	150.00	4.14	1.38	0.47	8,483	46.29	5.19	19.89	6.90
15	27.50	95.00	100.00	4.01	1.34	0.50	8,679	46.06	5.42	20.53	5.70
16	27.50	95.00	100.00	3.97	1.32	0.51	11,559	43.44	5.58	19.55	7.85
17	27.50	95.00	100.00	4.51	1.50	0.46	9,294	50.63	5.62	23.77	6.00

MC: moisture content, temp: temperature Exp: expansion, L* represents the lightness, a* (red/green) and b* (yellow/blue)

in a lower density and hardness of the products. The RSM surface plots of expansion ratio and hardness against feed moisture content and die end temperature are illustrated in Figure 2. Increase in extrudate hardness was observed with an increased moisture content and lower die-end temperature. The fiber in the feed ingredients could interrupt the starch network resulting in a lower extrudate expansion. The feed with lower expansion contained less porosity and resulted in higher hardness [14], [43]. Water activity (a_w) and moisture content of the swine feed extrudates ranged 0.43–0.57 and 4.05–7.85%, respectively, which indicated longer shelf life stability during storage. Products with higher expansion exhibited more porosity and light diffraction, result in higher L* values.

Regression analysis for expansion ratio and hardness at any feed moisture content (X_1), die end temperature (X_2), and screw speed (X_3) are shown in Equations (3)–(5). The coefficient of regression equations (R^2) determination coefficient for these process variables and product properties ranged from 0.80–0.95. These models can be applied for selecting the condition that yields the required properties of the extruded feed.

$$\text{Expansion ratio} = 0.948 - 0.024X_1 + 0.009X_2 + 0.002X_3 \quad R^2 = 0.80 \quad (3)$$

$$\text{Bulk density} = 1.476 - 0.036X_1 + 0.001X_2 - 0.003X_3 - 3.767 \times 10^{-5} X_1X_2 - 1.88 \times 10^{-5} X_1X_3 + 3.38 \times 10^{-5} X_2X_3 + 0.00X_1^2 - 4.947X_2^2 + 1.633 \times 10^{-6} X_3^2 \quad R^2 = 0.89 \quad (4)$$

$$\text{Hardness} = 203{,}664 - 3{,}146X_1 - 2{,}164X_2 - 650X_3 - 15.37\, X_1X_2 + 3.67X_2X_3 + 10.36X_1X_3 + 74.96X_1^2 + 9.17X_2^2 + 0.05X_3^2 \quad R^2 = 0.95 \quad (5)$$

The extruded swine feeds were compared with the commercial swine feeds available in the market. The commercial feeds produced by pelleting, exhibited the bulk density at around 0.36–0.50 g/cm^3 and hardness ranged 7,000–9,000 g. The conditions that yielded the swine feed with physical properties close to the commercial products from pelleting were feed moisture content at 18–21% (w/w), die end temperature of 95–120 °C, and screw speed at 100–150 rpm. Several combinations of the three variables could yield the bulk density and hardness appropriate for swine feeds, including run 4, 8, 9, 13, 14, 15, and 17. Extrusion run 9 with feed moisture content at 20% (w/w), 130 rpm of screw speed, and the die end temperature at 95 °C yielded the extrudates with expansion ratio at 1.58, bulk density 0.43 g/cm^3 and hardness at 6,888 g.

3.3 *Supplementation of pentosanase and enzyme stability*

The physical properties of swine feed supplemented with pentosanase at 0.5 g/kg extruded with two different die end temperatures, 95 °C, and 100 °C, are presented in Table 5. The extrudates showed the non-significant difference in diameter and expansion ratio, but the bulk density of the extrudates from 95 °C exhibited a higher bulk density (0.41 g/cm^3), and lower hardness (8,891 g) compared to those extrudates from 110 °C, 0.38 g/cm^3, and 6,972 g, respectively. Using a higher die end temperature led to a higher degree of gelatinization and

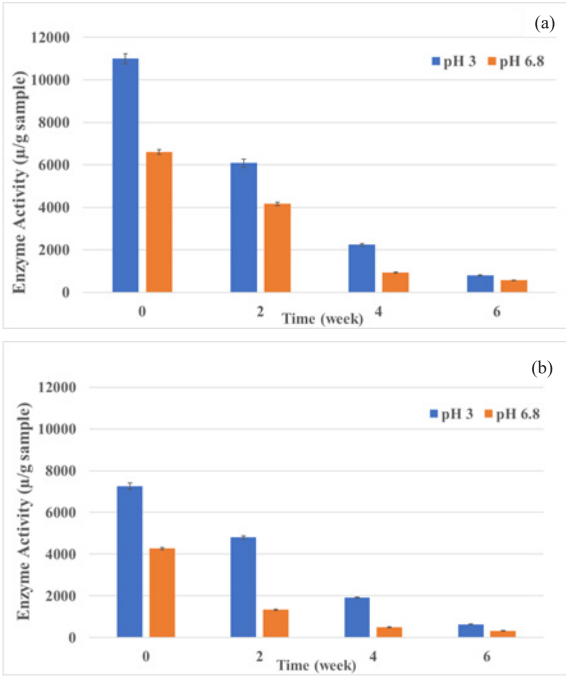


Figure 3: Enzyme activity (at pH 3.0, and 6.8) remaining in the swine feeds extruded using 2 different die end temperatures, during 4 weeks storage; (a) 95 °C, and (b) 110 °C.

the extent of vapor evaporation and degree of puffing as described above [14], [42].

However, as expected, the pentosanase was retained more in the extrudates made from the lower temperature (95 °C) than at higher temperature (110 °C). Generally, pentosanase is thermostable up to 75 °C for 2–5 min. The extrusion is an HTST process with a very short residence (a few seconds). Some activity loss occurred during the extrusion under high temperature. The xylanase activity in the pentosanase-added swine feeds from 95 °C was 11,000 U/ g samples (pH 3.0) and 6,599 U/g sample (pH 6.8) while those samples from 110 °C exhibited a lower enzyme activity at 7,259 U/g samples (pH 3.0) and 4,266 U/g sample (pH 6.8), respectively. After extrusion, the enzymes were stored at room temperature (30 ± 2 °C) for about 3 weeks, and the xylanase activity in the samples decreased to less than 50% of the initial activity presented in Figure 3. The xylanase activity at pH 3.0 was higher than the activity at pH 6.8, which indicated that xylan substrate in the feeds would be digested both in the pig's stomach and the small intestine. The pig stomach has acidic condition in which protein digestion starts in the stomach with pepsin in the stomach mucosa. The acidic condition in the stomach will be optimum for xylan digestion in monogastric animals [44]. Thacker and Baas [45] showed that the in vivo gastric situation was designed to mimic a low pH (2.5, 3.5) in the stomach and a high pH (4.5, 5.5, and 6.5) in the small intestine. Thus, incorporating pentosanase enzymes in the extruded swine feeds will potentially improve the digestion of the raw material and growth rate of the fed pigs. However, improving enzyme stability during the extrusion and storage is required. Improved stability by coating or encapsulating the enzyme or storage at lower temperatures could be considered in further study.

Table 5: Physical properties of swine feed supplemented with pentosanase at 0.5 g/Kg extruded with two different die end temperature

Die End Temp. (°C)	Diameter (mm)[ns]	Expansion Ratio[ns]	Bulk Density (g/cm^3)	Hardness (g)
95	4.68 ± 0.00	1.61 ± 0.00	0.41 ± 0.00[a]	8,891 ± 181[a]
110	4.69 ± 0.00	1.62 ± 0.00	0.38 ± 0.00[b]	6,972 ± 47[b]

Values are mean ± SD (n = 10) ns = means within a column with the same letter are not significantly different ($p > 0.05$).

4 Conclusions

Response surface methodology for optimal extrusion conditions for swine feeds from the main ingredients, including broken rice, ground corn, rice bran, soybean meal, and fish meal yielded the products with physical properties close to the commercial swine feeds produced by pelleting method. The supplementation of pentosanase for swine feeds at 0.5 g/ kg, with feed moisture content at 20% (w/w), die end temperature of 95 °C, and 110 °C, exhibited acceptable physical properties. However, lower temperatures showed the better retention of the enzyme during the storage. Xylanase activity was higher at pH 3.0 than at pH 6.8. However, the enzyme activities in the samples decreased to less than 50% after the storage at room temperature (30 ± 2 °C) for about 3 weeks. Further study on the improvement of enzyme stability during extrusion can help with pentosanase-supplementation for swine feed, resulting in higher feed energy values for higher pig growth rates.

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References

[1] O. J. Rojas and H. H. Stein, "Processing of ingredients and diets and effects on nutritional value for pigs," *Journal of Animal Science and Biotechnology*, vol. 8, pp. 48–60, 2017.

[2] K. J. Wondra, J. D. Hancock, K. C. Behnke, R. H. Hines, and C. R. Stark, "Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs," *Journal of Animal Science*, vol. 73, pp. 757–763, 1995.

[3] K. L. Herkelman, G. L. Cromwell, T. S. Stahly, T. W. Pfeiffer, and D. A. Knabe, "Apparent digestibility of amino-acids in raw and heated conventional and low-trypsin inhibitor soybeans for pigs," *Journal of Animal Science*, vol. 70, pp. 818–826, 1992.

[4] K. K.Lundblad, S. Issa, J. D. Hancock, K. C. Behnke, L. J. McKinney, S. Alavi, E. Prestløkken, J. Fledderus, and M. Sørensen, "Effects of steam conditioning at low and high temperature, expander conditioning and extruder processing prior to pelleting on growth performance and nutrient digestibility in nursery pigs and broiler chickens," *Animal Feed Science and Technology*, vol. 169, pp. 208–217, 2011.

[5] J. D. Hancock and K. C. Behnke, "Use of ingredient and diet processing technologies (grinding, mixing, pelleting, and extruding) to produce quality feeds for pigs," in *Swine Nutrition*, A. J. Lewis and L. L. Southern, Eds. Washington, DC: CRC Press, 2001, pp. 474–498.

[6] M. Thomas, D. J. van Zuilichem, and A. F. B. van der Poel, "Physical quality of pelleted animal feed. 2. contribution of processes and its conditions," *Animal Feed Science and Technology*, vol. 64, no. 2–4, pp. 173–192, 1997.

[7] M. Le Gall, M. Warpechowski, Y. Jaguelin-Peyraud, and J. Noblet, "Influence of dietary fibre level and pelleting on the digestibility of energy

and nutrients in growing pigs and adult sows," *Animal*, vol. 3, pp. 352–359, 2009.
[8] H. H. Stein and R. A. Bohlke, "The effects of thermal treatment of field peas (*Pisum sativum* L.) on nutrient and energy digestibility of growing pigs," *Journal of Animal Science*, vol. 85, pp. 1424–1431, 2007.
[9] R. T. Zijlstra, S. Tibble, and T. A. T. G. van Kempen, "Feed manufacturing technology and feed intake in young pigs," in *Voluntary Feed Intake in Pigs*, D. Torrallardona and E. Roura, Eds. Wageningen: Wageningen Academic Publishers, 2009, pp. 277–292.
[10] J. K. Spears and G. C. Fahey, "Resistant starch as related to companion animal nutrition," *Journal of AOAC International*, vol. 87, pp. 789–791, 2004.
[11] B. T. Richert and J. DeRouchey, "Swine feed processing and manufacturing," in *National Swine Nutrition Guide*. Iowa: Pork Center of Excellence, 2010.
[12] V. Rungsardthong, "Processing effects on some properties and functional components of extruded foods," in *Functional Foods, Dietary Supplements, Processing Effects and Health Benefits*, A. Noomhorm and I. Ahmad, Eds. UK: John Wiley & Sons, 2014, pp. 327–363.
[13] P. Sereewat, C. Suthipinittham, S. Sumathaluk, C. Puttanlek, D. Uttapap, and V. Rungsardthong, "Cooking properties and sensory acceptability of spaghetti made from rice flour and defatted soy flour," *LWT-Food Science and Technology*, vol. 60, no. 2, pp. 1061–1067, 2015.
[14] S. Korkerd, S. Wanlapa, C. Puttanlek, D. Uttapap, and V. Rungsardthong, "Expansion and functional properties of extruded snacks enriched with nutrition sources from food processing by-products," *Journal of Food Science and Technology*, vol. 53, no. 1, pp. 561–570, 2016.
[15] J. Wongsa, D. Uttapap, B.-P. Lamsal, C. Puttanlek, and V. Rungsardthong, "Effect of extrusion conditions, monoglyceride and gum arabic addition on physical and cooking properties of extruded instant rice," *KMUTNB International Journal of Applied Science and Technology*, vol. 10, no. 1, pp. 23–30, 2017, doi: 10.14416/j.ijast.2017.02.006.
[16] S. Grasso, "Extruded snacks from industrial by-products: A review," *Trends in Food Science and Technology*, vol. 99, pp. 284–294, 2020.
[17] A. Bouasla and A. Wójtowicz, "Gluten-free rice instant pasta: Effect of extrusion-cooking parameters on selected quality attributes and microstructure," *Process*, vol. 9, no. 4, 2021, Art. no. 693, doi: 10.3390/pr9040693.
[18] V. Rungsardthong, S. Wutthisilanon, T. Thongkum, T. Suthtinium, C. Puttanlek, and D. Uttapap, "Quality assessment of rice spaghetti made from jasmine rice flour and sweet potato flour supplemented with protein sources by direct extrusion," *Journal of Food Processing and Preservation*, vol. 45, no. 1, 2021, doi: 10.1111/jfpp.15450.
[19] S. Singh, S. Gamlath, and L. Wakeling, "Nutritional aspects of food extrusion: A review," *International Journal of Food Science and Technology*, vol. 42, no. 8, pp. 916–929, 2007.
[20] O. J. Rojas, E. Vinyeta, and H. H. Stein, "Effects of pelleting, extrusion, or extrusion and pelleting on energy and nutrient digestibility in diets containing different levels of fiber and fed to growing pigs," *Journal of Animal Science*, vol. 94, no. 5, pp. 1951–1960, 2016.
[21] K. Tanruean, W. Penkhrue, J. Kumla, N. Suwannarach, and S. Lumyong, "Valorization of lignocellulosic wastes to produce phytase and cellulolytic enzymes from a-thermophilic fungus, Thermoascus aurantiacus SL16W, under semi-solid-state fermentation," *Journal of Fungi*, vol. 7, no. 4, pp. 286–300, 2021.
[22] K. E. B. Knudsen, N. P. Norskov, A. K. Bolvig, M. S. Hedemann, and H. N. Larke, "Dietary fibers and associated phytochemicals in cereals," *Molecular Nutrition and Food Research*, vol. 61, no. 7, 2017, doi: 10.1002/mnfr.201600518.
[23] S. Dhawan and J. Kaur, "Microbial mannanases: An overview of production and applications," *Critical Reviews in Biotechnology*, vol. 27, pp. 197–216, 2007.
[24] K. R. Park, C. S. Park, and B. G. Kim, "An enzyme complex increases in vitro dry matter digestibility of corn and wheat in pigs," *Springer Plus*, vol. 5, 2016, Art. no. 598.
[25] M. R. Bedford and H. Schulze, "Exogenous enzymes for pigs and poultry," *Nutrition Research Reviews*, vol. 11, pp. 91–114, 1998.
[26] P. Pangsri and T. Wuttipornpun, "Mannanase and cellulase enzyme production from the agricultural

wastes by the Bacullus subtilis P 2-5 strain," *Applied Science and Engineering Progress*, vol.14, pp. 425–434, 2021, doi: 10.14416/j.asep.2020.05.002.
[27] H. Chen, S. Zhang, and S. W. Kim, "Effects of supplemental xylanase on health of the small intestine in nursery pigs fed diets with corn distillers' dried grains with solubles," *Journal of Animal Science*, vol. 98, no. 6, 2020, Art. no. 32497179, doi: 10.1093/jas/skaa185.
[28] N. Amornthewaphat and S. Attamangkune, "Extrusion and animal performance effects of extruded maize quality on digestibility and growth performance in rats and nursery pigs," *Animal Feed Science and Technology*, vol. 144, no. 3–4, pp. 292–305, 2008.
[29] D. E. Reese, R. C. Thaler, M. C. Brumm, A. J. Lewis, P. S. Miller, and G. W. Libal, "Swine nutrition guide," University of Nebraska, Nebraska, USA, 2000.
[30] G. E. P. Box and K. B. Wilson, "On the experimental attainment of optimum condition" *Journal of the Royal Statistical Society*, vol. 13, pp. 1–45, 1951.
[31] R. H. Myers and D. C. Montgomery, Response *Surface Methodology: Process and Product Optimization Using Designed Experiments*. New York: John Wiley and Sons, 1995.
[32] AOAC, *Official Methods of Analysis*, 17th ed. Maryland: Association of Official Analytical Chemists, 2000.
[33] AACC, *Approved Methods of Analysis*, 10th ed. Minnesota: American Association of Cereal Chemists, 2000.
[34] L. Alvarez-Martinez, K. P. Kondury, and J. M. Harper, "A general model for expansion of extruded products," *Journal of Food Science*, vol. 53, no. 2, pp. 609–615, 1988.
[35] X. Jiang, Y. Wang, Y. Wang, H. Huang, Y. Bai, X. Su, J. Zhang, B. Yao, T. Tu, and H. Luo, "Exploiting the activity–stability trade-off of glucose oxidase from Aspergillus niger using a simple approach to calculate thermostability of mutants," *Food Chemistry*, vol. 342, 2021, Art. no. 128270.
[36] H. K. Sharma, C. C. Xu, and W. Qin, "Isolation of bacterial strain with xylanase and xylose/glucose isomerase (GI) activity and whole cell immobilization for improved enzyme production," *Waste Biomass Valorization*, vol. 12, no. 2, pp. 833–845, 2021.
[37] J. Liu, S. Jin, H. Song, K. Huang, S. Li, X. Guan, and Y. Wang, "Effect of extrusion pretreatment on extraction, quality and antioxidant capacity of oat (*Avena Sativa* L.) bran oil," *Journal of Cereal Science*, vol. 95, 2020, Art. no. 102972.
[38] J. Ye, X. Hu, S. Luo, W. Liu, J. Chen, Z. Zeng, and C. Liu, "Properties of starch after extrusion: A review," *Starch-Stärke*, vol. 70, no. 11–12, 2018, Art. no. 1700110.
[39] S. Thymi, M. K. Krokida, A. Pappa, and Z. B. Maroulis, "Structural properties of extruded corn starch," *Journal of Food Engineering*, vol. 68, pp. 519–526, 2005.
[40] B. Vergnes, J. P. Villemaire, P. Colonna, and J. Tayeb, "Interrelationships between thermomechanical treatment and macromolecular degradation of maize starch in a novel rheometer with preshearing," *Journal of Cereal Science*, vol. 5, pp. 189–202, 1987.
[41] B. Launay and L. M. Lisch, "Twin screw extrusion cooking of starches: Flow behavior of starch pastes, expansion, and mechanical properties of extrudates," *Journal of Food Engineering*, vol. 2, pp. 259–280, 1983.
[42] M. M. Mohamad, R. A. Talib, F. S. Taip, N. L. Chin, R. Sulaiman, R. Shukri, and N. M .Z. Mohd, "Changes in the physical properties and specific mechanical energy of corn-mango peel extrudates," *CyTA-Journal of Food*, vol. 18, no. 1, pp. 417–426, 2020.
[43] M. D. P. Masli, B. A. Rasco, G. M. Ganjyal, "Composition and physicochemical characterization of fiber-rich food processing byproducts," *Journal of Food Science*, vol. 83, no. 4, pp. 956–965, 2018.
[44] P. G. Lawlor, P. B. Lynch, P. J. Caffrey, J. J. O'Reilly, and M. K. O'Connell, "Measurements of the acid-binding capacity of ingredients used in pig diets," *Irish Veterinary Journal*, vol. 58, no. 8, pp. 447–452, 2005.
[45] P. A. Thacker and T. C. Baas, "Effects of gastric pH on the activity of exogenous pentosanase and the effect of pentosanase supplementation of the diet on the performance of growing-finishing pigs," *Animal Feed Science and Technology*, vol. 63, no. 1–4, pp. 187–200, 1996.