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Chemical Composition, Physicochemical Properties, and *In Vitro* Digestibility of Pretreated Corn Grain for Use as Animal Feed

Sukanya Poolthajit[1], Suriyanee Takaeh[1], Waraporn Hahor[1], Nutt Nuntapong[2], Wanwisa Ngampongsai[3], and Karun Thongprajukaew[1]*

[1]Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand
[2]Kidchakan Supamattaya Aquatic Animal Health Research Center, Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110, Thailand
[3]Animal Production Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110, Thailand

ABSTRACT

Pretreatment techniques have been widely used to improve the quality of animal feed ingredients. In the current study, the chemical composition and the physicochemical properties of treated corn grain (extrusion, microwave irradiation, gamma irradiation, or NaOH hydrolysis) were investigated. The *in vitro* digestibility was evaluated using digestive enzyme extracts from Nile tilapia (*Oreochromis niloticus*) and broiler chicken (*Gallus gallus domesticus*), as well as the pepsin-cellulase technique of the ruminant model. There were notable changes in the chemical compositions ($p < 0.001$), as well as a nutritive profile assessment with Fourier transform infrared spectroscopy. The extrusion pretreatment significantly increased crude protein and ether extract contents, as well as gross energy. However, this method reduced ash and non-fiber carbohydrate contents while increasing the amount of neutral detergent fiber ($p < 0.001$). The determination of pH, water solubility, water absorption capacity, and thermal properties, and observation of diffraction patterns and microstructures indicated that the altered physicochemical properties of treated corn enhanced enzymatic hydrolysis. The results of carbohydrate digestibility testing suggested that microwave irradiation or extrusion were the best pretreatments for aquatic animals, while only extrusion was suitable for poultry. However, the pepsin-cellulase digestibility test revealed no differences in cellulase organic matter solubility, digestible organic matter, and metabolizable

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E-mail addresses:
spx_nu177@hotmail.com (Sukanya Poolthajit)
suriyanee.ta@gmail.com (Suriyanee Takaeh)
waraporn.hahor@gmail.com (Waraporn Hahor)
nutt.n@psu.ac.th (Nutt Nuntapong)
wanwisa.n@psu.ac.th (Wanwisa Ngampongsai)
karun.t@psu.ac.th (Karun Thongprajukaew)
*Corresponding author

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energy. Therefore, the most suitable pretreatment method for corn grain used in animal feed is dependent on the target animal group.

Keywords: Alkali treatment, aquatic animal, corn grain processing, gamma radiation, microwave treatment, poultry, pretreatment, ruminant

INTRODUCTION

Animal feed uses between 70 and 80% of the world's corn crop (Dozier et al., 2011). Pigs, poultry, and aquatic animals consume between 50 and 70% corn grain in their diets (Numoto et al., 2019). It serves as the primary source of energy and carotene in animal feed. In most countries, corn grain is the raw material most used as an energy supplement in cattle feed (Freitas et al., 2020). The different starches contained in cattle feed grains are digested at different rates and corn is an excellent grain to offer cattle because of the slow rumen solubility of its starch content. Dietary fiber must be reduced while available carbohydrates rise to increase the percentage of energy feed elements in animal diets. Therefore, increasing the efficiency of ruminant production requires improving the usage of corn, especially the utilization of carbohydrates.

Many previous studies have found that distinct changes occur in the chemical structure of grain during processing. For example, when processing grain by heating or alkaline treatment, proportions of amylopectin to amylose may affect chemical structures and physical morphology. Amylopectin component readily spreads in water to create a gel that retrogrades. Starch with a high amylose content forms a gel that undergoes stronger retrogradation (Silva et al., 2016). The morphology of starch granules changed during processing, suggesting that heat and alkaline treatments can both have an impact on starch granular structure. The strong pericarp and cuticle protein of corn seed was also broken by high temperatures and alkaline treatments, which improved ruminal degradation and increased solubility (Berger et al., 1981). In feed production, microwave and gamma irradiation can improve the quality of feed by modifying physicochemical properties to enhance enzymatic digestion (Sansuwan et al., 2017; Thongprajukaew et al., 2013). Gelatinization has been shown to increase starch availability, and gelatinized starch has been extruded and pelletized successfully in feed manufacturing (Sansuwan et al., 2017). This hydrothermal pretreatment has improved the quality of feed in a number of studies (Han et al., 2022; Karami et al., 2018; Ma et al., 2023).

The structure of the starch granule and the grain are directly impacted by the physical pretreatment techniques listed above (extrusion, microwave irradiation, gamma irradiation, and alkaline treatment). The animal feed industry accepts these techniques, which can be used to alter animal feeds in bulk at once. In general, the desirable effects of pretreatments not only affect proximate chemical compositions, but also provide qualitative changes,

involving physicochemical properties such as pH, water solubility, water absorption capacity (WAC), thermal properties, X-ray diffraction behaviors, relative crystallinity (RC) and microstructures. These characteristics are related to *in vitro* enzyme digestion, indicating whether the pretreated raw material is suitable for use as animal feed (Hahor et al., 2022; Thongprajukaew et al., 2015; Zhou et al., 2018).

Before performing an *in vivo* feeding trial, feed utilization can be examined using an *in vitro* digestibility test (Hahor et al., 2022; Thongprajukaew et al., 2015; Zhou et al., 2018). As a result, the purpose of this study was to determine how four pretreatment strategies (extrusion, microwave irradiation, gamma irradiation, or NaOH hydrolysis) affected the physicochemical characteristics and approximate chemical composition of corn grain. *In vitro* digestibility was investigated using digestive enzymes from the Nile tilapia (*Oreochromis niloticus*) and the broiler chicken (*Gallus gallus domesticus*), which were representative of aquatic animal and poultry models, respectively. Commercially available pepsin and cellulase were also used in an *in vitro* ruminant model. The obtained results could be applied for improving corn grain quality for animal feeds.

MATERIALS AND METHODS

Corn Grain Pretreatments

The yellow corn grain (CP 801; ≤ 14.5% moisture, ≥ 8 % crude protein) was purchased from Mitrkasetphand Co., Ltd. in Nakhon Pathom, Thailand. They were packed in sacks and stored at room temperature during distribution. No more than 4% of cracked grains were found during the selection and production standards. Fifty-kilogram samples were downsized to accommodate fifteen experimental units (1 kg each), derived from five treatments and three replications. The pretreatment methods were applied as used in previous studies on feed or food. The control was untreated corn (a). A pellet mill (Model 1112-4; California Pellet Mill, Crawfordsville, IN, USA) was used to extrude the corn mash through a 4.0 mm diameter ring die after it had been conditioned at 82°C for 45 s. The extrudates were separated and dried at 50 °C for 2 h (b). In a round plastic container of 23 cm in diameter by 10.5 cm in height, 300 g of corn grain was combined with distilled water (2: 1 *w*/*v*) for the microwave irradiation treatment. The mixture was exposed to radiation for 11 min at 700 W in a microwave oven (MW71B; Samsung, Kuala Lumpur, Malaysia) (Sansuwan et al., 2017) (c). In the treatment with gamma radiation, corn grain was irradiated at a dose of 30 kGy (Shawrang et al., 2008) using Co from a carrier-type gamma irradiator (JS 8900 IR-155; MDS Nordion, Ottawa, ON, Canada) (d). The grain was treated with 3.5% of concentrated NaOH (35% *w*/*v*) for 15 min as part of the alkaline pretreatment, as detailed by Takaeh et al. (2024). Loosely packed in a jar, the treated samples were spread out over a carpet and dried for 48 h at 60 °C (e). All the triplicate samples of

untreated and treated corn were freeze-dried for 24 h, ground, and sieved. Before being used for chemical analysis, the samples were stored in a desiccator.

Proximate Chemical Compositions and Nutritive Profiles

The chemical compositions of samples were determined as described by the Association of Official Analytical Chemists (1990), including dry matter (DM), crude protein (CP), ether extract (EE), and ash contents. The quantities of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the samples were measured using the Van Soest et al. (1991) method. The formula for determining non-fiber carbohydrate (NFC, %) was 100 – (% CP + % EE + % NDF + % ash). Gross energy (GE) was measured using an adiabatic bomb calorimeter (AC500; Leco, St. Joseph, MI, USA).

Fourier transform infrared (FTIR8400s; Shimadzu, Kyoto, Japan) spectroscopy was applied to untreated and treated samples to evaluate qualitative variations in the treated samples' nutritional values. Using an infrared tablet press (FW-4; Thermo Fisher Scientific, Waltham, MA, USA), two hundred milligrams of KBr were combined with two milligrams of dried corn, ground evenly, and then formed into a tablet. The spectra were obtained at a resolution of 4 cm^{-1} and in the mid-IR range of 4000 to 400 cm^{-1}.

Physicochemical Properties

pH

Distilled water (6.25 mL) was used to suspend 0.25 g of untreated and treated corn samples, which were then shaken for 10 min (Sokhey & Chinnaswamy, 1993). A pH meter (Five Easy F20; Mettler-Toledo GmbH, Greifensee, Switzerland) was used to measure the pH.

Water Solubility

Corn samples that were both untreated and treated were tested for water solubility using the protocol described by Chung et al. (2010). To summarize, 1 g of the material was mixed with 10 mL of water, shaken at 200 rpm for 1 h at room temperature, and then centrifuged for 10 min at 1500 × *g*. Following collection, the supernatant was weighed and dried for 48 h at 60 °C. The solubility of the sample was calculated by dividing the weight of the dissolved particles in the supernatant by the weight of the dry solids in the original sample.

Water Absorption Capacity (WAC)

WAC analysis was performed as described by Jitngarmkusol et al. (2008). In summary, 2 g samples of both untreated and treated corn were suspended in 5 mL of distilled water and allowed to stand at room temperature for 30 min. The suspended samples were centrifuged

for 10 min at 2000 × *g*. Following the supernatant's decantation, the sample was weighed again, and the WAC was expressed as grams of water absorbed per gram of material and was computed on a dry basis.

Differential Scanning Calorimeter (DSC)

The thermal characteristics of untreated and treated corn samples, including onset (T_o), peak (T_p) and conclusion (T_c) temperatures of starch gelatinization, temperature range (T_c–T_o), and transition enthalpy (ΔH), were determined by DSC (DSC7; Perkin Elmer, Waltham, MA, USA). Three milligrams of the sample were placed in an aluminum pan, which was sealed, allowed to acclimate for 1 h at room temperature, and then heated at a rate of 10 °C/min from 40 to 400 °C.

Diffraction Pattern and RC

Using an X-ray diffractometer (XRD, X′ Pert MPD; Philips, Netherlands), the RC and diffraction patterns of both untreated and treated corn samples were ascertained at 40 kV and 30 mA. Only a range of 4 to 40 ° was given; however, the angles were scanned from 4 to 90 ° (2θ). The RC (%) was estimated from (Area under peaks/total area) × 100.

Microstructure

Scanning electron microscopy (SEM, Quanta 400; FEI, Brno, Czech Republic) was used to examine samples of both untreated and treated corn. For the dried samples, double-sided sticky tape was used first, and then gold plating. Surface microstructures were captured at 250× and 1 500×.

In Vitro Digestibility Screening

Aquatic Animal and Poultry Models

Three specimens of four-month-old Nile tilapia and three specimens of forty-two-day-old broilers were acquired from a farm located within Prince of Songkla University. The Institutional Animal Care and Use Committee granted approval for the animal protocols (Code 2022-Sci11-018). The intestinal specimens of the investigated species were obtained and subjected to extraction using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA) and 0.2 M Na_2HPO_4-NaH_2PO_4 buffer at pH 8 (1:5 *w*/*v*). Following a 30-minute centrifugation of the homogenate at 4°C at 15000 × *g*, the recovered supernatant was dialyzed overnight against an extraction buffer. Until they were needed, the dialyzed enzymes were stored as tiny aliquots at –20 °C. The *in vitro* digestibility trial was carried out using Thongprajukaew et al. (2011)'s methodology. Five milligrams of dried corn, 10 mL of

50 mM Na_2HPO_4-NaH_2PO_4 buffer at pH 8, 50 µL of 0.5% chloramphenicol, and 125 µL of dialyzed crude enzyme extract were added to reaction mixtures, which were then incubated for 24 h at 25 °C. Protein digestibility *in vitro* was measured spectrophotometrically at 420 nm using the *DL*-alanine standard curve, and carbohydrate digestibility at 540 nm using the maltose standard curve.

Ruminant Model

The pepsin-cellulase method, as described by De Boever et al. (1986), was used to evaluate the digestibility of untreated and treated corn samples. A glass filter-crucible containing 300 mg of freeze-dried sample was filled with 30 mL of a pepsin-hydrochloric acid solution. Every five hours, the crucible was shaken during its 24-h incubation at 40 °C. After 45 minutes of immersion in a water bath at 80 °C, the crucible was rinsed with distilled water to remove any remaining residue, and the solution was aspirated. Cellulase buffer was added to 30 mL of solution at 40 °C, and the mixture was shaken every five hours for 24 h. The residue was washed with distilled water at 40 °C after the solution was aspirated. The cellulase organic matter solubility (COMS) was calculated by burning the residue at 550 °C after the digested fraction was dried at 103 °C to determine the dry matter value. The formula for COMS (%) is $(W_o–W_t/W_o) \times 100$, where W_o and W_t represent the weights of organic matter (OM) prior to and following incubation. According to De Boever et al. (1986), the metabolizable energy (ME) and digestible organic matter (DOM) of the untreated and treated corn samples were determined as follows: ME (MJ/kg DM) = (0.150 × COMS) + (0.214 × EE) – 0.99, and DOM (%) = (0.973 × COMS) – 2.49.

Statistical Analysis

There was a completely randomized design (CRD) for the studies. The presented data are the means ± standard error of mean (SEM). Significant differences between means were examined and ranked using One-Way Analysis of Variance and Duncan's Multiple Range Test (DMRT) at 95% confidence levels.

RESULTS

Chemical Compositions of Untreated and Treated Corn

Table 1 shows the proximate chemical contents of both untreated and treated corns. Relative to untreated corn, significantly decreased DM was observed in corn pretreated by extrusion and NaOH, while corn pretreated by microwave and gamma irradiation showed increased DM ($p < 0.05$). Decreased OM was observed only in the NaOH treatment. Extruded and gamma-irradiated corns showed higher CP contents, but the NaOH-treated group showed the opposite effect. The CP content of the untreated and microwave-irradiated groups did

not differ significantly. Improved EE contents were observed in all treatments, except for the NaOH treatment. Among the four treatments, large amounts of NDF and ADF were disrupted by gamma irradiation. The remaining treatments increased both indigestible components, with the exception of ADF in extruded corn. Ash content was significantly higher in the NaOH treatment relative to untreated corn, and lower in the extrusion, microwave irradiation, and gamma irradiation treatments. Increased NFC content was observed only in gamma-irradiated corn. The gross energy of the extruded sample increased, but the gamma-irradiated samples displayed the opposite pattern.

Although there were variations in peak heights and intensities, overall, the FTIR spectra were similar. At least eighteen bands (2924, 2852, 1743, 1652, 1542, 1462, 1373, 1240, 1157, 1059, 991, 927, 858, 765, 711, 574, 526, and 437 cm^{-1}) were observed in the range of 4000 to 400 cm^{-1} (Figure 1), showing qualitative alterations in nutritional profiles, particularly in the components of proteins, lipids, carbohydrates, and inorganic matter (Table S1).

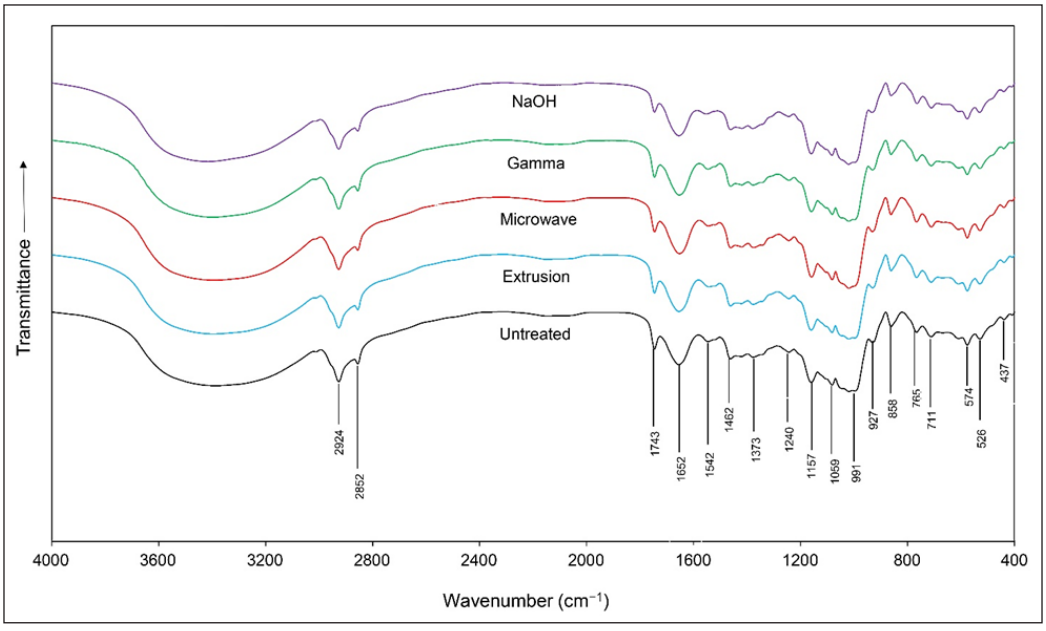

Figure 1. FTIR spectra of untreated, extruded, microwave-irradiated, gamma-irradiated, and NaOH-treated corn samples

Physicochemical Properties of Untreated and Treated Corn

pH

The pretreatment changed the pH of the corn samples ($p < 0.05$, Table 2). The corn that had been treated with NaOH had the highest pH, followed by the corn that had been microwave-

Table 1
The chemical compositions of dry matter of untreated and treated corn grains. Three separate samples were used to calculate the values

Chemical composition	Untreated	Extrusion	Microwave	Gamma	NaOH	*p*-value
Dry matter (%)	93.3 ± 0.6^{c}	82.4 ± 0.2^{e}	93.9 ± 0.1^{b}	94.4 ± 1.9^{a}	92.1 ± 0.5^{d}	<0.001
Organic matter (%)	98.5 ± 0.1^{b}	98.8 ± 0.1^{a}	98.8 ± 0.1^{a}	98.7 ± 0.1^{a}	94.7 ± 0.1^{c}	<0.001
Crude protein (%)	9.7 ± 0.1^{b}	12.3 ± 0.1^{a}	10.0 ± 0.1^{b}	12.1 ± 0.2^{a}	8.1 ± 0.1^{c}	<0.001
Ether extract (%)	4.16 ± 0.05^{c}	4.29 ± 0.01^{b}	4.52 ± 0.04^{a}	4.15 ± 0.02^{c}	2.75 ± 0.01^{d}	<0.001
Neutral detergent fiber (%)	21.3 ± 0.4^{d}	24.6 ± 0.5^{c}	28.2 ± 0.3^{b}	11.5 ± 0.3^{e}	31.9 ± 0.3^{a}	<0.001
Acid detergent fiber (%)	3.51 ± 0.03^{c}	3.49 ± 0.04^{c}	4.79 ± 0.03^{a}	2.98 ± 0.04^{d}	4.43 ± 0.02^{b}	<0.001
Ash (%)	1.66 ± 0.03^{b}	1.47 ± 0.01^{c}	1.33 ± 0.01^{c}	1.36 ± 0.01^{c}	5.74 ± 0.09^{a}	<0.001
Non-fiber carbohydrate (%)	63.1 ± 0.4^{b}	57.3 ± 0.5^{c}	55.9 ± 0.3^{d}	71.0 ± 0.3^{a}	51.5 ± 0.3^{e}	<0.001
Gross energy (kJ/g)	18.3 ± 0.1^{b}	19.0 ± 0.1^{a}	18.1 ± 0.1^{b}	18.2 ± 0.1^{c}	17.2 ± 0.1^{b}	<0.001

$^{a-e}$ Significant differences are indicated by means in the same row with different superscripts ($p < 0.05$)

Table 2
pH, water solubility, and water absorption capacity (WAC) of untreated and treated corn grains. Three separate samples were used to calculate the values

Item	Untreated	Extrusion	Microwave	Gamma	NaOH	*p*-value
pH	6.6 ± 0.1^{c}	6.3 ± 0.1^{d}	6.8 ± 0.1^{b}	6.3 ± 0.1^{d}	10.9 ± 0.1^{a}	<0.001
Water solubility (%)	6.9 ± 0.6^{c}	19.3 ± 0.24^{a}	5.8 ± 0.1^{c}	8.5 ± 1.9^{c}	12.8 ± 0.5^{b}	<0.001
WAC (g water/g feed)	0.26 ± 0.01^{bc}	0.23 ± 0.01^{c}	0.29 ± 0.01^{b}	0.27 ± 0.03^{bc}	0.37 ± 0.01^{a}	0.001

$^{a-d}$ Significant differences are indicated by means in the same row with different superscripts ($p < 0.05$)

irradiated. The pH values of extruded and gamma-irradiated corn were significantly reduced relative to untreated corn.

Water solubility and WAC

Pretreatment techniques had a substantial impact on water solubility and WAC ($p < 0.05$, Table 2). The corn that was extruded showed a maximum solubility, followed by NaOH-treated corn. Corn treated with NaOH showed the highest WAC, while the other treatments did not differ significantly compared to untreated corn.

Thermal Transition Properties

Starch gelatinization and phase change were quantified by DSC. Within the studied temperature range, all samples produced two transition peaks, except NaOH-treated corn, which produced only one peak (Table 3). Peaks I and II designated available nutrients and available nutrients in complex with other compartments. These peaks spanned temperature ranges from 45.4 to 159 °C and 264 to 286 °C, respectively. Overall, significant shifts in transition temperatures (T_o, T_p, and T_c) were observed in all pretreatments. In peak I, broader T_c–T_o ranges were observed in extruded, gamma-irradiated, and NaOH-treated corn, while ΔH did not differ significantly compared to untreated corn. For peak II, the T_c–T_o ranges in the gamma and extrusion pretreatments were wider and narrower, respectively. Corn pretreated by microwave irradiation exhibited the highest ΔH value. ΣΔH values were unaffected by pretreatment.

Diffraction Patterns

The main peaks in the diffraction patterns of the untreated and treated corn samples were similar (16.5, 18.3, and 24.6 º) (Figure 2). Nonetheless, minor variations were noted at the angles of 14.5 to 19.0 º and 22.0 to 23.5 º. Pretreatment had little effect on RC, which ranged from 21.2 to 22.7%.

Microstructure

Across the four corn grain treatments, there were a few minor variations in microstructure architectures. All of the samples had agglomerated irregular particles as their general morphology at low magnification. Smooth surfaces and swelling were observed at higher magnification. Untreated (Figures 3a and 3b), extruded (Figures 3c and 3d), and gamma-irradiated (Figures 3g and 3h) samples had similar general features. Fusion and aggregation of starch granules were seen in corn pretreated by microwave irradiation (Figures 3e and 3f). Rough, laminated, and abraded surfaces with shallow grooves were observed in corn pretreated by NaOH (Figures 3i and 3j).

Table 3
Thermal transition properties of untreated and treated corn grains. Three separate samples were used to calculate the values

Thermal parameter	Untreated	Extrusion	Microwave	Gamma	NaOH	*p*-value
Peak I						
T_o (°C)	46.8 ± 1.6	45.4 ± 1.3	46.3 ± 0.9	48.6 ± 0.6	46.1 ± 0.7	0.380
T_p (°C)	88.6 ± 0.9^{b}	90.3 ± 1.2^{ab}	88.7 ± 1.2^{b}	91.9 ± 0.5^{a}	92.5 ± 0.4^{a}	0.030
T_c (°C)	147 ± 1^{c}	157 ± 1^{ab}	149 ± 1^{c}	155 ± 1^{b}	159 ± 1^{a}	<0.001
T_c–T_o (°C)	100 ± 2^{c}	111 ± 1^{a}	103 ± 1^{c}	107 ± 1^{b}	113 ± 1^{a}	<0.001
ΔH (J/g)	207 ± 3^{ab}	180 ± 7^{b}	194 ± 1^{b}	198 ± 10^{ab}	224 ± 6^{a}	0.030
Peak II						
T_o (°C)	270 ± 1^{a}	270 ± 1^{a}	270 ± 1^{a}	264 ± 1^{b}	–	<0.001
T_p (°C)	277 ± 1^{b}	275 ± 1^{c}	278 ± 1^{a}	274 ± 1^{d}	–	<0.001
T_c (°C)	285 ± 1^{b}	280 ± 1^{d}	286 ± 1^{a}	281 ± 1^{c}	–	<0.001
T_c–T_o (°C)	15.1 ± 0.5^{b}	10.0 ± 0.2^{c}	15.9 ± 0.8^{ab}	16.8 ± 0.3^{a}	–	<0.001
ΔH (J/g)	6.13 ± 0.10^{b}	4.74 ± 0.21^{b}	7.57 ± 0.93^{a}	5.65 ± 0.26^{b}	–	<0.001
ΣΔH (J/g)	213 ± 3	185 ± 7	202 ± 12	204 ± 10	224 ± 6	0.060

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; T_c–T_o, melting temperature range, ΔH, enthalpy
$^{a–d}$ Significant differences are indicated by means in the same row with different superscripts ($p < 0.05$)

Table 4
The dry matter digestibility of untreated and treated corn grains using pepsin-cellulase for a ruminant model. Three separate samples were used to calculate the values

Digestibility	Untreated	Extrusion	Microwave	Gamma	NaOH	*p*-value
COMS (%)	94.1 ± 0.1	95.7 ± 0.1	95.9 ± 0.4	95.7 ± 0.2	96.4 ± 1.6	0.360
DOM (%)	89.1 ± 0.1	90.6 ± 0.1	90.8 ± 0.4	90.7 ± 0.19	91.3 ± 1.6	0.370
ME (MJ/kg)	14.0 ± 0.1	14.3 ± 0.1	14.4 ± 0.1	14.3 ± 0.1	14.1 ± 0.2	0.240

Note.
COMS, cellulase organic matter solubility; DOM, digestible organic matter; ME, metabolizable energy
Data are expressed as means ± SEM

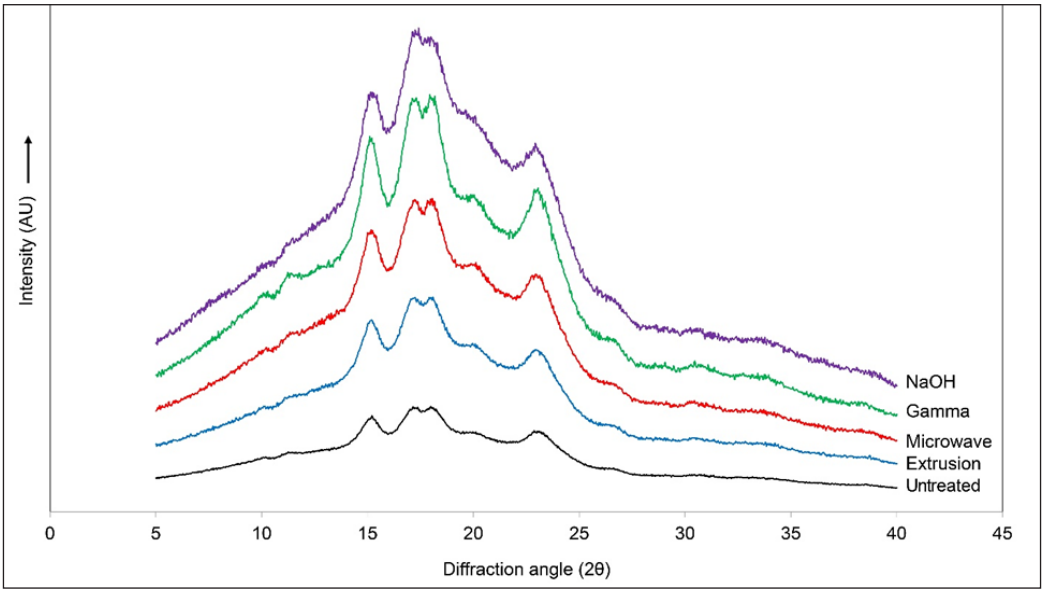

Figure 2. Diffraction patterns of untreated, extruded, microwave-irradiated, gamma-irradiated, and NaOH-treated corn samples

In Vitro Digestibility

Aquatic Animal and Poultry Models

Using Nile tilapia digestive enzymes, the corn treated with NaOH showed the maximum carbohydrate digestibility, followed by corn that was microwave-irradiated, extruded, and gamma-irradiated, respectively. All pretreated samples showed higher carbohydrate digestibility relative to untreated corn ($p < 0.05$; Figure 4a). There were no discernible variations in the protein digestibility between the untreated and treated samples ($p > 0.05$; Figure 4b). When using digestive enzymes from broiler chickens, the highest carbohydrate digestibility was observed in NaOH-treated corn, followed by extruded corn. Untreated corn, gamma-irradiated corn, and microwave-irradiated corn showed the lowest digestibility of carbohydrates ($p < 0.05$; Figure 4c), except for gamma-irradiated corn which showed no significant differences relative to extrusion ($p > 0.05$). Protein digestibility using broiler enzymes was unaffected by pretreatment ($p > 0.05$; Figure 4d).

Ruminant Model

No significant differences in COMS (94.1 to 96.4%), DOM (89.1 to 91.3%), and ME (14.0 to 14.4%) were observed among the four treatments and control ($p > 0.05$, Table 4).

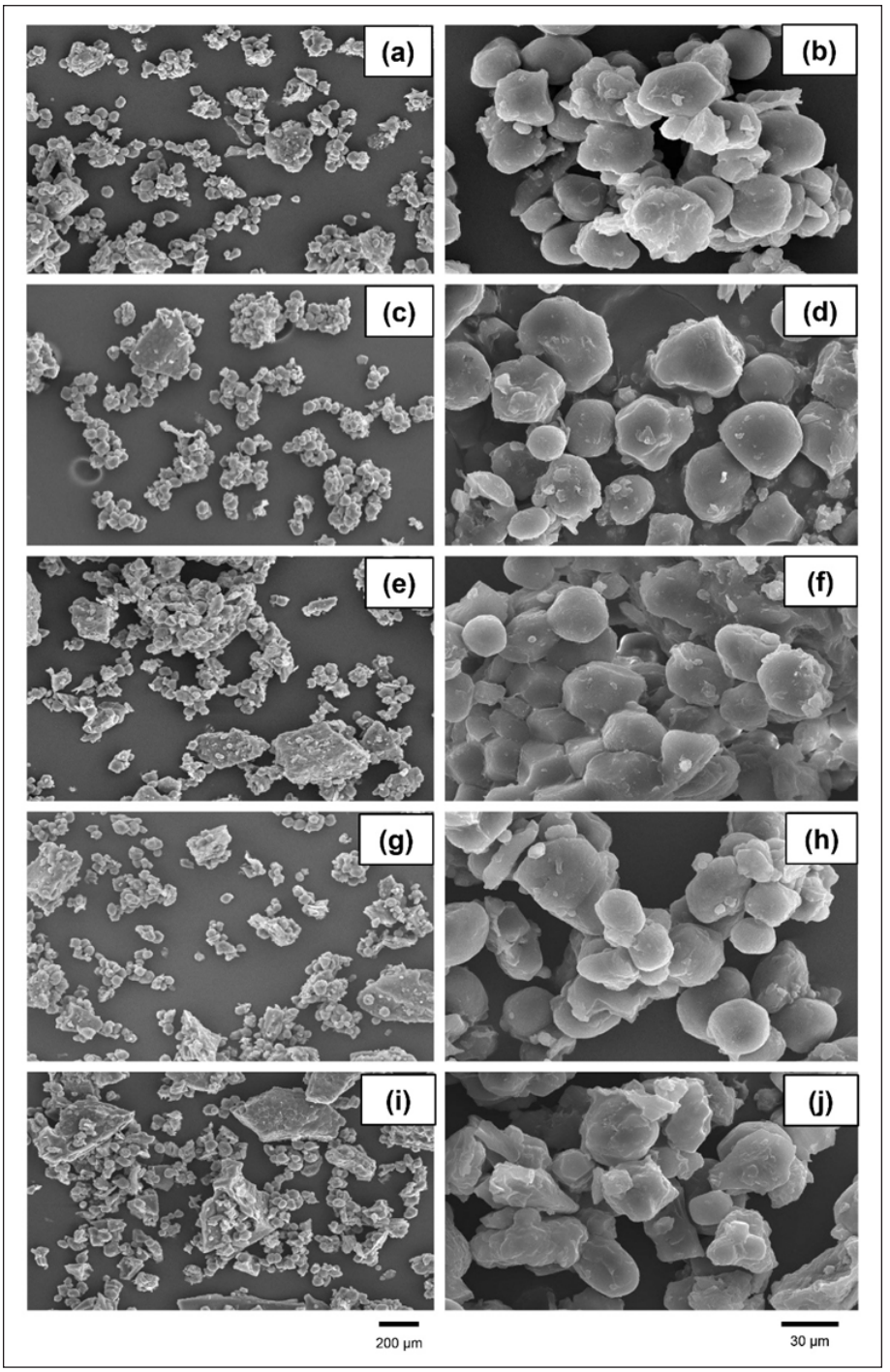

Figure 3. Microstructures of untreated (a and b), extruded (c and d), microwave-irradiated (e and f), gamma-irradiated (g and h), and NaOH-treated (i and j) corn. Micrographs were captured at 250× (left) and 1500× (right) magnifications

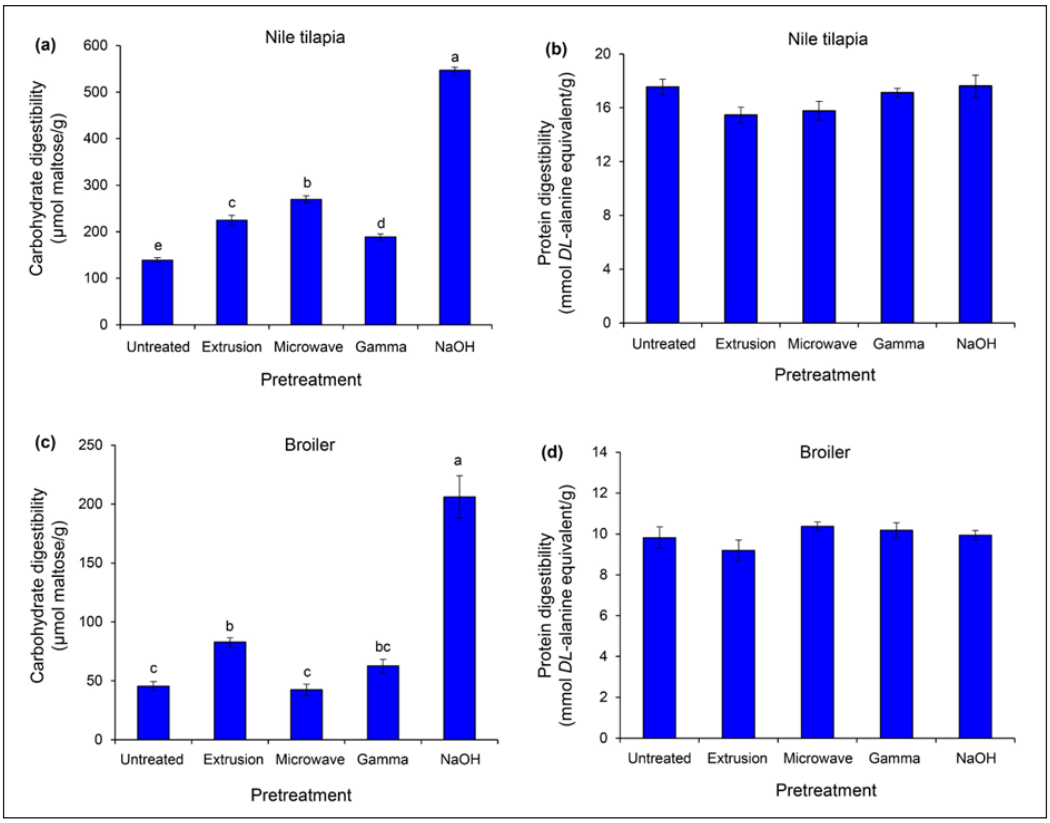

Figure 4. *In vitro* digestibility of untreated and treated corn samples for carbohydrates (µmol maltose/g sample, left) and protein (mmol *DL*-alanine equivalent/g sample, right). The Nile tilapia (a and b) and broiler (c and d) provided the enzymes for the *in vitro* tests. Means ± SEM ($n = 3$) are used to express the data. Different superscripts ($p < 0.05$) indicate significant differences between treatments

DISCUSSION

Chemical Compositions

The chemical compositions of the corn grain were significantly altered by the pretreatments. Changes in the chemical composition can be due to differences in dry matter content, or losses in some components, for example volatilization of organic matter can increase the ash content. CP and EE contents were lower in the NaOH treatment than in control and all other treatments. The average ranges of CP and EE (8.09 to 12.3% and 2.75 to 4.29%, respectively) were similar to previously reported ranges of 8.44 to 8.80% for CP and 4.70 to 4.95% for EE (Srakaew et al., 2021; Suksombat et al., 2007). In general, the thermo-alkaline NaOH process drastically changed the proteins, lipids and starch in the grain kernels, reducing the values of CP, EE, and NFC in treated samples (Palacios-Fonseca et al., 2013). These changes increased the amounts of NDF and ADF in the NaOH-treated corn. Furthermore, the results showed an increase in ash content when the samples were

treated with NaOH, perhaps due to Na is present in the oxide form. Nonetheless, grain corn contains very little ash and fibrous carbohydrates, so any loss will not have an impact on the composition of other ingredients or probably GE.

The increased CP in gamma-irradiated corn was consistent with findings reported for canola meal (Sekali et al., 2023) and palm kernel meal (Thongprajukaew et al., 2013). Proteins and other N-containing substances developed covalent cross-linkages upon exposure to gamma radiation, which changed them into greater molecular weight aggregates (Sadeghi & Shawrang, 2006). While NDF and ADF decreased, NFC increased, indicating the disruption of cell wall constituents, enabling the expansion of available carbohydrates (Thongprajukaew et al., 2013). The significant decrease in the ash content of gamma-irradiated corn is in agreement with studies of pretreatments of velvet bean seed (Bhat et al., 2008) and pigeon pea flour (Bamidele & Akanbi, 2013). In general, gamma irradiation can affect the amount of minerals and ash in certain materials. It might alter the mineral composition and impact the overall quantity of ash present, potentially leading to the amorphization of some phases (Hashim et al., 2024; Lowińska-Kluge & Piszora, 2008).

The proximate chemical composition of microwaved corn grain was different to the proximate chemical composition of gamma-irradiated corn. In general, EE-containing foods are sensitive to heating by microwave irradiation. However, since microwave irradiation can extract lipids, the increased EE contents in the present study might be due to improved extraction efficiency (Hahor et al., 2022). The creation of protein-fiber complexes during pretreatment may be the cause of the elevated NDF and ADF (Bressani, 1993). These changes would have a direct effect on the cellulose, hemicellulose, and lignin composition of the lignocellulosic barrier as well as the NFC (Thongprajukaew et al., 2015). Alteration in the structure and composition of cell walls and other components, such as the formation of new cell wall-bound protein or insoluble condensed tannin-protein polymers, can result from microwave treatment, increasing the amount of NDF and ADF in food (Jančík et al., 2017; Shishir et al., 2020). For hydrothermal extrusion, the amount of CP and EE in corn grain were increased by this treatment method. However, this method reduced the amount of ash and NFC while increasing NDF.

Differences among specific constituents between the four pretreatments did not affect GE in microwaved and NaOH-treated corns. However, FTIR spectral analysis indicated qualitative changes to corn grain after pretreatment. Changes in the characteristics of proteins, lipids, carbohydrates, and inorganic materials were found, as shown in the annotated spectra (Table S1).

Physicochemical Properties

Significantly increased pH was observed in corn pretreated with NaOH. NaOH contributes to the increased overall alkalinity of pretreated corn. Significant changes were also observed in the other treatments. The breakdown of larger molecules into smaller ones, particularly

proteins and carbohydrates, which results in the production of acidic carboxyl groups, is most likely the cause of the lowered pH of extruded and gamma-irradiated corn (Hahor et al., 2022; Thongprajukaew et al., 2015). Conversely, the possibility exists that the elevated pH in the microwave-irradiated corn results from the hydroxyl groups released during the decomposition of lignocellulosic components (Thongprajukaew et al., 2013).

The extruded and NaOH-treated corn had higher water solubility than other treatments. This characteristic is associated with the capacity of enzymes to hydrolyze substrates (Chung et al., 2010). Regarding interactions with water, the WAC of NaOH-treated corn was higher than control whereas WAC did not differ relative to control in the other treatments. The differences in fiber content might have impacted WAC since the fiber constituents, especially hydrophilic celluloses, contain a number of hydroxyl groups, producing a polarity which facilitates water absorption (Rashid et al., 2015).

The starch gelatinization is often quantified using DSC. In the present study, the T_p and T_c from Peak I showed upward trends, except for the corn pretreated by microwave irradiation. Amylose-amylose and amylose-amylopectin interactions, or the complexation between amylose and other substances may cause increased temperature (Caetano et al., 2019). However, similar changes in Peak II were found in the microwave-irradiated sample, suggesting that complexes form at higher temperatures. On the other hand, some thermal parameters in Peak II were lower than control in corn pretreated by extrusion or gamma irradiation. It is possible that these pretreatment methods disrupted the starch granule, which might be related to the lower temperature of gelatinization (Macarthur & Appolonia, 1984). Shifts in onset and conclusion points caused significantly changed $T_c - T_o$ ranges, indicating a wide range of cleaved polymer chain lengths after pretreatments (Thongprajukaew et al., 2015). Molecular transformation brought about by pretreatment can indicated by the ΔH. The reduced $\Sigma\Delta H$ of the extruded corn was significant, indicating the presence of low amounts of unaltered untreated corn. The hydrothermal extrusion pretreatment destroyed the ordered structure of starch granules. This characteristic is beneficial for enzymatic hydrolysis *in vitro*.

The crystal structure of the untreated and treated corn samples was determined by XRD analysis. The diffraction patterns exhibited intense peaks at 16.5, 18.3 and 24.6 °, respectively. This result was consistent with the intense peaks of corn starch at 15, 17, 18 and 23 ° reported by Wang et al. (2020) that indicated a classical A-type crystalline structure. However, this unique crystal structure can be altered by the pretreatment disruption of the crystallization region (Chang et al., 2013). Although some structural changes were observed via XRD, the four present pretreatment methods did not alter corn RC.

Surface roughness and the ability of enzymes to use feed components are directly related (Thongprajukaew et al., 2013). Fusion and aggregation characteristics in corn pretreated by microwave irradiation indicate the gelatinization of starch. Disruptions

causing rough, laminated, and abraded surfaces with shallow grooves were observed in corn treated with NaOH. On the other hand, extruded and gamma-irradiated corn samples showed similar characteristics to untreated corn, indicating small changes in the starch architecture. Based on overall physicochemical investigations, extrusion appears to be a suitable pretreatment method for corn grain.

In Vitro Digestibility

The qualitative changes in raw materials caused by the pretreatment process directly improve digestibility by digestive enzymes, whereas changes in proximate chemical composition have little impact on *in vitro* digestibility (Thongprajukaew et al., 2013). Significant improvements in carbohydrate digestibility were observed in the aquatic animal and poultry *in vitro* models. NaOH pretreatment increased carbohydrate digestibility most in both models, but NaOH can have hazardous effects and there is a lack of information about the consumption of corn pretreated in this way by monogastric animals (Han et al., 2022). The hydrothermal extrusion treatment appears to be suitable for these two model species, while microwave irradiation gave good results with digestive enzymes from Nile tilapia. Improved nutrient digestibility or feed utilization have been reported for these pretreatments in a number of studies (Hahor et al., 2022; Sansuwan et al., 2017; Thongprajukaew et al., 2015). Nutritive profiles and physicochemical changes support the increased *in vitro* carbohydrate digestibility in both models.

For ruminant model, we observed no differences in digestibility values between untreated and treated corn samples. Nevertheless, there are not many published comparisons of the molecular structures of variously treated corn. Xu et al. (2018) reported that steam-flaked corn had higher rumen degradable DM and starch, but lower rumen degradable protein, compared to untreated corn. Furthermore, Karami et al. (2018) used an *in vitro* gas test with two types of thermally treated corn demonstrating that the OM digestibility and ME of extruded corn were higher than those of steam-flaked corn. Moreover, based on the degradation of starch, the study of Han et al. (2022) found that steam flaking and extrusion were more beneficial than grinding. The starch gelatinization was increased during both processes (Boroojeni et al., 2016), possibly due to the swelling during steam flaking and extrusion. However, starch digestibility was not assessed in the present study and it is possible that physicochemical changes may have direct effects on starch digestibility, and classification by degrees of digestibility might clarify the results.

CONCLUSION

All of the observed proximate chemical compositions and nutritional values of corn grain were substantially altered by the pretreatment methods. Among four pretreatments, hydrothermal extrusion increased crude protein and ether extract contents but reduced ash

and non-fiber carbohydrate contents. In ways that were expected to increase enzymatic hydrolysis, pretreatment techniques improved physicochemical qualities. *In vitro* carbohydrate digestibility indicates that either hydrothermal extrusion or microwave irradiation should be utilized to prepare corn grain for aquatic animals, even if only extrusion was suitable for chicken feed. The use of pretreatment to improve corn grain for ruminant feed was supported by better chemical composition, nutritional values, and physicochemical features, even though the four pretreatment techniques did not affect the observed digestibility values. The data obtained in this study can be used to produce high-quality raw materials for sale to consumers or to prepare meals for animal farms. Nevertheless, this investigation altered raw materials under particular circumstances mentioned in previous studies. Changes to the pretreatment circumstances may also have an impact on the chemical composition, physicochemical characteristics, and digestibility.

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Table S1
Tentative assignments of FTIR spectral peaks found in unprocessed (native) and pretreated corn grains

Wavenumber (cm^{-1})	Tentative band assignment	Macromolecule	References
2924	v_{as} (CH_2) stretching of methylene	Lipid	Lewis and McElhaney (1996) and Stuart (1997)
2852	v_s (CH_2) stretching of methylene	Lipid	Lewis and McElhaney (1996) and Stuart (1997)
1743	v (C=O) stretching of esters	Lipid	Giordano et al. (2001)
1652	v_s (C=O) stretching of amide I	Protein	Dean et al. (2008)
1542	δ (N-H) bending and v (C-N) stretching of amide II	Protein	Stuart (1997)
1462	δ_{as} (CH_2) bending of methyl	Lipid	Lewis and McElhaney (1996) and Stuart (1997)
	δ_{as} (CH_3) bending of methyl	Protein	Giordano et al. (2001)
1373	v_s (COO-) stretching of amino acid salt	Protein	Guzman et al. (2001)
	v_s (C=O) stretching vibrations of carboxylate	Carbohydrate	Falkeborg et al. (2014)
1240	N(C=O) stretching and (C-OH) bending of deprotonated amino acid	Protein	Li et al. (2009)
1157	v (C-O-C) stretching of polysaccharide	Carbohydrate	Brandenburg and Seydel (1996)
1059	v (C-O-C) stretching of polysaccharide	Carbohydrate	Brandenburg and Seydel (1996)
991	v (C-O-C) stretching of polysaccharide	Carbohydrate	Brandenburg and Seydel (1996)
927	C=C bending of alkene	Alkene	Guzman et al. (2001)
858	v_{as} (PO_4^{3-}) P-O asymmetric stretching of lipids	Lipid	Dean et al. (2007)
765	δ (CO_3^{2-}) Out of O-C=O bending of oxalate	Inorganic	Giordano et al. (2001)
711	(CH^{2-}), C-H rocking of lipids	Lipid	Stuart (1997)
574	C-Br stretching of halo compound	Inorganic	Wong et al. (1993)
526	N-C=O of amides	Amides	Maquelin et al. (2002)
437	v_4 (PO_4^{3-}) P-O stretching of tetrahedral inorganic molecules	Inorganic	Benning et al. (2004)