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Particle size distribution of hammer-milled maize and soybean meal, its nutrient composition and *in vitro* digestion characteristics

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ABSTRACT

The mean particle size of ground grains influences both pig growth performance and gastric health although this effect is not consistent and the underlying reasons are not fully understood. The inhomogeneous nutrient content distribution in particles of different sizes and the characteristics of particles may be reasons for inconsistent findings. The objective of the present study was to determine the nutrient content and physical characteristics of individual size fractions of hammer-milled maize and soybean meal (SBM), and to relate it to *in vitro* digestibility. Maize and SBM were hammer-milled over a 6- and 2-mm size screen, respectively, and were sieved into seven fractions. Particle characteristics of the hammer-milled material were determined by dry sieving, wet sieving and image analysis methods; the nutrient composition including dry matter, ash, crude fibre (CF), crude fat (CFat), crude protein (CP), starch and *in vitro* digestibility of organic matter (OM), CP (SBM) and starch (maize) were measured and nitrogen-free-extract was calculated. The results show that the nutrient composition differed among fractions of ground maize and SBM ($P < 0.001$). A large difference in starch levels (754.2 *vs* 578.9 g/kg) of maize was observed between the various sieve size fractions whereas the CP content of SBM increased with larger sieve sizes. The *in vitro* digestibility of OM and CP was different ($P < 0.001$) among the various particle size fractions for both ingredients. However, the *in vitro* digestibility of starch did not differ between each size fraction in maize ($P = 0.060$). The regression models relating the nutrient composition and *in vitro* digestibility show that the digestibility of OM was positively related to the starch level ($P < 0.001$). As for SBM, CF (negatively) and CFat (positively) were correlated with OM digestibility ($P < 0.001$); ash and CF had a negative effect on the digestibility of CP, though CFat had a positive relation with the CP digestibility ($P < 0.05$). Using image analysis, the OM digestibility of different fractions of maize and SBM could be related to the projected perimeter ($R^2 = 0.933$) and solidity ($R^2 = 0.704$) of particles in a linear model. The presented data show that the nutrient composition and physical characteristics of materials among various size fractions of hammer-milled maize and SBM differ and may explain pig growth performance differences observed in commercial production.

Abbreviations: CFat, crude fat; CF, crude fibre; CP, crude protein; DM, dry matter; EPS, equivalent particle size; GMD, geometric mean diameter; GSD, geometric standard deviation; NFE, nitrogen-free-extract; OM, organic matter; PSD, particle size distribution; SBM, soybean meal.

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1. Introduction

Feed and feed ingredient particle size are important in both feed manufacturing and the nutrition of pigs (Laurinen et al., 2000; Fastinger and Mahan, 2003; Huang et al., 2015; Zhao et al., 2019) and poultry (Xu et al., 2015; Zaefarian et al., 2016; Mtei et al., 2019; Bozkurt et al., 2019). Grinding, as a standard procedure in feed manufacturing, benefits mixing, conditioning (standard and pressure conditioning methods) and pelleting (Goodband et al., 2002). By reducing the mean particle size of feed ingredients/diets, the digestibility of nutrients and growth performance in pigs can be increased (Kim et al., 2005; Lahaye et al., 2008; Ball et al., 2015; Rojas and Stein, 2015) due to an increased surface area to volume ratio exposing more nutrients to digestive enzymes (Wondra et al., 1993). This influence of particle size on pig growth performance is, however, not consistent and the reasons are not fully understood. Lawrence et al. (2003) found that growth performance of nursery pigs was not affected by the particle size of soybean meal (SBM). Li et al. (2018) reported that for weanling and growing pigs, when the particle size of brown rice in the diet decreased from 800 to 600 μm, the apparent total tract digestibility of gross energy, dry matter (DM) and crude protein (CP) increased, however, with no further improvements observed in the 400 μm diet.

In feed manufacturing, particle size is routinely determined *via* the dry sieving method and usually expressed as the geometric mean diameter (GMD) with a geometric standard deviation (GSD), both calculated from the particle size distribution (PSD) (ASABE, 2008). Wet sieving, using water to wash down dust-like particles, is another method to determine PSD and is commonly used for feces or pelleted feed. Research on the relationship between particle shape characteristics, such as circularity, projected area and aspect ratio on nutrient digestion in animals is limited and may be important to more fully explain the influence of feed particles on animal performance. Sidwell et al. (2017) reported that low sphericity pellets (0.87 *vs* 0.97) release more drugs than high sphericity pellets. Equivalent particle size (EPS) is the diameter that equals an irregular shaped particle to a sphere based on a characteristic as for instance surface area, volume, weight or any other physical characteristic. Lyu et al. (2020) indicated that the estimation of a specific EPS for nutritional purposes may be superior to the industry standard GMD when relating feed particle size to gain to feed ratio.

Although many studies have been published examining the effect of mean particle size of ingredients or diets on digestion, the information regarding particle size fractions, its nutrient level and its digestion is still limited. Studies measuring the latter two properties in different size classes of particles focused mainly on starch in sorghum, barley (Sundberg et al., 1995a, 1995b; Al-Rabadi, 2009, 2012) and rice flour (De la Hera et al., 2013). The physical characteristics and nutrient content of fractionated particles and their digestibility warrant further investigation and may provide data to explain the variation in pig growth performance caused by changes in particle size characteristics due to grinding. We hypothesize that hammer milling will result in size fractions differing in nutrient levels and *in vitro* digestibility.

The aim of the present study was to determine the nutrient composition within fractionated particles, its physical characteristics and *in vitro* digestion of maize and SBM. In addition, the possibilities of other particle size determination and expression methods and their correlation to *in vitro* OM digestibility were explored.

2. Materials and methods

Two commonly used ingredients in pig feeds, maize and SBM were hammer-milled and sieved into different size fractions. Particle size and characteristics were determined using dry sieving, wet sieving and image analysis. The latter was used to obtain fractionated particle characteristics, *e.g.* circularity, projected perimeter, and relate these characteristics to *in vitro* digestion. The nutrient composition including starch, CP, ash, DM, crude fibre (CF) and crude fat (CFat) were analyzed and nitrogen-free-extract (NFE) was calculated in the various particle size classes and *in vitro* digestibility of organic matter (OM), starch and CP was determined.

2.1. Sample preparation

Two 20 kg batches, one of maize and one of Brazilian SBM (purchased from Research Diet Service B.V., Wijk bij Duurstede, The Netherlands) were ground using a hammer mill (Engl hammer mill, Dongen, The Netherlands, type 30, with 7.5 kW motor) employing a half-open bunker at a fixed running speed of 1500 rpm. For practical reasons, 6- and 2-mm screen-sized plate sieves were selected for maize and SBM, respectively. Ground ingredients were first divided with a multi-slot divider (Mooij-Argo, Hegelsom, the Netherlands) to obtain identical subsamples (1.25 kg for PSD analysis and 3.75 kg for further sieving).

Hammer-milled ingredients were dry sieved into seven fractions using six sieves (1.190, 0.841, 0.595, 0.297, 0.149 and 0.074 mm) and a pan for SBM and six sieves (3.360, 2.380, 1.680, 0.841, 0.420 and 0.210 mm) and a pan for maize. These fractions were selected from the full PSD determination sieve set based on the yield of the individual particle size fractions. Dry sieving was performed for 10 min using a 3-D throwing motion sieve shaker (AS 200 Control, Retsch, Haan, Germany) with an amplitude of 2.0 mm at intervals of 6 s. Four rubber balls (Ø 20 mm) were used as sieving aid on each sieve layer where the aperture size was less than 300 μm. To obtain enough material (>70 g) for analyses, multiple sievings were conducted with material collected from the same sieve/pan for each ingredient pooled, and kept at room temperature until physical, chemical and *in vitro* analysis.

2.2. Regrinding of samples prior to analysis

The material of each size class was analyzed for its nutrient content, *in vitro* digestibility, and morphology characteristics (*e.g.* circularity, solidity, aspect ratio, projected area). For the analysis of the chemical composition and *in vitro* digestibility, and according to the current standard protocols, all samples should pass a 1.0 mm sieve to obtain homogenous samples for analyses (Boisen and

Fernández, 1995; Chen et al., 2019). For this reason, unsieved maize and SBM as well as the samples with a GMD larger than 1.0 mm, were reground on a laboratory mill using a 1.0 mm sieve with trapezoidal holes (ZM200, Retsch GmbH, Hann, Germany) at 12,000 rpm. To obtain identical samples, the rotary divider (Retsch, Haan, Germany) was used.

2.3. Particle size determination and expression

Particle size distribution of hammer-milled maize and SBM was determined by dry and wet sieving in duplicate. The dry sieving was conducted using 14 sieves and a pan according to ASABE (2008). The wet sieving was performed according to the method described by Wolf et al. (2010) with modifications using six sieves (3.360, 2.380, 1.680, 0.841, 0.420 and 0.210 mm for maize and 1.190, 0.841, 0.595, 0.297, 0.149 and 0.074 mm for SBM) and a pan. Approximately 25 g samples were soaked in 500 ml water for 45 min before the suspension was quantitatively poured onto the sieve tower using water. The tower was closed, and water was added before the start of sieving at an amplitude of 2.0 mm without intervals. After 10 min the water was drained and the procedure repeated 3 times, before material on each sieve was collected and quantitatively transferred onto previous dried and weighed coffee filters (No. 4), dried for 4 h at 103 °C and weighed. The PSD was determined based on the mass fraction after drying.

The particle size of hammer-milled material was calculated according to ASABE (2008) and expressed as a GMD and GSD. The GMD of the material retained on the i^{th} sieve layer was taken as the geometric mean size of the two consecutive sieves (d_i, d_{i+1}):

$$\overline{d_i} = (d_i \times d_{i+1})^{\frac{1}{2}} \tag{1}$$

where d_i = nominal sieve aperture size of the i^{th} sieve in mm, d_{i+1} = nominal sieve aperture size in next larger than i^{th} sieve (just above in a set) in mm and $\overline{d_i}$ = GMD of the material retained on the i^{th} sieve layer in mm (Eq. (1)).

Equivalent particle size such as arithmetic mean diameter, mean surface area diameter, mean volume diameter, mean volume - surface area diameter and weight mean diameter were used to further evaluate particle characteristics and were calculated as follows (Lachman et al., 1987):

$$d_{ari} = \frac{\sum_{i=1}^{n}\left(\overline{d_i} \times w_i\right)}{\sum w_i} \tag{2}$$

$$d_s = \left(\frac{\sum_{i=1}^{n}\left(\overline{d_i}^2 \times w_i\right)}{\sum w_i}\right)^{\frac{1}{2}} \tag{3}$$

$$d_v = \left(\frac{\sum_{i=1}^{n}\left(\overline{d_i}^3 \times w_i\right)}{\sum w_i}\right)^{\frac{1}{3}} \tag{4}$$

$$d_{v-s} = \frac{\sum_{i=1}^{n}\left(\overline{d_i}^3 \times w_i\right)}{\sum_{i=1}^{n}\left(\overline{d_i}^2 \times w_i\right)} \tag{5}$$

$$d_w = \frac{\sum_{i=1}^{n}\left(\overline{d_i}^4 \times w_i\right)}{\sum_{i=1}^{n}\left(\overline{d_i}^3 \times w_i\right)} \tag{6}$$

where w_i = mass on i^{th} sieve in g, n = number of sieves + 1 (pan), d_{ari}= arithmetic mean diameter in mm (Eq. (2)), d_s = surface mean diameter in mm (Eq. (3)), d_v = volume mean diameter in mm (Eq. (4)), $d_{v\text{-}s}$= volume-to-surface mean diameter in mm (Eq. (5)) and d_w = weighted mean diameter in mm (Eq. (6)). The calculated mean values as given by Eqs. (2)–(6) are the diameters of a single sphere representing the entire distribution, based on different traits like the arithmetic mean, the surface, the volume, the surface to volume ratio and the mass distribution of the sample, respectively.

Images of the particles were obtained using a laboratory microscope combined with a digital camera (Bresser, microcam 3.0, megapixel, software version 7.2.1.7) according to the method described by Rezvani et al. (2019) with modifications. A teaspoon of sample was dispersed on a clean petri dish and placed on a black background under optimal lighting. Twenty images were captured for each sample and each image was obtained by rotating the petri dish manually. All images of particles above 212 (maize) or 75 (SBM) μm were analyzed using Image J (1.51f) software. For the finest particles smaller than 212 (maize) or 75 (SBM) μm, microscopical resolution was insufficient to obtain clear images. For these smaller particles, additional image analyses were conducted using a Morphologi 4 rapid, automated particle size and particle shape analysis system (Malvern Panalytical Ltd, Almelo, The Netherlands). Approximately 19 mm^3 of sample was placed in the dry sample dispersion unit with low pressure, with the microscope set at 2.5 times

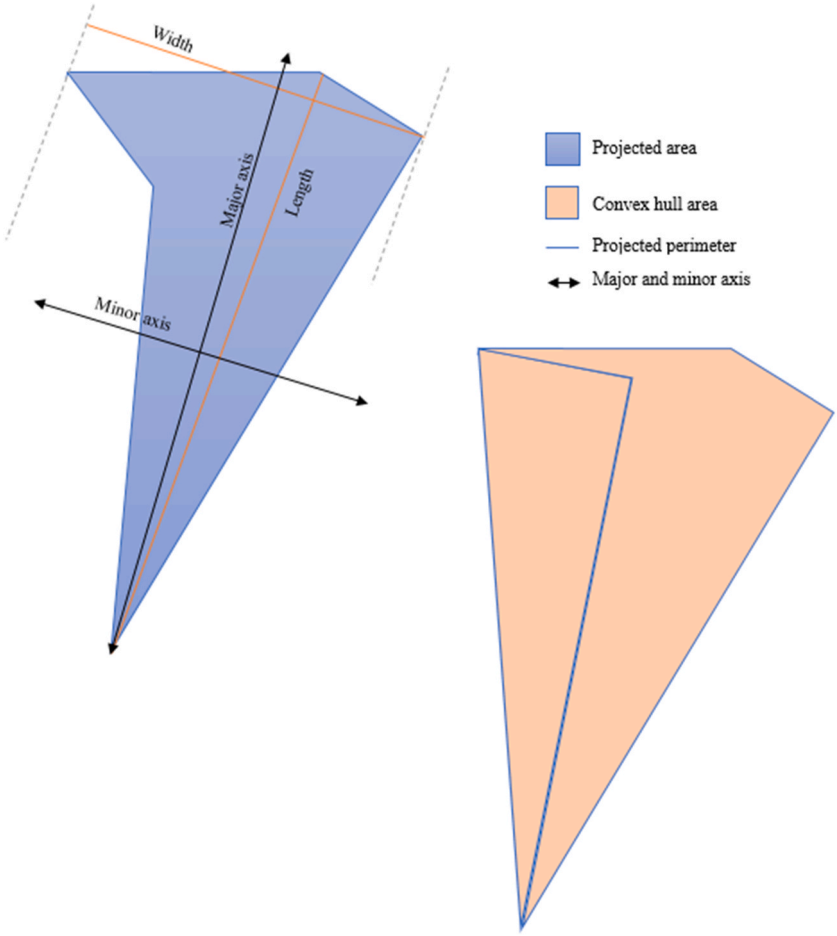

Fig. 1. Illustration of particle physical characteristics. Perimeter is the length of the outside boundary of the selection. Circularity $= 4\pi \times \frac{area}{perimeter^2}$, ranges from 0 (infinitely elongated polygon) to 1 (perfect circle). Aspect ratio $= \frac{major\ axis}{minor\ axis}$, is the aspect ratio of the particle's fitted ellipse. Major and Minor are the primary and secondary axis of the best fitting ellipse. Roundness $= 4 \times \frac{area}{\pi \times major\ axis^2}$ Solidity $= \frac{projected\ area}{convex\ hull\ area}$.

(8.5–1300 μm) magnification. The images were automatically analyzed by Morphologi G3 Particle Characterization Software (version 10.21). The measured physical characteristics included the projected area, projected perimeter, circularity, aspect ratio, roundness and solidity. An illustration of how these characteristics are calculated is shown in Fig. 1.

2.4. Chemical analysis

Samples were dried in an air circulation oven at 103 °C for 4 h to determine the DM content (ISO, 6496, 1999), with ash content determined after combustion at 550 °C for 3 h in a muffle furnace (ISO, 5984, 2002). Crude fibre content was determined according to (ISO, 6865, 2000) and CFat by (ISO, 6492, 1999). Nitrogen content was determined by the DUMAS technique (ISO, 16634-1, 2008), and CP was calculated by multiplying the nitrogen content by 6.25. Starch content was determined using enzymic method as specified in ISO 15914 (2004). All chemical analyses were performed in duplicate. Nitrogen-free-extract (NFE) was calculated as DM – ash – CF – CFat – CP (g/kg DM).

2.5. In vitro digestibility

The *in vitro* digestion of OM (in both maize and SBM), CP (in SBM) and starch (in maize) was determined according to the method as described by Boisen and Fernández (1995) with modifications. 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)

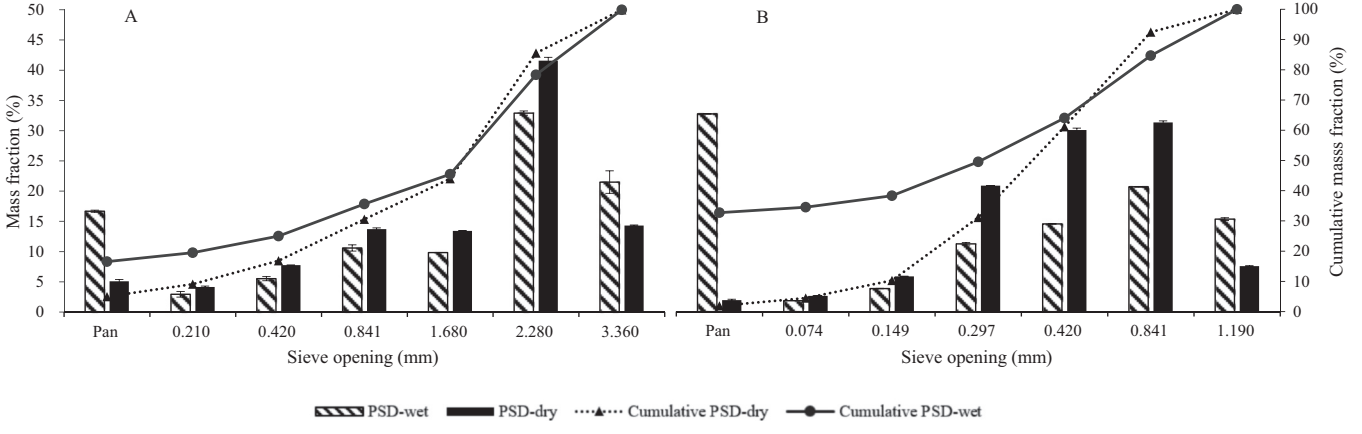

Fig. 2. Mass and cumulative mass distribution (dry base) of the dry and wet sieving of hammer-milled maize (A) and soybean meal (B). Error bars represent standard deviations.

Table 1
Mean (± SEM) of various particle size (μm) expressions of hammer-milled maize and soybean meal determined by dry and wet sieving.

Ingredient	Sieving method	Geometric mean diameter	Arithmetic mean diameter	Mean surface area diameter	Mean volume diameter	Mean volume-surface area diameter	Weight mean diameter
Maize	dry	1766 ± 2.4*	2260 ± 16.9	2533 ± 11.7	2713 ± 8.6	3110 ± 1.4*	3294 ± 0.0*
	wet	1316 ± 3.7	2170 ± 54.5	2574 ± 49.5	2806 ± 45.5	3337 ± 35.0	3506 ± 30.6
Soybean meal	dry	643 ± 1.9*	746 ± 0.5*	816 ± 0.2	872 ± 0.6	995 ± 1.5*	1082 ± 2.3*
	wet	323 ± 3.7	608 ± 11.6	780 ± 2.4	888 ± 2.5	1151 ± 2.5	1228 ± 2.2

SEM: standard error of the mean
* Significantly different ($P < 0.05$) to corresponding wet sieving value

Table 2
Geometric mean diameter (GMD) and image analysis parameters of particles retained on the various sieves after dry sieving of hammer-milled maize and soybean meal.

Ingredient	Fraction† (mm)	GMD (mm)	Projected area (μm^2)	Projected perimeter (μm)	Circularity	Aspect ratio	Roundness	Solidity
Maize	0.0 (pan)*	0.096	1296^{e}	130^{f}	0.860^{a}	1.402^{b}	–	0.928^{b}
	0.210	0.297	7423^{e}	355^{ef}	0.716^{b}	1.503^{ab}	0.697^{ab}	0.942^{ab}
	0.420	0.594	32847^{e}	815^{e}	0.631^{c}	1.587^{a}	0.676^{b}	0.930^{b}
	0.841	1.189	156308^{d}	1779^{d}	0.614^{cd}	1.509^{ab}	0.690^{ab}	0.939^{ab}
	1.680	2.000	354790^{c}	2851^{c}	0.567^{cde}	1.471^{ab}	0.702^{ab}	0.938^{ab}
	2.380	2.828	648063^{b}	3924^{b}	0.552^{de}	1.456^{ab}	0.708^{ab}	0.955^{a}
	3.360	3.999	968342^{a}	5060^{a}	0.527^{e}	1.397^{b}	0.739^{a}	0.949^{a}
SEM			130696.2	659.7	0.0406	0.0232	0.0073	0.0034
P value			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Soybean meal	0.0 (pan)*	0.057	406^{e}	65^{g}	0.910^{a}	1.445^{bc}	–	0.972^{a}
	0.074	0.105	852^{e}	123^{f}	0.717^{b}	1.630^{a}	0.657^{c}	0.908^{e}
	0.149	0.210	2291^{de}	200^{e}	0.707^{bc}	1.496^{ab}	0.706^{bc}	0.933^{d}
	0.297	0.353	7646^{d}	369^{d}	0.698^{bc}	1.459^{bc}	0.718^{bc}	0.943^{cd}
	0.595	0.707	23090^{c}	652^{c}	0.685^{bc}	1.446^{bc}	0.724^{b}	0.946^{bc}
	0.841	1.000	41445^{b}	871^{b}	0.683^{c}	1.342^{cd}	0.762^{ab}	0.952^{bc}
	1.190	1.414	138336^{a}	1595^{a}	0.687^{bc}	1.255^{d}	0.808^{a}	0.955^{b}
SEM			17441.5	190.5	0.0286	0.0413	0.0177	0.0071
P value			< 0.001	< 0.001	0.051	< 0.001	< 0.001	< 0.001

Values with different superscripts within column per ingredient are significantly different ($P < 0.05$).
- no data available.
† Size of the sieve opening.
* Physical characteristics of this fraction particles were analysed by Morphology 4 and remaining fractions were analysed with microscope and image J software

using a vacuum pump. After sequential washing of all material with 10 ml of 70% ethanol and acetone, the residues were dried overnight in an oven at 70 °C. Dry matter, ash, CP and starch were determined using the methods described above. Digestibility was calculated according to the difference in nutrient content before and after digestion.

2.6. Statistical analysis

R (3.6.1) was used to analyze the data (R Core Team, 2019). Nutrient content of sieve fractionated particles was analyzed by one-way analysis of variance using the 'lm' function and 'HSD.test' function in 'agricolae' package (De Mendiburu, 2020) for Tukey's multiple comparisons. Duplicate analysis of the nutrient content and *in vitro* digestibility results was used as experimental units in the analysis of the single hammer mill runs on maize and SBM. Regression models were derived to predict the *in vitro* digestibility of OM and CP (for SBM) from its nutrient composition. In order to formulate the models, CF, CFat, CP and starch were considered as factors to predict the OM digestibility. For the prediction, CP digestibility, ash, CF, CFat and CP were used. Factor selection was done using the step wise method based on the 'stepAIC' in both directions in 'MASS' package (Venables and Ripley, 2002).

3. Results

3.1. Particle size determinations and expressions

In total, 99.7 ± 0.04% of material was recovered during dry sieving. In order to compare the results of dry and wet sieving, the PSD obtained from dry sieving was recalculated into the 7 fractions used for wet sieving. Dry and wet sieving of the hammer-milled SBM and maize resulted in different (cumulative) mass distribution patterns (Fig. 2) where a larger percentage of material was retained on the coarsest sieve and dissolved and colloidal matter accumulated in the water used in wet sieving. The cumulative mass fraction for

Table 3
Nutrient content and *in vitro* digestibility of retained particles on the various sieves after dry sieving of hammer-milled maize and soybean meal.

Ingredient	Fraction[†] (mm)	DM (g/kg)	Nutrient composition (g/kg dry matter)						*In vitro* digestibility coefficient	
			Ash	CF	CFat	CP	Starch	NFE	Starch or CP[‡]	OM
Maize	Unsieved	876.4	13.9	26.6	40.8	91.7	694.0	703.4	0.959	0.870
	0.0 (pan)	885.0^{a}	14.2^{c}	19.4^{c}	46.2^{b}	70.1^{f}	754.2^{a}	735.2^{a}	0.991	0.935^{a}
	0.210	887.1^{a}	18.9^{a}	43.3^{b}	55.5^{a}	95.8^{c}	597.9^{b}	673.6^{c}	0.986	0.798^{b}
	0.420	884.4^{a}	16.8^{b}	49.6^{ab}	43.5^{b}	97.7^{b}	578.9^{b}	676.7^{c}	0.922	0.710^{c}
	0.841	886.5^{a}	16.8^{b}	53.8^{a}	32.6^{c}	104.5^{a}	597.3^{b}	678.8^{c}	0.971	0.798^{b}
	1.680	879.0^{b}	10.4^{d}	22.8^{c}	28.2^{c}	89.0^{d}	733.5^{a}	728.6^{a}	0.955	0.888^{a}
	2.380	879.3^{b}	13.8^{c}	19.3^{c}	42.6^{b}	89.4^{d}	742.6^{a}	714.2^{b}	0.961	0.907^{a}
	3.360	877.4^{b}	11.3^{d}	18.9^{c}	33.7^{c}	86.9^{e}	729.4^{a}	726.5^{ab}	0.959	0.907^{a}
SEM		1.52	1.02	5.21	3.08	3.56	23.29	8.96	0.0075	0.0269
P value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.060	< 0.001
Soybean meal	Unsieved	884.3	69.4	49.9	11.9	549.7	–	203.4	0.948	0.809
	0.0 (pan)	908.1^{a}	94.2^{a}	34.7^{e}	28.5^{a}	480.3^{f}	–	270.4^{a}	0.961^{a}	0.858^{a}
	0.074	902.4^{b}	74.2^{b}	51.0^{bc}	22.0^{b}	509.6^{e}	–	245.6^{b}	0.952^{a}	0.811^{b}
	0.149	893.3^{c}	69.8^{c}	64.0^{a}	18.0^{bc}	523.2^{d}	–	218.3^{c}	0.939^{ab}	0.778^{c}
	0.297	888.7^{d}	69.3^{c}	56.5^{b}	12.9 ^{cd}	546.2^{c}	–	203.8^{cd}	0.919^{bc}	0.774^{c}
	0.595	887.9^{d}	68.6^{c}	48.8^{cd}	10.4^{d}	559.9^{bc}	–	200.1^{d}	0.913^{c}	0.775^{c}
	0.841	888.0^{d}	68.9^{c}	42.7^{d}	8.9^{d}	568.0^{ab}	–	199.5^{d}	0.885^{d}	0.761^{c}
	1.190	890.7 ^{cd}	68.5^{c}	35.2^{e}	12.8^{cd}	574.2^{a}	–	200.0^{d}	0.954^{a}	0.830^{b}
SEM		2.89	3.11	3.56	2.37	3.11		9.36	0.0092	0.0118
P value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001

Values (exclusive unground) with different superscripts within column per ingredient are significantly different ($P < 0.05$). SEM, Standard error of the mean; DM, dry matter; CF, crude fibre; CFat, crude fat; CP, crude protein; OM, organic matter; NFE, nitrogen-free-extract.
- not determined.
[†] Size of the sieve opening in mm. Fractions with a geometric mean diameter greater than 1.0 mm were ground to pass a 1.0 mm sieve prior to chemical and *in vitro* digestibility analysis conform the analytical protocol.
[‡] Starch for maize and CP for soybean meal.

both reached 50% at a sieve size at 1.68 mm for maize and 70% at 0.595 mm for SBM. As a result of different mass distributions between dry and wet sieving, particle size characteristics also showed differences (Table 1). In maize, dry sieving provided a greater GMD (1.766 *vs* 1.316 mm) compared with wet sieving, with the arithmetic mean diameter (2.260 *vs* 2.170 mm) being not different between the two methods. Mean surface area diameter and mean volume diameter were not different between dry and wet sieving for both maize and SBM. Mean volume-surface diameter and mean weight diameter of particles were greater for maize and smaller for SBM (dry sieved materials *vs* wet sieved) ($P < 0.05$). The GMD when measured using dry sieving was almost twice as large compared with the wet sieving for SBM (0.643 *vs* 0.323 mm, Table 1).

3.2. Physical characteristics of different size fractions

The various measurements of the shape of particles using image analysis (Table 2) showed differences ($P < 0.001$) among sieve size classes except for circularity in SBM. Coarser particles had a greater projected area and projected perimeter but smaller aspect ratio for both ingredients. In maize, the circularity of particles decreased from 0.860 to 0.527 and the roundness ranged from 0.676 (0.420 mm sieve) to 0.739 (3.360 mm sieve). The greatest particle solidity (0.955) was associated with maize retained on the 2.380 mm sieve, while ground maize on the 0.420 mm sieve showed the lowest solidity value (0.930). For SBM, no differences ($P = 0.051$) were observed in the circularity among size classes. Roundness increased from 0.657 to 0.808 with increasing particle size. The largest difference in solidity was observed in the pan and 0.074 mm sieve fractions (0.972 *vs* 0.908).

3.3. Nutrient content and in vitro digestibility

Differences ($P < 0.001$) in the nutrient composition and *in vitro* digestibility of the different particle size fractions were observed for both hammer-milled maize and SBM (Table 3). In maize, lower CF, DM and higher starch and NFE contents were especially observed in larger particle size fractions (1.680–3.360 mm), although the smallest fraction (pan) also contained less CF ($P < 0.001$) and more ($P < 0.001$) starch and NFE. The material retained on the 1.680 mm sieve showed the highest ash (18.9 g/kg) and CFat (55.5 g/kg) content, while the lowest ash (10.4 g/kg) and CFat (28.2 g/kg) content was recorded in the pan fraction. As for the SBM, the difference in DM and ash content was mainly present between the material with the two smallest size fractions (0.074 mm and pan) and the material collected from the other five larger sieves. Along with the increase in sieve sizes, the CF content first increased and then decreased, reaching the highest value of 64.0 g/kg on sieve 0.149 mm ($P < 0.05$). Furthermore, a steady decreasing trend in CFat content was observed with an increasing particle size, though the largest particle size fraction did not follow this trend. Conversely, the CP content increased with increasing particle size.

Among the various particle size fractions, the *in vitro* digestibility of OM (for both maize and SBM) and CP (SBM) were different ($P < 0.001$). The digestibility of starch in the different maize size classes was high (> 0.922) and no difference ($P > 0.05$) was

Table 4
Relationships between *in vitro* digestibility coefficient of organic matter and starch in maize and organic matter and crude protein in soybean meal with the nutrient composition (g/kg) of these ingredients.

Ingredients	Regression equation*	Adjusted R^2	P value	RMSE
Maize	Digestibility OM = 0.19 (± 0.067) + 0.001 (± 0.00010) × Starch	0.88	< 0.001	0.026
Soybean meal	Digestibility OM = 0.83 (± 0.0217) – 0.002 (± 0.0004) × CF + 0.003 (± 0.0006) × Cfat	0.83	< 0.001	0.013
	Digestibility CP = 1.13 (± 0.089) - 0.003 (± 0.0012) × Ash – 0.001 (± 0.0005) × CF + 0.006 (± 0.0015) × CFat	0.69	< 0.05	0.013

CF, crude fibre; CFat, crude fat; CP, crude protein; DM, dry matter; OM, organic matter; RMSE, residual mean square error.
* Variables (nutrient composition) were selected into the model by the stepwise procedure with a probability value of 0.05 as the significance level.

observed. The largest *in vitro* OM digestibility in maize and SBM was obtained in the finest fraction (0.935 and 0.858, respectively), as well as the digestibility of starch (0.991) in maize although other fractions had values which were not different ($P = 0.060$). The digestibility of CP in SBM decreased from 0.961 to 0.885 with increasing sieve size from the finest fraction (pan) to sieve size of 0.841 mm, with the coarsest fraction breaking this trend with a value of 0.954.

Regression models, of the relationship between nutrient composition and *in vitro* digestibility parameters over the various particle size fractions were provided in Table 4. In maize, the *in vitro* digestibility of OM was positively ($P < 0.001$) related to the starch content. As for the SBM, a relationship ($P < 0.001$) was observed between CF and CFat and *in vitro* OM digestibility. Ash and CF had a negative effect on the digestibility of CP, though CFat showed a positive influence on the *in vitro* CP digestibility ($P < 0.05$).

4. Discussion

Hammer-milled maize and SBM, as routinely used ingredients in pig feeds were examined for their fractionated nutrient composition and *in vitro* digestibility in the present study. Dry and wet sieving were used to obtain the data on PSD. From these data, various values were calculated *e.g.* mean volume-surface area diameter, arithmetic mean diameter. In addition, image analysis was used to determine morphological characteristics of fractionated particles and these characteristics were related to the *in vitro* digestion.

4.1. Particle size determination and expression

Different determination and expression methods have been used to illustrate various characteristics of particles (Lyu et al., 2020). The dry sieving method is widely used because of its simplicity and low cost, with the more complex and labor intensive wet sieving method considered to be more suitable for analysing the PSD of pelleted feeds (Wolf et al., 2010) or digesta and feces (Uden and Van Soest, 1982; Dixon and Milligan, 1985). In the present study, dry and wet sieving clearly showed a different PSD resulting in a different GMD. With wet sieving, a larger mass percentage of material was calculated to be part of the smallest fraction, which is in line with the results of Dirkzwager et al. (1998) and Wolf et al. (2010). Probably, the very fine particles/dust that might stick to the sieves during dry sieving were washed down with the water during the wet sieving procedure. Also, larger particles may break down to smaller particles, leading to an increase in mass of finer material. A larger amount of material was also retained to a higher degree on the largest sieve using the wet method, likely due to particle swelling and agglomeration during the soak and sieving procedure.

Geometric mean diameter is normally used to indicate the particle size of ingredients or diets (Ball et al., 2015; Rojas and Stein, 2015). Other EPS indicators such as mean volume-surface area diameter showed further possibilities of describing particle sizes, that may be relevant especially when related to pig performance indicators (Lyu et al., 2020). In the studies of Wondra et al. (1995b), the importance of a uniform of PSD was stressed while results of Lawrence et al. (2003) and Li et al. (2018) showed that reducing mean particle sizes (*e.g.* GMD) did not improve the pig performance or increase the digestibility of energy and nutrients in pigs as expected. These studies support investigation of constituent fractions in explaining nutrient digestibility.

4.2. Characteristics of fractionated particles

Breaking behavior of feed raw materials can, in part, be assessed by analyzing morphological characteristics of fractionated particles. A pre-requisite is that these characteristics have a relationship with grinding properties and nutritional value of ingredients.

In the present study, the average morphology of particles in the seven fractions was different, which are similar to those obtained by Maaroufi et al. (2000). As expected, the projected area and projected perimeter of particles were increased with increasing sieve size. Circularity, is defined as the degree to which the particle is similar to a circle, taking into consideration the smoothness of the perimeter (Ostadhassan et al., 2018). For both ingredients, a trend for a decline in circularity and aspect ratio with particle size increase was observed, which is in line with the results of Ogden et al. (2010) who found that a larger screen size leads to less circular particles in ground maize. This might be because smaller sized particles were more likely to have resided in the grinding chamber longer increasing the chance of being hit by other particles and hammers. It should be noted that the aspect ratio of the finest particles (pan) did not follow the trend of a decrease with increasing particle size. This was also observed in solidity of SBM particles: material with a higher GMD had a greater solidity except for the particles collected in the pan. This might be because the material of these two fractions was analyzed by a different device from the other fractions (Malvern M4 device), that dispersed the sample by pressured air, and has less adhesion among particles compared with other fractions, which were dispersed manually.

Roundness is the measure of the sharpness of a particle's edges and corners, which is largely dependent on the sharpness of angular protrusions (convexities) and indentations (concavities) from the object (Cruz-Matías et al., 2019). The roundness of particles of hammer-milled SBM and maize increased with increased particle size, but the aspect ratio showed the opposite. This is reasonable when we consider the calculation of these two parameters, which are both related to the major axis. According to the formula of aspect ratio and roundness, a small aspect ratio means the major axis is short resulting in a large roundness (Takashimizu and Iiyoshi, 2016).

4.3. Nutrient composition in different particle size fractions

The nutrient composition among particle size fractions has been previously reported for barley (Sundberg et al., 1995a, 1995b), sorghum (Al-Rabadi et al., 2009, 2012), rice grains (De la Hera et al., 2013) and peas (Maaroufi et al., 2000) but limited data was available for maize and SBM, which are the two most used feed ingredients in diets for pigs and poultry (Healy et al., 1994; Wondra et al., 1995b, 1995a; Lawrence et al., 2003; Ball et al., 2015; Huang et al., 2015; Rojas and Stein, 2015; Shi et al., 2017). The sieving of

Table 5
Coefficients of determination* (R^2) of a linear model relating *in vitro* organic matter digestibility coefficient to various physical characteristics of particles retained on various sieves of hammer-milled maize and soybean meal.

Ingredient	GMD	Projected area	Projected perimeter	Circularity	Aspect ratio	Roundness	Solidity
Maize	0.882	0.818	0.933	0.908	0.655	0.555	0.438
Soybean meal	0.198	0.018	0.109	0.608	0.414	0.336	0.704

* Pan fraction was excluded from the linear model since its content was analyzed by a different method.
GMD, geometric mean diameter.

hammer-milled maize and SBM in the present study, showed that the nutrient composition differed among size classes, results consistent with the above-mentioned other ingredients. Sundberg et al. (1995a), (1995b) reported that the CP, starch, CFat and dietary fibre content were different in barley fractions after milling and air-classification. Similar results were obtained by Al-Rabadi et al., (2009, 2012), who found that the starch and aNDF (neutral detergent fibre expressed inclusive of residual ash) content varied among size classes in both barley and sorghum. In the study of Acosta et al. (2019), maize was hammer-milled to target particle size of 300, 500 and 700 μm, and then sieved into 6 fractions. The finest fraction (sieve opening 210 μm) contained the highest DM, and lowest CP content, which is in line with the results of the present study. De la Hera et al. (2013) also found the lowest CP content in the finest particle size fraction when hammer milling rice, although the highest CP content was observed in the finest fraction of hammer-milled peas reported by Maaroufi et al. (2000). In the present study, the pan fraction contained the largest amount of starch. Whilst, in the study of Acosta et al. (2019), this was only observed for maize ground at 500 μm rather than the maize milled at 300 and 700 μm. This indicated that for the same batch of maize the screen size of the hammer mill (target GMD) affects the nutrient content distribution. Starch content of fractionated maize ranged from 578.9 to 754.2 g/kg in the current study, which is higher than results obtained by Acosta et al. (2019) ranging from 556 to 683 g/kg. This may be due to the nutrient composition that was expressed on an as-is basis by Acosta et al. (2019) instead of DM basis in the present study.

In maize, bonds between starch and protein are relatively strong (Delcour, 2010), and they are mainly present in the endosperm of the maize kernel (Eckhoff and Paulsen, 1996). According to Maaroufi et al. (2000), different constituents of a seed adhere to different comminution laws. This means that the soft endosperm is more likely milled into fine particles, which might be the reason why the finest fraction has the highest starch content. The high ash and CFat content of the material retained on the 0.210 mm sieve (Table 3) indicates that the germ is more likely to be ground into this fraction.

Different from a whole grain kernel, SBM is a co-product which has been processed for oil extraction and desolventizing/toasting. A second size reduction of SBM at the feed mill, therefore, does not follow the same breakage behavior as whole seed, making it difficult to associate morphological structures to its grinding characteristics after its first or second grinding run. In the current experiment, we analyzed seven size fractions of SBM for its nutrient composition to have a first and detailed view on the relation between nutrient composition and particle size (Table 3).

4.4. In vitro digestibility of nutrients

In maize, the *in vitro* digestibility of OM was lowest in the fraction with a GMD of 0.594 mm (sieve size 0.420 mm) and increased when particles became smaller and larger. Similar results were obtained for SBM fractions with the lowest digestibility obtained for particles with a GMD of 1.000 mm (sieve size 0.841 mm) with values increasing when the GMD de- or increased. The digestibility of starch in maize fractions was not different among size fractions (Table 3). This might be because the starch digestibility in each fraction was relatively high (average of 0.964).

Step-wise linear regression was used to investigate the relationship between nutrient composition and *in vitro* digestibility among various particle size fractions. Since the nutrient composition is listed per particle size fraction, and NFE is calculated as the difference from the other components, a separate regressor per particle size would be a confounding factor in the regression model, and therefore GMD and NFE were not included in the model.

The *in vitro* OM digestibility in maize is highly related to the starch content while in SBM it is related to the CF and CFat content. These results differ from results of Noblet and Jaguelin-Peyraud (2007) who used data from the Boisen and Fernández (1997) assay. They found that the prediction of *in vitro* OM digestibility is more accurate ($R^2 = 0.9$) when the equation includes digestible OM, acid detergent fibre or CF and ash in mash compound feeds. This might be because of the high starch content relatively to other nutrients in maize, which leads to large differences in numbers among various particle size fractions. In the current study, the ash content was excluded as a predictor in the regression model as OM is calculated from the ash content and as such ash cannot be considered an independent variable. As for CF content decreasing the OM digestibility, this appears to be logical as CF is largely indigestible. In SBM, CF also was negatively related to CP digestibility, which is in line with results obtained by Noblet and Perez (1993). In addition, CFat was positively related to the CP digestibility with similar results obtained by Li and Sauer (1994) in *in vivo* trials. These authors found that the apparent ileal digestibility of most of the amino acids increased linearly ($P < 0.05$) with increasing dietary fat levels. The *in vitro* digestibility of starch of maize was not different among fractionated particle size, and, therefore, no regression model was developed.

In terms of the influence of particle size on *in vivo* digestibility, Wondra et al. (1993, Al-Rabadi et al. (2009) explained the effects to be related to the enzyme interaction with the nutrients released from various surface area of particles. In the present study, possibilities of relating some other physical characteristics of particles to *in vitro* digestibility using the standard Boisen and Fernández (1995)

Table 6
In vitro digestibility coefficient of organic matter of hammer-milled maize and soybean meal samples with a geometric mean diameter (GMD) greater than 1.0 mm and the same samples additionally ground to pass a 1 mm sieve for chemical analyses.

Ingredient, sieve size (mm) (GMD, mm)	*In vitro* digestibility coefficient	
	Hammer-milled	Hammer-milled + reground
Maize, 0.841 (1.189)	0.476	0.798
Maize, 1.680 (2.000)	0.302	0.888
Maize, 2.380 (2.828)	0.212	0.907
Maize, 3.360 (3.999)	0.161	0.907
Soybean meal, 1.190 (1.414)	0.783	0.830

assay, are provided. For hammer-milled maize, Table 5 shows that the digestibility of OM is highly related to the GMD, projected area, projected perimeter and circularity of particles with R^2 ranging from 0.818 to 0.933. In SBM, when relating particle characteristics to *in vitro* OM digestibility, particle solidity showed the highest correlation with an R^2 of 0.704 and OM *in vitro* digestibility increased with solidity decreasing. This may be because particles with lower solidity are more likely to be broken into smaller particles during the digestion procedure and therefore the digestibility was improved. The wet sieving data in the present study appear to support this observation that more than 30% of particles were observed in the pan fraction (Fig. 2). The *in vitro* digestibility of OM and CP in the SBM fraction with the largest particles did not follow the declining trend as observed for the other fractions. This might because, unlike the other SBM fractions, this fraction was additionally ground to pass a 1 mm sieve as prescribed by the assay of Boison and Fernández (1995; 1997) with the reduction in particle size leading to an increase in digestibility of nutrients. The highest *in vitro* OM digestibility was observed for the finest SBM fraction, which may be due to the finest particle size or may also because of the lowest CF and the highest CFat content (Table 4).

Fractions with a GMD larger than 1.0 mm were ground to pass a 1-mm sieve according to the assay described by Boison and Fernández (1995; 1997). It has been reported that smaller particles have a higher nutrient digestibility as a result of increased surface area for enzymes to act upon (Blasel et al., 2006; Healy et al., 1994; Livesey et al., 1995; Wondra et al., 1995a). Considering that the reduced particle size as a result of additional grinding may influence the *in vitro* digestibility, the original hammer-milled material was additionally analyzed for its *in vitro* OM digestibility. It was observed that the *in vitro* OM digestibility of these fractions (Table 6) yielded far lower values as a result of not grinding below 1 mm. This decrease in the digestibility of OM in maize and SBM indicates that the physical characteristics (size) of particles appears to be highly important in the *in vitro* digestibility assay. Boisen and Fernández (1997) showed that the *in vitro* total tract OM digestibility of maize and SBM was reduced by 1.4% and 0.4% units, respectively when ingredients were ground at 3 instead of 1 mm. The effect of grinding ingredients over sieves finer than 1 mm on *in vitro* digestibility values, is unknown. Next to the specification that ingredients should be finely (< 1 mm) ground, the *in vitro* digestibility assay of Boisen and Fernández (1995, 1997) also calls for the filtration of undigested material on crucibles with a pore size of 40–90 μm with undigested material < 40–90 μm considered to be digested. As such, the analysis of ingredients/material where the particles have a lower GMD or size reduction of particles occurs due to the assay conditions to < 40–90 μm, a greater digestibility value would be found. The wet sieving data in the present study appears to support particle size reduction due to digestion (Fig. 2). Further investigations are warranted to determine the influence of particle size and as such the grinding over a 1 mm sieve of ingredients, on the *in vitro* digestibility assay of Boisen and Fernández (1995, 1997). Therefore, PSD should be determined and reported for material/ingredients analyzed by the *in vitro* assay of Boisen and Fernández (1995, 1997).

5. Conclusions

New measurements of particle characteristics were introduced which may prove to be applicable in future evaluation of physical feed characteristics. Nutrient composition of fractionated particles after hammer-milling of maize and soybean meal differs, and the *in vitro* digestibility of nutrients of fractionated materials is related to both physical (particle size and circularity) and chemical (nutrient composition) characteristics of particles. Results of the present study also provides an indication that particles size reduction in the widely used *in vitro* digestibility assay of Boisen and Fernández (1995, 1997) may be influenced by particle size distribution of the ingredient under investigation. Knowing that the nutrient composition and the *in vitro* digestibility of diet ingredients differs along with the particle size distribution, it is possible to grind ingredients into a specified size class to realize a higher digestibility of nutrients in feed.

CRediT authorship contribution statement

F. Lyu: Investigation, Formal analysis, Project administration, Writing – original draft. **A. F. B. van der Poel**: Writing – review & editing, Funding acquisition, supervision. **W. H. Hendriks**: Writing – review & editing, Resources, Funding acquisition, supervision. **M. Thomas**: Conceptualization, Methodology, Writing – review & editing, supervision.

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Conflict of interest

The authors declare that none of them have a conflict of interest regarding this manuscript.

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