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Breaking behaviour and interactions in maize and soybean meal while grinding of a hammer mill

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ABSTRACT

The objective of the present study was to investigate whether mixing ratio of maize and soybean meal (SBM) affects the breaking behaviour during hammer-milling in terms of the nutrient properties and in vitro digestibility of fractionated particles. Mixtures of maize and SBM with different proportions (% Maize:SBM; 0:100, 25:75, 50:50, 75:25, 100:0) were hammer milled using a 2-mm screen. The obtained powder was sieved into seven fractions with size ranges from 0.149 to 1.190 mm. Results show that energy consumption of grinding mixtures increased from 3.8 to 48.4 kJ/kg with the maize proportion increasing from zero to 100%. Mixing proportion of maize and SBM showed significant effects on nutrient content of fractionated material. For hammer milled material <595 µm, the in vitro digestibility of crude protein (CP) and organic matter (OM) of fractionated material decreased with increasing particle size. Additionally grinding fractionated particles ≥595 µm over a 1-mm sized screen before in vitro digestion analysis increased the digestibility of OM and CP. Equivalent particle size (EPS) and geometric standard deviation (GSD) of hammer milled maize and SBM and their mixtures correlated better than geometric mean diameter (GMD) to OM and CP in vitro digestibility in a linear regression model. In summary, the mixing ratio of maize and SBM had a significant effect on the breaking behaviour of ingredients and in vitro digestibility of CP and OM of the isolated fractions. Mixing ingredients before grinding is suggested in terms of saving energy consumption. The GSD/EPS of ground material should be considered while studying the effects of particle size distribution on the in vitro digestibility of nutrients.

1. Introduction

Grinding is an essential technological process in feed manufacturing, as it determines the particle size of ingredients, which plays an important role not only in downstream processing procedures (e.g. conditioning, pelleting, extrusion) in the feed industry but also relates to the digestibility of nutrients and growth performance of animals. Svihus et al. [1] reported that smaller particle size achieves a higher degree of starch gelatinization and improves pellet durability. Numerous studies found that reducing the particle size of ingredients/diets increases the digestibility of nutrients and, as a result, better growth performance in pigs can be found [2–7]. Coarse particles can help reduce gastric ulcers in pigs and maintain gastrointestinal health [8–13]. Because of the impact of particle size on animal health and productivity, finding the optimized particle size of ground ingredients or diets has attracted significant attention. On one hand, research has been conducted regarding hammer-milling conditions to improve grinding performance including variables such as mill type, mill method (dry, wet), sieve opening, (tip) speed of hammers, feed rate, the geometry of the grinding chamber concerning position and number of breaker plates [14–18]. On the other hand, various studies have been conducted investigating the properties of various feed materials to be ground such as initial particle size and moisture content [19–23].

In addition, other studies have investigated fractions of ground ingredients and feeds to characterize particle properties (physically and chemically), as well as the in vitro digestibility of

Abbreviations: CP, crude protein; DM, dry matter; EPD, equivalent particle size; GMD, geometric mean diameter; GSD, geometric standard deviation; NDF, neutral detergent fibre; OM, organic matter; PSD, particle size distribution; SBM, soybean meal.

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nutrients/components or digestion rate of starch [24–29]. The latter investigations [26,28,29] provide a better understanding of the breaking behaviour of feed ingredients and their interaction with digestive processes within the animal. All these studies, however, focused on the grinding of single ingredients rather than that of a mixture of feed materials. The novelty of this research is looking into breaking behaviour of ingredient mixtures, which has a more instructive significance to practical feed production, since grinding ingredients after dosing and mixing is more common in practical feed manufacturing.

The objective of the present study was to investigate the effects of mixing ratio of maize and soybean meal (SBM) on breakage behaviour during hammer-milling in terms of nutrient properties and in vitro digestibility of feed particles. Maize and SBM were mixed in different proportions (% maize:SBM; 0:100, 25:75, 50:50, 75:25, 100:0) and hammer milled with a screen of 2 mm. Energy consumption during grinding was recorded, and particle size distribution (PSD), nutrient content and in vitro digestibility of OM and CP of hammer milled material was determined. After hammer-milling, the material was also sieved into different size fractions, and then were analyzed for nutrient content and in vitro OM and CP digestibility.

2. Material and method

2.1. Sample preparation

2.1.1. Material mixture

Whole French maize (200 kg) and Brazilian SBM (Research Diet Service B.V., Wijk Bij Duurstede, The Netherlands) (200 kg) each originating from a single batch was purchased in ten bags of 20 kg per ingredient. Maize and SBM were mixed in a paddle mixer (Forberg, type F60, Larvik-Norway) for 120 s in a ratio (%) of (maize:SBM) 0:100, 25:75, 50:50, 75:25 and 100:0 (M0S100, M25S75, M50S50, M75S25 and M100S0, respectively). Batches of 20 kg per treatment were prepared. Care was taken to prepare each mixing ratio by using two bags of each raw material as duplicates. The resulting 10 bags (20 kg each) were individually air-tight sealed in plastic bags and kept at 4 °C. At least 12 h before hammer-milling, each sealed bag was acclimatized to room temperature.

2.1.2. Grinding

A separate batch of 20 kg maize was first ground to warm up the hammer mill (Engl hammer mill, Dongen, The Netherlands, type 30, with 7.5 kW motor) before the first randomly selected mixture was milled. Subsequent mixtures were randomly selected with repeats following each other and thorough cleaning of the hammer mill (feeder, milling chamber, outlet) between runs. For each grinding, the material was poured into a feeding hopper and the adjustable inlet was opened to a fixed 80% position when the tip speed reached 1500 rpm. Each mixture was hammer milled at a fixed running speed of 1500 rpm over a 2 mm screen-sized plate sieve. A data-logger (Hiflex, OPT-2-2USB485-OBUS, The Netherlands) was used to record the various parameters of the hammer mill (e.g. motor current, motor voltage, grinding time) every second during grinding. The effective energy consumption was calculated by subtracting the idle load from the total load (kJ/kg) energy consumption during grinding. The energy consumption of grinding maize, SBM and the mixtures of the two ingredients were also estimated according to Kick's law [30]:

$$SME = K_k \times \ln(\frac{d_i}{d_f})$$

where SME (kJ/kg) is the effective energy consumption, K_k is the Kick's constant (kJ/kg), d_f is the final (mean) particle size (mm) and d_i is the initial (mean) particle size (mm).

With measured SME, and the GMD of maize and SBM before and after grinding, the Kick's constant can be calculated. The values obtained in these calculations were 22.75 kJ/kg for maize and 19.16 kJ/kg for SBM. Kick's constants for the mixtures of maize and SBM were linearly interpolated between the values for Maize and SBM using the following formula:

$$K_m = 22.75 \times x + 19.16 \times (1 - x)$$

where K_m is the Kick's constant for the mixture of maize and SBM; x is the fraction of maize in the mixture, equal to 0.25, 0.50 or 0.75.

In addition, Kicks constants were calculated for the mixtures by calculating the initial mean particle sized based on a weighted average of the original PSD's of Maize and SBM (d_i) and estimated for the materials after grinding from the sieve analysis (d_f). In combination with the energy consumption Kick's constants for the mixtures is calculated as indicated above.

2.1.3. Sampling and sieving

After hammer-milling, approximately 5 kg of a representative subsample was collected from the 20 kg using the quartering and coning method [31]. After this ~1.25 kg of the subsample was collected using a multi-slot divider (Mooij-Argo, Hegelsom, the Netherlands) to determine PSD, nutrient content and in vitro digestibility of OM and CP of hammer milled maize, SBM and their mixtures. The PSD was determined using the 15-sieve method in duplicate [32]. The sieve shaker (AS 200 Control, Retsch, Haan, Germany) employed a 3-D throwing motion for 10 min with an amplitude of 2 mm and an interval time of 6 s shaking. Two rubber 20 mm-diameter balls were used as sieving aid on each sieve where the sieve opening was smaller than 300 μm. Geometric mean diameter (GMD) and geometric standard deviation (GSD) were calculated based on the PSD according to ASABE (2008). The equivalent particle size (EPS) including arithmetic mean diameter, mean surface area diameter, mean volume diameter, mean volume-surface area diameter and weight mean diameter were calculated according to Lachman et al. [33]. The individual EPS, GMD and GSD were all related to the in vitro digestibility data in a linear regression model.

The remainder of the hammer milled material (~3.75 kg) was used to obtain seven fractions for each of the treatments by sieving. The selection of six sieves was determined from PSD data to yield fractions of relatively evenly distributed mass. The sieve opening for these sieves included 1.190, 0.841, 0.595, 0.420, 0.297, 0.149 mm and the pan. In the present study, the term particle size refers to particles that were retained on a particular sieve. For example, F0.595 means that the particles in that fraction passed the 0.841 mm sieve and were retained on the 0.595 mm sieve. Multiple sievings were performed to obtain enough material (at least 70 g for each fraction) for chemical- and in vitro digestion analysis. The sieved material on each sieve layer was collected from each sieving, pooled per treatment, and kept at −20 °C until further analysis.

2.1.4. Additionally grinding of samples before analysis

The material of each size class was analyzed for its nutrient content and in vitro digestibility of organic matter (OM) and crude protein (CP). As prescribed for the in vitro digestion analysis protocol of Boisen and Fernández (1995; 1997) [34,35], samples should pass a 1.0 mm sieve to obtain homogenous samples for analysis. For this reason, samples with a GMD greater than 1.0 mm (particles retained on the 1.190- and 0.841-mm sieves) were additionally ground in a laboratory mill (ZM200, Retsch GmbH, Hann, Germany) using a 1.0 mm sieve with trapezoidal holes at 12,000 rpm.

2.2. Chemical composition analysis

All the samples were analyzed in simplo. The dry matter (DM) content of all samples was determined after drying in an air circulation oven at 103 °C for 4 h [36], with ash content determined after combustion at 550 °C for 3 h in a muffle furnace [37]. Neutral detergent fiber (NDF) was determined with heat-stable amylase (thermamyl) and alcalase, using the standard procedure of Van Soest et al. [38]. Nitrogen (N) content was determined by the DUMAS technique [39], and CP was calculated by multiplying the N content by 6.25. The starch content of maize was determined using the enzymic method [40].

2.3. In vitro digestibility analysis

The in vitro digestibility was determined as per the method described by Lyu et al. [41] which is based on the method described by Boisen and Fernández (1995) [34]. Briefly, 10 g of sample was mixed with 250 ml phosphate buffer (0.1 M, pH 6.0) and 20 ml HCl solution (1 M) in a 600 ml beaker before being incubated with freshly prepared pepsin solution (10 ml, 10 g/l) at pH 3.5 and 39 ℃ for 90 min under constant magnetic stirring. To mimic small intestine digestion, 100 ml phosphate buffer (0.2 M, pH 6.8) and 30 ml NaOH (1 M) were added to the mixture, followed by incubation with freshly prepared pancreatin solution (10 ml, 100 g/l) and bile solution (10 ml, 150 g/l) at pH 6.8 and 39 °C for 210 min under constant magnetic stirring. The undigested residues were then collected by filtration through nylon gaze with a pore size of 40 μm and porosity of 0.30 (PA 40/30, Nybolt, Switzerland) using a vacuum pump. After sequential washing of all material with 70% ethanol and acetone, residues were dried overnight in an oven at 70 °C before determination of DM, ash, and CP.

2.4. Statistical analysis

Data on nutrient content and in vitro digestibility of particle size fractions for the different mixing ratios were analyzed by two-way analysis of variance using the general linear model in R 3.6.1 [42], followed by Tukey's multiple comparisons using 'HSD. test' function in 'agricolae' package [43]. The statistical model used was:

$$\gamma_{ijk} = \mu_0 + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ijk}$$

where γ_{ijk} = response variable, (k = 1,2, the number of measurements), μ_0 = overall mean, α_i = effect of mixing ratio i = 1…5, β_j = effect of fraction j = 1…7, $(\alpha \times \beta)_{ij}$ = interaction of mixing ratio i and fraction j and ε_{ijk} = residual error with a mean of 0 and variance σ^2. α_i β_j was the fixed effect and the minimum significance threshold was set at 0.05. It should be noticed that the fractions of F0.841 and F1.190 were ground over 1 mm sized screen before in vitro digestibility analysis as prescribed for the protocol of Boisen and Fernández (1995; 1997) [34,35], and the statistical model cannot account for this additional grinding effect.

3. Results

3.1. Grinding performance

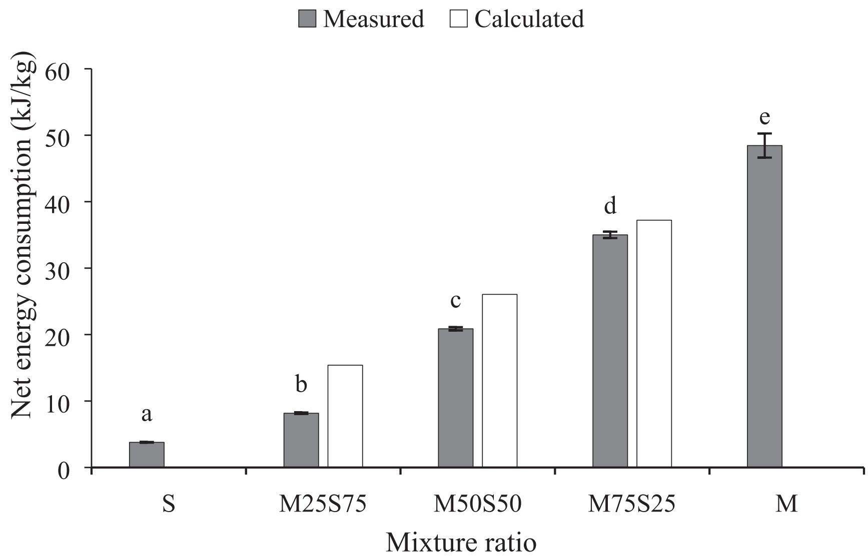
The energy consumption (as measured) during the hammer-milling of maize and SBM mixtures is presented in Fig. 1. The energy consumption for grinding increased linearly (P < 0.05) from 3.8 to 48.4 kJ/kg with the maize proportion rising from 0% to 100%. The calculated energy consumption for grinding the M25S75, M50S50 and M75S25 samples were 7.23, 5.18 and 2.21 kJ/kg more respectively than the measured energy consumption.

The particle size (GMD), distribution width as measured by GSD and EPSs of the different hammer milled maize and SBM mixtures are reported in Table 1. Geometric mean diameter, GSD and EPS were significantly different (P < 0.05) among mixtures (Table 1). The significant difference of GMD was only observed between the single SBM of 658.0 μm and the other three mixtures M25S75, M50S50 and M75S25 of 587.7, 577.0 and 577.4 μm, and maize of 594.6 μm. The uniformity of ground material was decreased (increasing GSD) with an increased maize level in the mixture. The EPSs of maize were the largest compared to the EPS of materials studied in this experiment. The mean volume-surface area diameter and weight mean diameter of milled mixtures becomes larger with an increasing proportion of maize. After grinding, the OM and CP in vitro digestibility of single maize, SBM and the mixtures of the two were analyzed (Table 1). With maize proportion increasing in the material, the in vitro digestibility of OM increased from 0.80 to 0.85, yet the CP in vitro digestibility decreased from 0.94 to 0.86. The EPSs, GMD and GSD were correlated to the OM and CP in vitro digestibility. Weight mean diameter, mean volume-surface area diameter and GSD fit well with OM in vitro digestibility with R^2 > 0.9. While the CP in vitro digestibility correlated to mean surface area diameter and mean volume diameter most, with an R^2 of 0.85 and 0.82 respectively.

On average, the recovery of the hammer milled material on the various sieves + pan to determine the PSD was more than 99.9%. The PSD and cumulative PSD as affected by the mixing ratio are presented in Fig. 2. The cumulative mass fraction for all ground ingredients reached 50% at F0.420 and F0.595. Approximately 60% of the mass of material was found in the range F0.595 – F1.190. For the F0.595 and F0.841, the mass of material decreased with an increase in maize level in the mixture, while in F1.680, the opposite trend was observed.

3.2. Nutrient content

The recovery of nutrient (ash, CP, starch, NDF) quantity of hammer milled maize and SBM and their mixtures on the various sieves + pan was 86.92–99.97%. Within the mixtures, marked differences in nutrient content of fractions were observed. Also, the nutrient content within fractions was also significantly affected by the maize and SBM mixing ratio (Fig. 3). In M0S100, the DM content decreased from 902.8 to 889.1 g/kg at F0.595 and then increased to 893.7 g/kg at F1.190. As for the other mixtures, the highest DM content was observed for material in the pan fraction without significant differences between mixtures. The ash content first increased and then decreased with an increasing particle size in M25S75, M50S50 and M75S25, and reached the highest value of 65.0, 55.4, and 40.0 g/kg DM at F0.595, respectively. As for the single ingredients, maize and SBM (M0S100 and M100S0), a decreasing trend was observed in the ash content for the pan fraction, with the largest amount of 86.1 and 27.5 g/kg DM, respectively. In each fraction, both the ash and CP content decreased with an increasing SBM level. In M25S75, M50S50 and M75S25, the CP content increased first and reached its highest value of 520.9, 435.0 and 317.2 g/kg DM, respectively in F0.595 and then decreased with increasing particle size. As for SBM (M0S100), the CP content increased with sieve opening increasing, and the highest value of 571.7 g/kg DM was recorded at F1.190. The CP content of maize (M100S0) differed less among fractions with an average of 96.1 g/kg across the various fractions. In the smallest and largest size fraction, NDF decreased as the maize level in the mixture increased. For M75S25 and M100S0, the NDF content increased and then decreased with an increasing particle size. These two



Material	S	M25S75	M50S50	M75S25	M
Interpolation (K_m; kJ/kg)		20.06	20.95	21.85	
Calculation (K_k; kJ/kg)	19.16	10.64	16.79	20.55	22.75

Fig. 1. Net energy consumption of hammer-milled maize (M) and soybean meal (S) and calculated energy consumption according to Kick's law. Measured values with different letters are significantly different ($P < 0.05$). Kick's constant of materials (kJ/kg) are provided in the table.

Table 1
Equivalent particle size and in vitro digestibility of hammer-milled maize (M), soybean meal (S) and the three respective mixtures.

Parameter	% M:S					SEM	P	R^2 (OM/CP)
	0:100	25:75	50:50	75:25	100:0			
Geometric mean diameter (μm)	658.0^a	587.7b^a	577.0^b	577.4^b	594.6^b	15.13	0.023	0.46/0.03
Geometric standard deviation (μm)	436.6^a	467.1^a	467.3^a	529.8^b	552.8^b	21.70	<0.001	0.91/0.70
Arithmetic mean diameter (μm)	758.4^a	716.8^a	715.9^a	746.3^a	775.4^b	11.66	0.012	0.11/0.54
Mean surface area diameter (μm)	828.0^a	807.1^a	819.9^a	866.9^b	904.6^c	17.87	<0.001	0.65/0.85
Mean volume diameter (μm)	884.5^a	877.7^a	901.5^a	957.4^b	999.0^c	23.40	<0.001	0.82/0.82
Mean volume-surface area diameter (μm)	1009.5^a	1037.8^b	1090.0^c	1167.7^d	1218.4^e	39.15	<0.001	0.95/0.71
Weight mean diameter (μm)	1100.4^a	1139.8^b	1201.6^c	1278.4^d	1323.2^e	41.53	<0.001	0.97/0.66
In vitro coefficient digestibility of organic matter[1]	0.80^a	0.81^{ab}	0.83^{abc}	0.85^{bc}	0.85^c	0.82	0.010	
In vitro coefficient digestibility of crude protein[1]	0.94^a	0.94^a	0.94^a	0.92^a	0.86^b	0.11	0.002	

R^2, regression coefficients of the parameter and in vitro digestibility of organic matter and crude protein in a linear model.
[a,b]Values with different superscripts within a row are significantly different ($P < 0.05$).
SEM, standard error of the mean.
[1] The in vitro coefficient digestibility was calculated based on dry matter.

mixtures contained the highest level of NDF of 155.2 and 140.1 g/kg DM, respectively at F0.595. For NDF, a decreasing trend was observed in SBM (M0S100) with increased sieve opening. For M25S75 and M50S50, the highest level of NDF was obtained in F0.149 and F0.420 being 117.5 and 118.1 g/kg DM. The starch content in various size fractions increased with the level of maize in the mixtures. Starch content of all mixtures decreased with particle size and reached the lowest point at F0.595 with 7.3, 81.4, 195.8, 374.1 and 621.7 g/kg DM in M0S100, M25S75, M50S50, M75S25 and M100S0, respectively, and then increased to the highest amount observed for all fractions including maize in F1.190.

3.3. In vitro digestibility of organic matter and crude protein

The OM and CP in vitro digestibility of mixtures of maize and SBM were significantly different within particle size fractions and between the different maize and SBM mixing ratios (Fig. 4). All the samples showed the lowest CP in vitro digestibility in F0.595, in which M100S0 had the lowest in vitro CP digestibility of 0.67, followed by M75S25 of 0.85. For M50S50, M75S25 and M100S0, the CP in vitro digestibility was decreased first and then increased with sieve openings, and a decreased trend was also observed in each size fraction with the decreasing content of SBM in the mixtures. Generally, the in vitro digestibility of OM decreased and then increased with increasing sieve openings. The differences of OM in vitro digestibility in SBM were enlarged with an increased proportion of maize in the mixtures: a significant drop was shown in M75S25 and M100S0 in F0.595 with 0.69 and 0.71 respectively. The highest in vitro digestibility of OM was obtained in the pan fraction and increased from 816.0 to 922.9 g/kg DM with more SBM in the mixture.

4. Discussion

4.1. Effect of mixing ratio of maize and SBM on grinding behaviour

The mixing proportions of maize and SBM affect the grinding behaviour of the mixtures. The more maize there was in the mix-

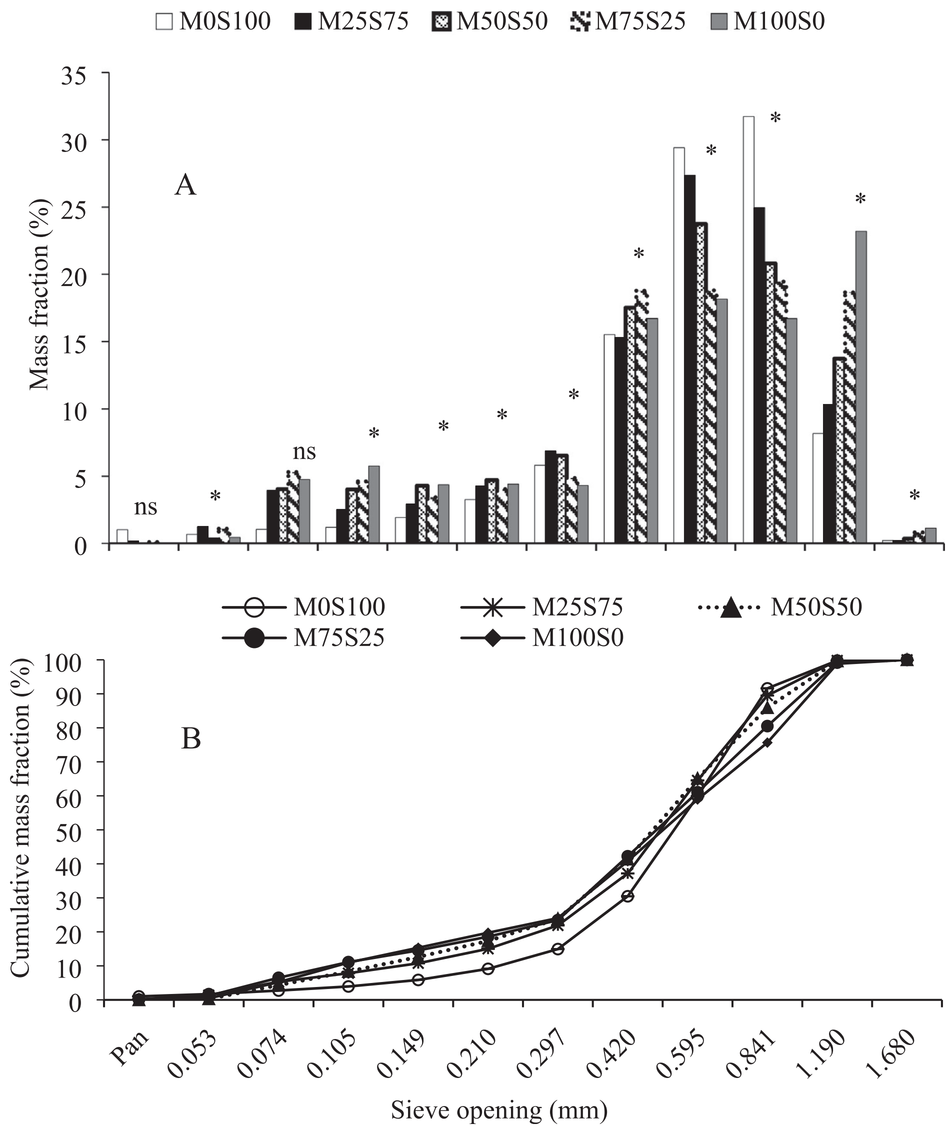
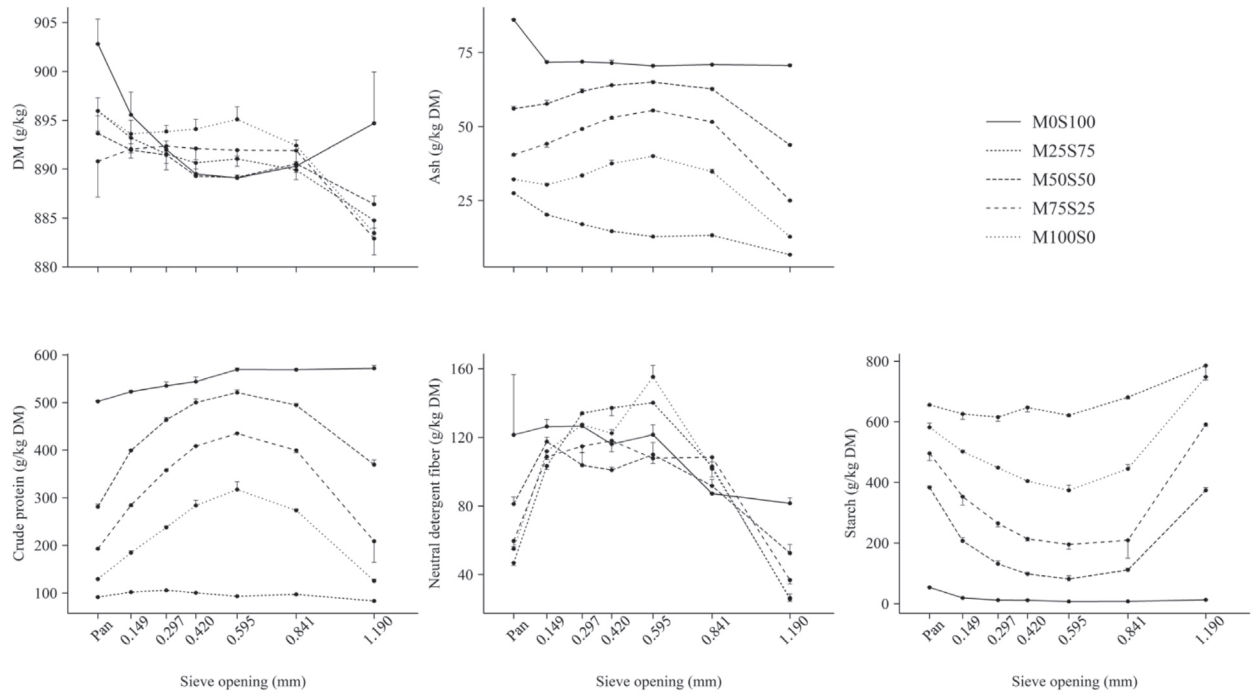


Fig. 2. Mass (A) and cumulative mass (B) distribution of hammer-milled maize (M) and soybean meal (S) and three mixtures (% M:S; 25:75, 50:50, 75:25:0) of the two. Significant differences (P < 0.05) between mixtures within each sieve are indicated by *. ns = not significant (P > 0.05).

ture, the more electric energy was consumed (P < 0.05). This could be because of the maize kernel having a larger size, and having different elasticity properties [44], which makes it more difficult to be ground and pass through a 2-mm screen in hammer mill resulting in a longer residence time in the hammer mill chamber. Goodband et al. [45] reported that round maize with a 4.8-mm screen size would have a finer particle size than milo or wheat, because maize kernels must be fragmented more often before they can pass through the screen, however, milo or wheat may fall through the opening intact because of their smaller kernel size. Another possible reason could be due to the degree of fill or active volume in the mill chamber. Material with a larger particle size has a lower angle of repose and better flow ability [45], which results in a larger degree of fill of the milling chamber. In the present study, with maize proportion increasing, the particle size increased, and this may have resulted in a higher level of fill leading to a higher energy consumption.

If it is assumed that there is no interactions among particles (or assumed to be as if ground separately), then the Kick's constant (K_m) can be calculated using interpolation method and the energy consumption can be predicted (Fig. 1). The measured energy consumption of grinding mixtures of M25S75, M50S50 and M75S25 was lower than the predicted ones, with the K_k value for the M25S75 mixture being about half the size of the interpolated value (K_m), and in the two remaining mixtures, the difference between (K_m) and (K_k) values were less pronounced. The K_k value for M25S75 and M50S50 was lower than either value of the K_k for 100% SBM or maize, indicating the presence of an interaction in grinding with a reduction in energy consumption as a result, when SBM and maize are ground as a mix. From these results it follows that Kick's constant for mixtures cannot be calculated as a linear relationship of the single grinding ingredients. Further experiments with different ingredients and or differing in mixing ratio should verify this finding. Based on the results obtained in the current study, grinding ingredients combined is suggested compared to grinding ingredients separately from an energy saving perspective.

Nutrient content of maize and SBM mixtures differed (P < 0.001) over the various fractions (Fig. 3). This is in agreement with studies Sundberg et al. [24,25], Maaroufi et al. [26,27], Al-Rabadi et al. [28,46], Al-Rabadi [47] and Lyu et al. [41,44], which focused on the nutrient content of single ingredients, such as peas,



Variable	Ash	Crude protein	Neutral detergent fiber	Dry matter
Mixture	<0.001	<0.001	<0.001	<0.001
Fraction	<0.001	<0.001	<0.001	<0.001
Mixture × Fraction	<0.001	<0.001	<0.001	<0.001
Adjusted R^2	0.9995	0.9972	0.955	0.8201
Residual standard error	0.5032	9.094	7.02	1.643

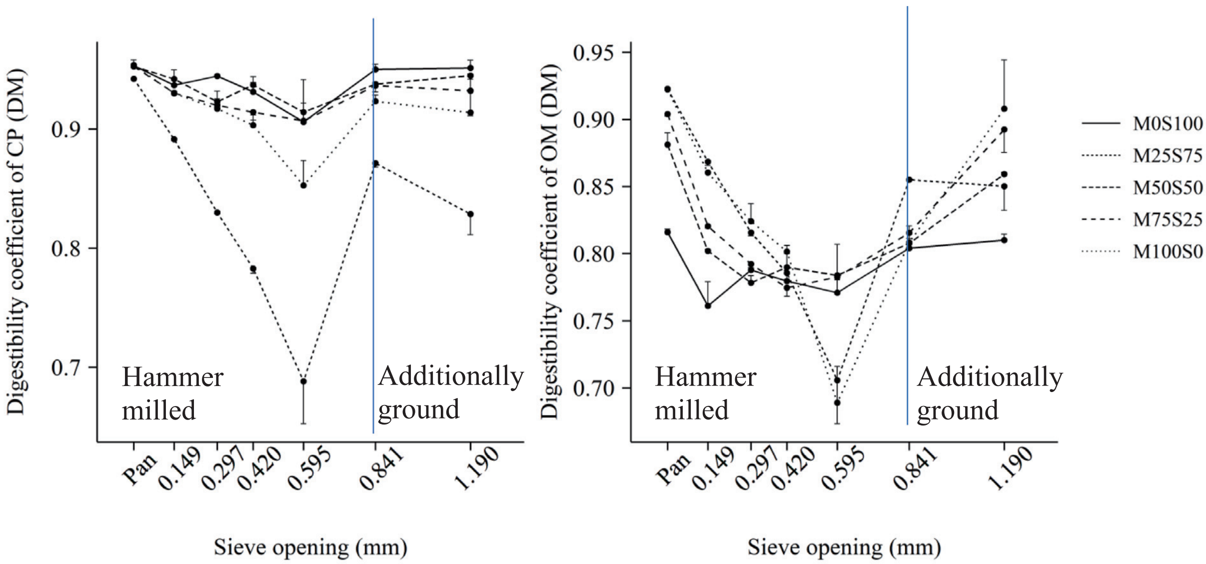
Fig. 3. Nutrient content per unit dry matter (DM) of particles retained on different sized sieves of hammer-milled maize (M), soybean meal (S) and three mixtures (%M:S; 25:75, 50:50, 75:25) of the two. Error bars represent the standard deviation of duplicate measurements. P-values of effects are provided in the table.

barley, sorghum, wheat, maize and SBM. These studies, however, did not investigate nutrient content of the fractionated mixtures of such ingredients. Dry matter content distributed differently among various size fractions were observed with different mixing ratios (Fig. 3). This could be due to the moisture content being distributed unevenly in the maize kernel. Zhang et al. [48] presented an uneven distribution of moisture in maize kernels, with a higher moisture content in the endosperm and a lower moisture content in the hull. Ash and CP content were increasing with SBM proportion increasing in the mixture. This is because SBM contains much more ash and CP than maize. Lyu et al. [41] showed that for the maize and SBM batches under investigation, the ash and CP content of SBM is more than 72 and 500 g/kg DM, respectively in all size fractions, while in maize, the ash and CP content is mostly around 25 and 110 g/kg. Similarly, the starch content increased with an increasing level of maize due to the higher starch content in maize than that in SBM. Adding maize to SBM leads to an increased NDF content in the largest particle size fraction, and a lower content in the pan fraction. This could be due to the NDF distribution in maize kernels, where fiber content is mainly found in the seed coat [49].

Soybean meal tends to end up in the middle sized fractions and maize in the coarse and fine fractions when looking at the PSD, starch and CP content: the mixture with more SBM made up a higher percentage of CP but lower percentage of starch in F0.595 and F0.841. Soybean meal, as a by-product of oil extraction, was processed before starting the current experiment. Therefore, after hammer-milling, the PSD of the ground mixture with a high proportion of SBM has a higher uniformity with lower GSD. In SBM, with an increasing particle size, the NDF content decreased, while in maize NDF increased first and then decreased. This agrees with the results of Lyu et al. (2021b). However, the amount of NDF differs between these two studies, which may be explained by the different sieve openings used in the two studies.

4.2. Effect of mixing ratio of maize and SBM on in vitro digestibility

Among various size fractions, the in vitro digestibility of CP was decreased with an increasing particle size and reached the lowest point at F0.595 and then showed a higher digestibility in the other two larger size fractions F0.841 and F1.190, after additionally grinding. This is as expected, because it is general accepted that smaller particle sizes result in a higher digestibility. Reducing particle size increases the surface area of particles for digestive enzymes to interact [10,13,50,51]. As for the increased digestibility in the larger fractions (F0.841 and F1.190) this could be due to the additional grinding of samples for the preparation of the in vitro determinations as per the protocol of Boisen and Fernández [34]. Particle size has been shown to have a significant effect on in vitro digestibility of OM and CP, and additionally grinding particles larger than 1 mm as per laboratory protocol will improve the in vitro digestibility [41,44]. It was also noticed that the decrease in in vitro digestibility is more pronounced for the mixtures with a higher maize level. This could be because maize kernels are larger than SBM particles and after hammer-milling coarse particles were mainly originating from maize in the mixture, therefore, more



Variable	Crude protein	Organic matter
Mixture	<0.001	<0.001
Fraction	<0.001	<0.001
Mixture × Fraction	<0.001	<0.001
Adjusted R^2	0.9533	0.9466
Residual standard error	0.1201	0.01274

Fig. 4. In vitro coefficient digestibility of organic matter and crude protein per unit dry matter (DM) of particles retained on different sized sieves of hammer-milled maize (M) and soybean meal (S) and three mixtures (%M:S; 25:75, 50:50, 75:25) of the two. Sieve openings ≥0.841 mm fractions were additionally ground over a 1.0 mm sieve as per the assay requirements. Error bars represent the standard deviations of duplicate measurements. P-values of effects are provided in the table. The in vitro coefficient digestibility was calculated based on dry matter.

maize in the mixture reduces the digestibility of OM and CP more significantly.

4.3. Relationship between particle size and in vitro digestibility

Particle size of ingredients/diets showed significant effects on the digestibility of nutrients both in vitro and in vivo, and generally reducing GMD of ingredients or diets increases the digestibility of nutrients [52–56]. In the present study, GMD of 100% maize and the mixtures were not significantly different. However, the in vitro OM and CP digestibility of SBM and the mixtures were significantly different (Table 1). A reason to this might be that the GMD was calculated based on the weight percentage of material on each layer of sieve and was not discriminative enough to capture the other characteristics of particles such as the surface area, volume or the ratio of volume and surface area. As Table 1 presented, the EPSs and GSD fit the OM and CP in vitro digestibility better than GMD. The metanalysis done by Lyu et al. [11] also demonstrated that EPS was better correlated to feed conversion ratio than GMD. In addition, comparing to GMD, GSD is superior in relating to digestive data with R^2 equals 0.91 vs 0.46 for OM, and 0.70 vs 0.03 for CP (Table 1). Wondra et al. [57] also reported that a more uniform PSD of corn (ground with hammer mill and roller mill) improved the apparent in vivo digestibility of DM, nitrogen and gross energy in corn and soybean meal-based diets although the GMD are all approximately 850 μm. The above conclusions indicate that more emphasis should be paid to the EPSs/GSD of ground materials as opposed to the GMD while studying the effects of particle size in the feed processing industry and animal nutrition. In addition, GMD, EPS or GSD cannot provide information about the unevenly distributed nutrient content over the particle size classes, which might be one of the reasons for the differences in OM and CP in vitro digestibility. The effect of nutrient content distribution over size classes might also contribute to animal performance (in vivo), which could be investigated in further research.

5. Conclusion

The novelty research on grinding mixture of ingredients showed that energy consumption increases with increasing maize levels when grinding maize and SBM mixtures in the hammer mill. Interactions between maize and SBM during hammer-milling were observed and mixing ingredients before grinding saves energy compared to grinding separately and then mix. The uniformity (GSD) of ground material increases the in vitro digestibility of nutrients although these materials had a similar GMD. Equivalent particle size, especially mean-volume and weight-mean diameter and GSD correlated better to OM and CP in vitro digestibility than GMD in a linear regression model. Mixing of maize and SBM has a significant effect on the nutrient distribution and in vitro digestibility of CP and OM among the various particle size fractions obtained after grinding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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