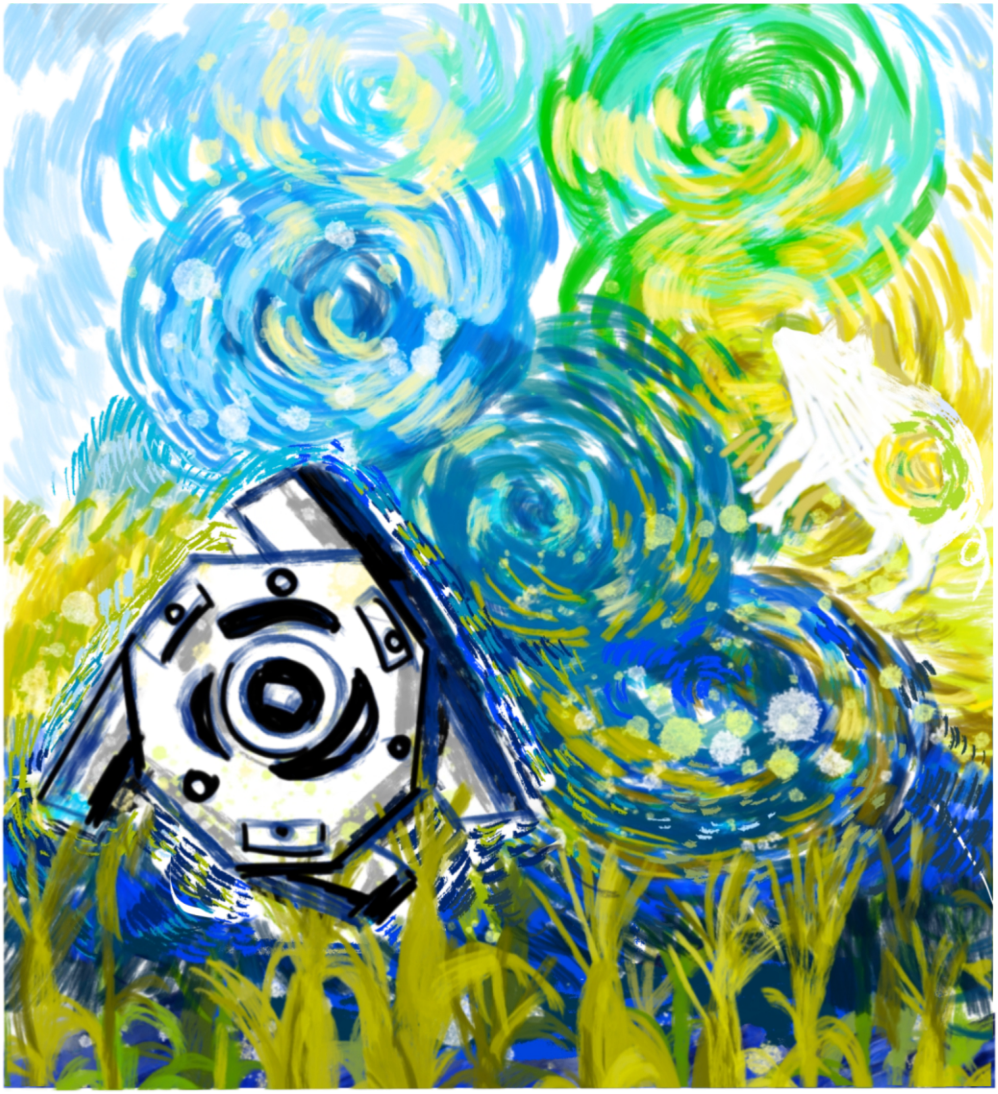
Hammer-milling Maize and Soybean Meal

Physical and Nutritional Characteristics of Particles



Fang Lyu

Propositions

1. ‘Chinese whispers’ through scientific publications also affected the *in vitro* digestibility assay which requires particle diameter to be < 595 μm.

(this thesis)

2. Particle size distribution of ground feed ingredients is an undervalued and interfering factor in animal nutrition.

(this thesis)

3. Limited data transparency and limited data interpretation turned Covid-19 into Covid-20 and Covid-21.

4. Every uncovered fraud by scientists should be rewarded.

5. The unwritten hierarchy which exists between professionals of different disciplines also applies to PhD candidates and their projects.

6. Before starting a PhD, candidates should ensure all their supervisors have an adequate mobile data plan.

Propositions belonging to the thesis, entitled

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Fang Lyu

Wageningen, 15 November 2021

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Table of contents

“A thousand miles begins with a single step”

--- Lao-Tzu

Chapter 1 **General introduction**

1.1 Background

The global human population is expected to continue to grow from 7.7 billion in 2019 to around 8.5 billion in 2030, 9.7 billion in 2050, and 10.9 billion in 2100 (UN, 2019). As such, the demand for plant- and animal-based food products will increase in the future. Such continued rapid population growth and increased demand for food are presenting challenges for the sustainable development of the global population and its environment. Therefore, using ingredients efficiently and improving animal production and its efficiency will continue to increase in importance. Feed processing is an essential part of bridging ingredient usage and animal farming. Before feeding, various ingredients are often processed to increase the value of the final compound feed in terms of nutrition, hygiene, safety, and handling. Feed processing includes many operations, such as grinding, proportioning, mixing, conditioning, pelleting, coating, and cooling, that apply physical, chemical, and thermal treatments to the ingredient or to ingredient mixtures. Often as a result of feed processing, nutrient availability of ingredients and performance of animals can be improved.

As a standard feed processing procedure, grinding reduces the particle size of feed ingredients, which increases the available surface area for digestive enzymes to act upon and improve the nutritional value of the raw material by improving nutrient and energy digestibility. In addition, feed ingredients come in many different forms and sizes. Grinding enables the particle size of all different ingredients to be restricted to more limited size ranges, so that the following process such as conditioning, extrusion, and pelleting achieve the expected effects in terms of pellet- (Thomas, 1998) and nutritional quality. In the last decades, researchers have evaluated the optimum particle size for swine nutrition based on animal performance, animal health and processing costs (Patience, 2012). Such studies, however, mainly focused on the mean particle size (MPS) e.g. geometric mean diameter (GMD) of ground materials, which only provides limited information for examining the relationship between particle size distribution (PSD), nutrient digestibility and animal growth performance. Capturing other characteristics of particles (e.g. surface area, circularity) using other methods of determination and expression can in this respect potentially provide a better understanding of breaking behaviour of feed ingredients. In addition, such methods may be more correlated to *in vivo* performance and explain observed effects with respect to animal performance.

1.2 Feed ingredients

There are many cereals and co-products that can be used as feed ingredients. Maize and soybean meal (SBM) are two common feed ingredients in simple-stomached animal's diets (e.g. swine and poultry), which have been widely used for many decades in compound feed manufacturing. Maize (Fig. 1.1) contains ~70% starch, ~4% oil, ~9% protein and has a low fibre content. Maize can contribute up to ~30% protein, ~60% energy and ~98% of the starch in diets for simple-stomached production animals (Dado, 1999). Soybean meal (Fig. 1.2) is one of the most widely used and important protein sources in swine and poultry diets as it contains >40% protein (Kwon and Kim, 2015). Soybean meal is produced primarily by solvent extraction of oil from soybeans with the oil being predominantly used in human nutrition.

Fig. 1.1 Maize

Fig. 1.2 Soybean meal

1.3 Hammer-milling

Grinding is an essential technology in the feed processing industry because the reduction of particle size enables improved feed material mixing that may benefit material flow, conditioning, extrusion, and pelleting. In feed processing, many devices can be used for grinding, among which, hammer mills are widely used because of their high grinding capacity and ability to take a wide range of particle sizes as an input and still obtain a satisfactory particle size distribution in the outgoing product. In a hammer mill, the material is crushed or shattered by a combination of repeated impacts by the hammer, collisions with the wall/screen of the grinding chamber, and particle on particle impacts. The screen is usually fitted at the bottom of the mill, which allows materials to pass as finished product once the particles are small enough to escape via the sieve openings while the coarse particles are retained for further grinding. Air is sucked through the grinding chamber to further assist in removing fine material from the mill. Hammer mills are widely used in milling of a mixture of feed ingredients for livestock because they are easy to operate and have a high capacity in the relatively fine grinding of a variety of diet ingredients compared to other milling devices (Scholten and McEllhiney, 1985; Islam and

Matzen, 1988; Thomas et al., 2018). Hammer mills, however, consume more energy comparing to other particle size reduction equipment such as a roller mill or multicracker (Thomas et al., 2018). It is known that grinding is the second largest energy cost in terms of electricity after the cost of pelleting (Reece et al., 1985) in the production of feeds. Optimal grinding is achieved when a controlled and consistent PSD is obtained using the minimum amount of energy. There are many factors affecting the grinding performance of a hammer mill, for example, feed rate, rotor speed, the size of the screen opening (determining the residence time in the grinding chamber), design of the hammers, as well as the characteristics of feed material, for instance, the initial particle size, plasticity or the moisture content. Al-Rabadi (2013) found that while hammer-milling sorghum, energy consumption increased from 8.5 to 14.0 Wh/kg with the GMD being decreased from 0.78 to 0.53 mm when the screen size decreased from 6.0 to 2.0 mm. Similar results were also reported for the hammer-milling of corn (Dabbour et al., 2015). In the study of Mugabi et al. (2017) where corn bran was hammer milled, it was found that the energy consumption increased when the feed rate and hammer tip speed were increased. Increasing hammer tip speed also resulted in a smaller particle size (Heiman, 2005; Mugabi et al., 2017) and increased grinding capacity (Yancey et al., 2013). El Shal et al. (2010) found that when the thickness of hammers increased from 1.5 to 5.0 mm, the percentage of fine corn particles increased. Many studies found that a higher moisture content of the material to be ground resulted in increased energy consumption and larger particle size during hammer-milling of, amongst others, maize (Armstrong et al., 2007; El Shal et al., 2010; Dabbour et al., 2015), corn bran (Mugabi et al., 2017), soybeans (Lee et al., 2013) and biomass including corn stover, switchgrass, *Miscanthus* and sorghum stover (Yancey et al., 2013).

1.4 Feed particles and its expression

After grinding, the material is made up of a plethora of particles with different sizes and shapes (Figures 1.3 and 1.4). Numerous studies have been conducted to investigate the effects of particle size on pig growth performance (Ohh et al., 1983; Hedde et al., 1985; Goodband and Hines, 1988; Healy et al., 1994; Wondra et al., 1995a; Callan et al., 2007; Lahaye et al., 2008; Ball et al., 2015; Rojas and Stein, 2015; Bao et al., 2016; Al-Rabadi et al., 2017) and gastro-intestinal tract health (Potkins et al., 1992; Cappai et al., 2013) as well as nutrient utilization, growth performance, gizzard development and feed passage rate in broilers (Gabriel et al., 2003a,b; Amerah et al., 2007a,b; Amerah et al., 2008). The particle size reported in these studies, however, is the MPS (e.g. GMD), which provides only limited information about particle characteristics. In fact, there are many other methods to determine and express particle size and

Fig. 1.3 Ground maize particles.

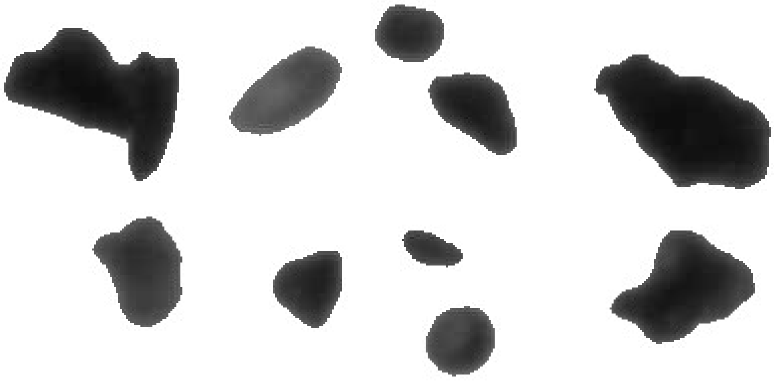

Fig. 1.4 Pictures of irregular shaped soybean meal particles analysed by image analysis technique (Lyu et al., 2021).

shape, which may be more suitable for particle size determination or be closer related to animal performance compared to the MPS. For example, compared to dry sieving, wet sieving enables the PSD determination of digesta, faecal or pelleted samples (Fritz et al., 2012; Gao et al., 2019), because material is soaked and washed. Image analysis can capture the shape of particles and describe the characteristics of particles with different parameters in addition to just size. These characteristics comprise parameters such as circularity, aspect ratio, solidity and projected area. In addition, laser diffraction as a further method of size analysis detects the volume of the particles and expresses the particle size with an equivalent particle size (EPS): EPS is a virtual diameter of a sphere to represent the size of a given irregular particle and is calculated based on the same volume, surface or weight, etc. Equivalent particle size can also be estimated when GMD and geometric standard deviation of particles are known (Hatch and Choate, 1929). The so-called EPS parameters provide more possibilities to express the characteristics of particles and may better relate to pig performance than MPS. The uniformity of PSD (geometric standard deviation, GSD) is also an important parameter. Wondra et al. (1995b) reported that pigs fed diet with smaller GSD (2.7 *vs* 2.3 *vs* 2.0 mm) had a greater apparent nutrient digestibility, and tended to have less severe stomach keratinization, although the growth performance was not affected.

1.5 *In vitro* digestion

In vitro digestibility assays are an inexpensive, compared to *in vivo* testing, and rapid method which have been developed to evaluate the nutritional value of feed ingredients and have been shown to accurately predict *in vivo* digestible energy and crude protein (Furuya et al., 1979; Löwgren et al., 1989; Boisen and Fernández, 1995; 1997; Huang et al., 2003; Noblet and Jaguelin-Peyraud, 2007). The *in vitro* methods developed by Boisen and Fernández (1995; 1997) have been widely used in pig feed evaluation systems because of its accuracy and repeatability. In their studies, a two-step method using pepsin and pancreatin to simulate gastrointestinal conditions was developed to predict the apparent ileal digestibility of crude protein (CP) and amino acids. In a third step, adding multi-enzyme complex is used to predict the total tract digestibility of organic matter (OM) and energy.

Generally, *in vitro* assays estimating protein digestibility are found to yield higher values when compared to actual *in vivo* values (Cone and van der Poel, 1993; Boisen and Fernández, 1995; Huang et al., 2003). An explanation for this effect may be that the *in vitro* methods do not take into account the effect of antinutritional factors or fibre content on the endogenous protein losses. The effects of changes in the incubation condition on the *in vitro* digestibility of OM was investigated by Boisen and Fernández (1997), including the variation in particle size, sample weight, stirring condition, addition of extra cellulase and EDTA. Boisen and Fernández (1997) also established the prediction equation of *in vivo* digestibility from *in vitro* digestibility data. Noblet and Jaguelin-Peyraud (2007) subsequently found that the accuracy of predictions could be improved when the ash or fibre content of feed is included in the equations.

1.6 Particle size, nutrition and animal performance

The relationships between particle size, nutrients and pig performance are fundamental to animal nutrition and should be taken into account when formulating diets for animals. Reducing the particle size of ingredients, however, mainly changes the physical characteristics of the material and does not alter the gross nutrient composition (e.g. protein, starch and fat) of ingredients. A smaller particle size, however, does result in an increase in the ratio of surface area to volume providing a greater surface area for digestive enzymes to act upon. The latter may lead to an improvement in nutrient digestion and potentially better growth performance of pigs (Wondra et al., 1995a; Lahaye et al., 2008; Ball et al., 2015; Rojas and Stein, 2016). Particle size reduction, however, has been shown to have a negative effect on stomach morphology of pigs (Morel and Cottam, 2007; Grosse Liesner et al., 2009). Increased lesions and keratinization of pars esphagea were observed when pigs were fed a smaller particle sized corn-soybean meal-based diet (Wondra et al., 1995a). Mavromichalis et al. (2000) also observed more ulcers and keratinization in pigs when wheat particle size were reduced from 1200 to 600 µm. Fibre was shown to aid in preventing gastric ulcerations (Potkins et al., 1992) and reduce the frequency of macroscopic lesion scores in gastric mucosa integrity (Millet et al., 2012) and further reduced the frequency of lesions when large particles are used (Millet et al., 2012). In the latter study, coarse grinding of feed ingredients for a growing pig diet with a high content of crude fibre reduced macroscopic lesions of the pars oesophageal although such a diet was accompanied by inferior carcass yield. In addition, the coarsely ground diet decreases the survival of *Salmonella* during passage through the stomach (Mikkelsen et al., 2004) compared to fine grinding.

In poultry, the effect of particle size on broiler performance varies depending on the age of broilers, the feed physical form (mash or pellet) and grain type. In general, medium and coarse grindings are advantageous to improve the performance of broilers fed mash diets and that these beneficial effects are greater in diets of better particle uniformity (Amerah et al., 2007a). Nir et al. (1994) reported that no significant differences in performance were observed in 7-day old chicks, whilst performance was improved in 21-day old birds that consumed medium and coarse mash diets compared with those that were fed the fine mash diet. This may be due to the fact that the underdeveloped gizzard of young broilers are not able to utilise the coarse particles efficiently. In addition, the effect of particle size on broiler performance is more critical in mash diet than that in the pelleted diet, becasue the differences in PSD can be evened out during the pelleting process (Svihus et al., 2004; Amerah et al., 2007a, 2008; Abdollahi et al., 2011). As for the interaction between particle size and grain type, in the study of Amerah et al. (2008), higher weight gain and and heavier gizzard weights were obsered when broilers were fed coarsely ground diets in corn-based diets, such influence, however, was not observed in wheat-based diets.

1.7 Knowledge gap

Up till now, researchers have mainly investigated the relevance between the MPS and pig performance. There have been many studies that showed that reducing mean particle size of feed ingredients/meal can improve the digestibility of nutrients and result in a better animal growth performance (Wondra et al., 1995a; Lahaye et al., 2008; Ball et al., 2015; Rojas and Stein, 2015). However, the influence of feed particle size on pig growth performance is not consistent in the scientific literature (Lawrence et al., 2003; Morel and Cottam, 2007; Li et al., 2019), and the underlying reasons are not fully understood.

A number of other methods to determine and express particle size, in addition to MPS, can provide further information on the characteristics of particles. Many of these methods have not been used in the field of animal feeding, which may provide the possibility for a better interpretation of the observed inconsistent results of particles size and its effects on animal performance. On the other hand, grinding feed ingredients not only produces particles with an uneven size distribution, but also heterogeneous chemical composition within the PSD due to the inherent structure of feed ingredients (Maaroufi et al., 2000). Whereas previous grinding studies mainly focused on particle size reduction and energy consumption, investigations into the nutrient distribution along with the PSD are scarce. Nutrient distribution and particle properties could be indicators for the breaking behaviour of feed ingredients as breakage can be expected to occur depending on the various morphological structures within the ingredient. Studies measuring the physical or chemical properties in different size classes of particles focused mainly on sorghum, barley (Sundberg et al., 1995a,b; Al-Rabadi et al., 2009, 2012) and rice flour (De La Hera et al., 2013). Other routinely and more important feed ingredients for simple stomached animals, such as maize and SBM have not been studied to date. It was found that the moisture content affects the breaking behaviour of corn bran (Mugabi et al., 2017) and biomass (Miu et al., 2006). Information on the effects of moisture content on the breaking behaviour of maize and SBM is limited.

Combining different ingredients before grinding is a routine strategy employed in the feed factory. The effect of such a strategy on breaking behaviour of the mixture has, however, not been studied. A further question is, in what way the heterogenous nutrient distribution across particles in ground feed ingredients or diets affects the digestion of these nutrients by simple stomached animals. Additional studies into particle characteristics and their digestibility warrant further investigation, as it can provide data that potentially provide an explanation for the variation in pig growth performance caused by change in particle size characteristics due to grinding.

1.8 Thesis objectives and outline

The objective of studies reported in this thesis was to gain insight into the breaking behaviour of maize and SBM during hammer-milling, via the physical and chemical characterisation of size-fractionated particles, relating to the *in vitro* digestibility of nutrients. The effect of water addition in a practical range, and the effect of mixing ratio of maize and SBM on the breaking behaviour of the two ingredients on a hammer mill were also investigated. Besides particle size, other physical properties of particles such as shape, area and circularity were investigated using various methods (e.g. image analysis, wet sieving). Models were used to describe the breaking behaviour of maize and SBM as affected by moisture content and by using different mixing ratios of the two ingredients in the hammer mill. **Chapter 2** consists of a literature review that describes the advantages and disadvantages of particle size reduction devices used in feed industry. In addition, particle size determination and expression methods, development of breakage equations as well as the effect of particle size reduction in downstream processes (pelleting, extrusion) are discussed.

To investigate potential underlying reasons for the inconsistent effects of MPS on pig growth performance and gastric health, the nutrient content and physical characteristics of individual size fractions of hammer milled maize and SBM, and their relation to *in vitro* digestibility were determined. Particles of individual size fractions were characterised by dry sieving, wet sieving and image analysis, and expressed with EPS (**Chapter 3**). In the study reported in Chapter 3, it was found that the additional grinding of the fractionated particles over a 1 mm screen for *in vitro* analysis, significantly improved the digestibility of OM and CP. This serendipitous finding resulted in a redirection of the focus of the work from building models, to investigating the effect of grinding before *in vitro* digestion on the evaluation of digestibility of nutrients. First the observed effects were conformed within the research described in Chapter 4 and additional research into the effect of the required grinding procedure before *in vitro* analysis on the digestibility of nutrients was conducted (**Chapter 6**). In **Chapter 4** and **5**, the effect of moisture content and the mixing ratio of maize and SBM on the breaking behaviour of maize and SBM were investigated. Finally, **Chapter 7** summarizes and discusses the results of all previous chapters.

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Chapter 2 **Size reduction in feed technology and methods for determining, expressing and predicting particle size: A review**

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Abstract

Particle size of diets or ingredients plays an important role in pig growth and gut health. The way the size of particles is measured and expressed, however, is limited in explaining pig growth performance differences. This review explores new possibilities to determine, express and predict particle size. Different grinding methods, including the use of roller mills, hammer mills, multicracker and multi-stage grinding were reviewed. Roller milling tends to produce a more uniform particle size distribution (PSD) and consumes less energy, whilst hammer mills have a greater grinding capacity and a higher reduction ratio compared to roller mill. The multicracker system, a more recently developed technology, can be considered cost-effective and ensures grinding capacity. Since the effects of different grinding methods vary, multi-stage grinding, combining different grinding methods, might be a solution to obtain a defined PSD. Particle size determination techniques, including dry/wet sieving, laser diffraction, microscopy, and static/dynamic image analysis are described and compared. It is concluded that more characteristics of particles (e.g. shape, volume or surface area) should be investigated. Besides geometric mean diameter (GMD), particle size can also be expressed with parameters such as D_{50}, $D_{4,3}$ and span of PSD. Equivalent particle size (EPS) is introduced as a mean of describing the size of particles related to a functional trait of the particles. A meta-analysis was performed by collecting particle size and pig performance data from scientific studies examining the effect of recalculated EPS on pig performance (feed conversion ratio, FCR). Regression/linear modelling shows that recalculated EPS was not better than GMD in explaining pig performance differences due to the high variation among studies. Different expressions of PSD may result in different conclusions. An introduction of describing the breaking behaviour of diet ingredients via mathematical models is provided. The development in breakage functions of wheat in roller milling in food preparations indicates that breakage functions are applicable in predicting the output PSD. Functions may also be extended to diet ingredients to be ground in animal feed manufacture. In feed manufacturing diagrams, particle size reduction in downstream processes (e.g. pelleting, extruding, expander processing) should be taken into account when the relationship between pig performance and particle size of diets is investigated. In conclusion, the determination, expression, and prediction of particle size can be a new direction for controlling the grinding process in the feed mill to better explain its relationship with pig performance.

Keywords: pig feed; particle size; size reduction; pelleting; breakage function

2.1 Introduction

In the field of feed manufacturing, grinding is a standard procedure for particle size reduction to make ingredients suitable for animal feeding and digestion. The principle of grinding methods is applying force to the ingredients, so that the bonds between various physical structures in feed materials are broken. For diet ingredients, the energy input and the extent of particle size reduction are important factors in feed manufacturing practices. Reducing particle size of ingredients can bring many benefits for feed processing and animals. According to Goodband et al. (2002), decreasing particle size of raw materials can improve mixing characteristics by reducing segregation with other ingredients in the mixture, and improve the pelleting capacity as well as the pellet quality. In addition, grinding increases the available surface area for the digestive enzymes to interact (Mavromichalis et al., 2000).

The effects of the particle size of ingredients or diets on pig performance and health (stomach and gut functioning) have been widely discussed. Smaller particles of ingredients or diets can improve nutrient digestion in animals (Wondra et al., 1993; Kim et al., 2005) and, therefore, improve pig performance (Ball et al., 2015; Huang et al., 2015; Nemechek et al., 2016). Wondra et al. (1995a) investigated effects of corn with a particle size ranging from 1,000 to 400 μm in both mash and pellet form on pig performance. By decreasing the particle size, the average daily feed intake increased and the feed conversion ratio (FCR) improved. These authors also found that the apparent total tract digestibility (ATTD) of dry matter, nitrogen and gross energy was increased by particle size reduction. Ileal digestibility of dry matter, organic matter and energy was improved by lowering the particle size of wheat in pig diets (Lahaye et al., 2008). The results were explained by a better digestion of starch due to fine grinding, so amylase has a better access to the starch (Al-Rabadi et al., 2009). Moreover, reducing particle size of corn improved the apparent ileal digestibility of starch and gross energy (Rojas and Stein, 2015) as well as ATTD of gross energy. Kim et al. (2005) also found that ATTD of starch was significantly increased when particle size of wheat was reduced. In addition to earlier reported influences on digestibility parameters, also crude protein digestibility improved when smaller particles were used (Ball et al., 2015).

However, too finely ground ingredients can increase the risk of gastric ulcer development in pigs (Healy et al., 1994; Wondra et al., 1995a; Grosse Liesner et al., 2009; Ulens et al., 2015), which is considered the main cause of sudden death of pigs on farms, leading to economic losses. Grosse Liesner et al. (2009) showed that if 30% of the particles were smaller than 400 μm, piglets would have a high risk for ulcerations. According to Bao et al. (2016), the number of beneficial bacteria increased and suppressed bacterial pathogens at the same time when particle size of wheat increased from 430 to 470 µm. Mavromichalis et al. (2000) reported that a particle size of 400 µm of wheat increases the development of stomach lesions. Meanwhile, coarsely ground meal had almost no negative effect on the gastric wall of pigs (Nielsen and Ingvartsen, 2000). Similar results were obtained by Millet et al. (2012), indicating that coarse particles may also be essential for animals to maintain gut health.

The relationship between particle size of diet or feed ingredients and pig performance seems obvious: finely ground particles can enhance animal performance and the coarse fraction of particles can aid in maintaining gut health in pigs. So, the optimal particle size distribution (PSD) of animal diets has been of great research interest over the last decades. Although optimal PSD of pig diet is difficult to define, there is a general consensus among researchers that optimal particle sized feed for animals should be evaluated on the basis of animal performance, animal health and processing costs (Stark, 2012). In other words, optimal PSD should maximize the utilization of nutrients, improve animal performance under the precondition of ensuring health (Vukmirović et al., 2017), maintaining pellet quality and a proper grinding efficiency (Amerah et al., 2007). It should be noted that many studies control fine/coarse particles of diet/ingredients by changing the screen size of the grinding machine (Dirkzwager et al., 1998; Ball et al., 2015), or report PSD values based on unstandardized sieve sizes (Nielsen and Ingvartsen, 2000; Grosse Liesner et al., 2009; Millet et al., 2012). In this case, conclusions about how particle size influences pig performance and digestion of nutrients can only be obtained within their own study and cannot be compared with other studies.

Generally, smaller particles lead to better pig performance, and larger particles are essential for pig health. In some cases, different results were also observed. In the study of Kim et al. (2005), significant effects of particle size of wheat on average daily gain (ADG), feed intake and feed efficiency were not observed. Similarly, ADG was not affected when particle size of sorghum reduced from 724 to 319 µm (Paulk et al., 2015). An increase in growth performance of pigs was observed with course ground corn compared to fine ground corn (Huang et al., 2015), which contradict with the results of Nemecheck et al. (2016). As such, particle size obviously cannot sufficiently explain differences in pig performance and digestion (Paulk et al., 2015).

Particle size of diets and feed ingredients in animal feed manufacturing is usually determined by dry sieving, and reported as PSD or GMD (ASABE, 2008). The difference in results among studies may be because of a poor correlation between the results of dry sieving method and digestive process and pig performance. A further reason is that the dry sieving selects particles based on two dimensions: maximum width and maximum thickness when particles pass the sieves (Allen, 1981). This means the actual shape of particles is not always determining the mass of a particle size class when dry sieving. The challenges then can be found in a more complete characterization of particles (e.g. volume, surface area, hardness, brittleness, hydratability) and different methods of determining and expressing particle size. An additional step is to obtain a better understanding of the breaking behaviour of various feed ingredients using mathematical formulas (breakage functions). These functions are the result of the interaction between ingredients and mills and can be used to predict PSD.

The objective of this review is to provide new insights into particle reduction technology related to pig feed ingredients based on fundamental theory analysis. Alternative ways to measure particle size are reviewed and the use of breakage functions as an innovative method to predict PSD is discussed.

2.2 Particle size reduction in the grinding process

Particle size reduction is accomplished by grinding for which different types of mills can be used. Currently, the hammer mill and roller mill are the most commonly used mills in the pig feed industry. Thomas et al. (2012; 2018) also investigated the possibility for the use of a multicracker device. Mill choices are usually based on ingredient type, energy consumption, grinding capacity as well as the animal species. Multi-stage grinding, which combines the advantages of different mills, may be a possible solution to obtain an ideal PSD with less cost (Lucht et al., 2011).

2.2.1 Hammer mill

Hammer mills consist of a series of hammers suspended from a central shaft and enclosed within a rigid metal casing. Particle size reduction in a hammer mill occurs as a result of the impaction/attrition between the rapidly moving hammers and relatively slow-moving particles (Koch, 2002; van der Poel et al., 2018). A screen fitted in the milling chamber allows appropriately sized materials to pass as finished product.

The extent to which the material will be broken down in a mill can be characterized by their reduction ratio. This ratio is calculated by dividing the average input particle size of a whole ingredient by the average output particle size of the ground sample (Thomas et al., 2012). The hammer mill has the highest reduction ratio of 5.95 for coarsely ground corn, followed by the roller mill, whilst the multicracker system (see the description of multicracker in 2.3) has the smallest reduction ratio of 1.60 for coarsely ground wheat. The operational variables, however, may strongly affect the reduction ratio (Thomas et al., 2018). The high grinding capacity, less maintenance cost and ease of operations also makes the hammer mill a preferred grinding method. In addition, in feed manufacturing, the hammer mill is highly suitable for grinding fibrous materials such as hulls (oat, barley, wheat bran) compared to the roller mill and multicracker system.

Compared to the roller mill and multicracker system, the hammer mill requires more energy. For a similar grinding intensity (particle size reduction), the hammer mill consumes more specific mechanical energy (kJ/kg) compared to the roller mill (Wondra et al., 1993; Vukmirović et al., 2016, Thomas et al., 2018). Moreover, a hammer mill tends to produce more fine particles and dust, which can have a negative impact on gut health in pig (Nielsen and Ingvartsen, 2000).

Many factors influence the milling output of hammer mills. For a similar percentage of screen openings, large screen openings result in less screen area leading to less collisions of particles in the hammer mill, therefore, the yield of coarse particles increased (Islam and Matzen, 1988); larger openings reduce the residence time in the grinding chamber (Martin, 1981). The impact of particles on the screen area is of great importance for the breakage of cereals, in addition to the impact of the hammers. A large screen area generates additional heat, which can reduce the efficiency of the hammer mill (Guo et al., 2016). Applying air flow through the hammer mill aids to improve the capacity of the mill and to achieve a more uniform PSD. Moisture content of feed ingredients influences breaking behaviour (Jindal and Austin, 1976; Adapa et al., 2011), as it affects the minimum cutting blade speed to ensure breakage of the material. When moisture content is low, material becomes harder and more brittle (Jindal and Austin, 1976). They also found that both less loading or overloading results in a different absolute rate of breakage, being also the case when using laboratory mills. This can be explained since a higher loading can result in a decrease in speed of the particles compared to one another and, therefore, leads to a lower breakage rate and higher energy consumption. Both the shape and the material of the hammer is of influence. Bochat et al. (2015) used a new rotor design with hammers in the shape of a circle section with an angle 45° and concluded that the grinding efficiency of cereals is improved by primarily reducing the time needed for grinding. The material of hammers was shown to have different wear mechanism during grinding process (Bao et al., 2011). Finally, the tip speed of hammers is found a factor of influence: Islam and Matzen (1988) found that when the tip speed is decreased, the percentage of coarse particles will increase under identical operating conditions. In the study of Dey et al. (2013), the reduction ratio as well as the energy consumption increases when the rotor speed increases from

1000 to 1400 rpm. Today's hammer mills have been developed to control PSD by adjusting tip speed and screen openings.

2.2.2 Roller mill

Particle size reduction in a roller mill is achieved by compression (same roll rotating speed) or shear (different roll rotating speed) forces and the roll design features (grooves and corrugations) (Koch, 2002; Vukmirović et al., 2017). This has made the roller mill suitable for very accurate controlled milling of the product, although it has difficulty in milling fibrous materials or hulls. Roller mills have better energy efficiency compared to hammer mill and multicracker devices (Wondra et al., 1993; Vukmirović et al., 2016; Thomas et al., 2018). Grain type can also be a factor affecting energy consumption. Healy et al. (1994) indicated that grinding sorghum took less energy than grinding corn when roller milling was applied. Coarse grinding of full fat soybeans requires more effective specific mechanical energy, followed by wheat and maize (Thomas et al., 2018).

Thacker (2006) suggests that pig producers consider the use of a roller mill rather than a hammer mill for grinding ingredients because pig performance and carcass traits were not affected by the grinding method; a roller mill however requires less energy, has lower maintenance costs, is a quieter operation and has a more exact control of particle size. Particles processed by roller milling had a better uniformity, and rolled feed contained less fine and more coarse particles than hammer milled feed (Nielsen and Ingvartsen, 2000; Svihus et al., 2004). In the study of Wondra et al. (1993) such results were also observed when corn was ground to 800 µm. These results correspond well with the research of Vukmirović et al. (2016) who found that coarsely rolled corn was more uniform than hammer milled corn. On the other hand, roller mills tend to produce more irregular particles (Vukmirović et al., 2017) due to the difference in packing of same sized material (Koch, 2002). For a unit mass of particles, irregular particles usually indicate a larger surface area, and this may aid enzyme accessibility in the gastrointestinal tract of pigs.

For the development of breakage equations with roller mill, many factors have been identified that influence the breakage process of diet ingredients. From roller mill studies, it became evident that kernel size, milling ratio (the ratio of the roller gap to the input particle size) and the hardness of wheat for example have their influence on the quantity of larger and smaller particles in the output of the PSD. It has been shown that the number of large particles increased when kernel size decreased (Fistes and Tanovic, 2006), the milling ratio increased (Campbell et al., 2001) or softer wheat seeds were milled (Campbell et al., 2007; Campbell et al., 2012). Furthermore, roll disposition influences the uniformity of the PSD. The disposition of the rollers, resulting from used grooves and corrugations, determines the kind of force affecting the particle: a sharp-sharp (S-S) disposition makes use of shearing or cutting force, while a dull-dull (D-D) disposition makes more use of compression (see Fig. 2.1). Using a S-S position will result in a less uniform PSD than using a D-D disposition (Fang and Campbell, 2002). Also, moisture content is of influence (Fang and Campbell, 2003b). Moisture contents lower than 10% show a more uniform distribution in comparison to wheat kernels with a moisture content of 20%. On the other hand, lower moisture kernels are easier to grind and require less energy for breakage (Dziki, 2007).

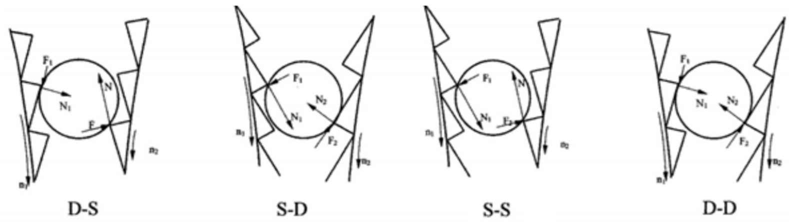

Fig. 2.1 The four possible break roll dispositions: dull-to-sharp (D-S), sharp-to-dull (S-D), sharp-to-sharp (S-S) and dull-to-dull (D-D) and its forces acting on the particle. (Fang and Campbell, 2002).

2.2.3 Multicracker system

The multicracker was introduced in 2005 as an alternative method to the conventional particle size reduction systems, such as hammer and roller mills. This technology comminutes ingredients by a method of cracking/cutting-edge crushing by two rows of special discs. Thomas et al. (2012) showed a schematic diagram of a multicracker system (see Fig. 2.2), with discs forming two contra-revolving rows. This system is relatively energy efficient and ensures grinding capacity at the same time. In the study of Thomas et al. (2018), compared to the hammer mill, the multicracker consumed less total specific mechanical energy to realize a similar mean particle size for coarsely ground maize, soybeans and wheat. In this comparative study between comminution devices, the roller mill used the lowest quantity of energy although it was not significantly different from the multicracker. Thomas et al. (2012) also investigated two factors affecting the grinding performance of different feed ingredients in a multicracker system. Disc type was considered to be an essential variable that significantly affects particle characteristics, as well as the use of specific mechanism energy. Compared to ceramic discs, steel discs had lower energy consumption and a lower reduction ratio. The gap between the discs also affected the mean particle size: a smaller opening between the discs led to a more uniformity PSD. In addition, a narrower width of PSD was observed, and the smallest particles were generated when a higher disc speed was applied.

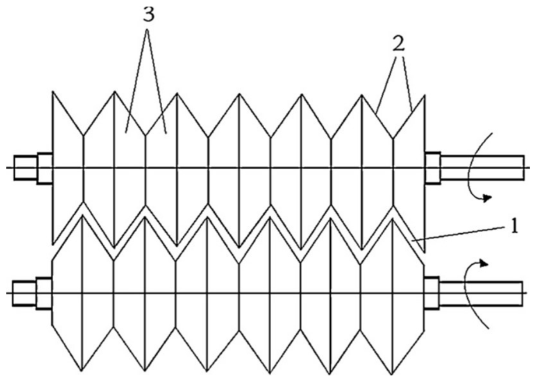

Fig. 2.2 Schematic diagram of twin parallel shafts in the multicracker grinding system: view from above. 1. Adjustable gap, 2. disc surface, 3. discs; →, shaft rotation direction (Thomas et al., 2012).

2.2.4 Multi-stage grinding

A desired pig feed structure is considered to contain the highest possible amount of medium- sized particles, with a low fraction of fine particles as well as coarse or very coarse particles (Healy et al., 1994; Wondra et al., 1995a,b; Lucht et al., 2011, Cappai et al., 2013). A roller mill produces a lower content of fines and is more efficient in energy consumption, while a hammer mill is more suitable for grinding husks. As such, different milling techniques can be combined to achieve a better grind and this multi-stage grinding is often applied (Rojas and Stein, 2015). Multi-stage grinding combines a hammer mill with a roller mill to maximize grinding efficiency and minimizing PSD variation. Multi-stage grinding often involves a sieving step after first grinding to sieve all particles of undesired size. Al-Rabadi et al. (2017) reported that regrinding the coarse fraction of grains (barley and sorghum) in a two-stage hammer mill system significantly improved the FCR in pigs by 6.6%. They suggested that regrinding coarse particles may be an alternative method to the pelleting process, due to the similar feed efficiency in pig growth performance. Similar results were reported by Black and Gidley (2017) in regrinding large particles of ground cereals. Lucht et al. (2011) compared four combined grinding methods to determine which combination can produce a well-structured pig diet in which 30% barley was included with the objective to have a low fine fraction (max. 25% < 0.5 mm), and where husks were ground efficiently. The four variants used by Lucht et al. (2011) were: 1. a two-stage crushing roller mill without intermediate sieving, 2. two hammer mills with pre-mill/ post-mill and intermediate sieving, 3. a hammer mill + one stage crushing roller mill with intermediate sieving and 4. a hammer mill + laboratory grinder with intermediate sieving. The most uniform feed structure was achieved by the combined hammer mill and roller mill, which resulted in the largest quantity of medium-sized (0.5 -1.6 mm) particles (60%), and the lowest quantity (lower than 25%) of fine (< 0.5 mm) particles as intended set-point values. Energy consumption was 30% lower compared to the use of two hammer mills.

2.3 Particle size determination

Methods of measuring and expressing particle size are different; in order to describe particle size or PSD, we therefore have to make a choice among available methods. Particles used in pig feed are normally larger than 100 μm, meaning that methods like sedimentation, electro zone sensing and dynamic light scattering systems are not routinely included in pig feed particle size research, since they are mainly used for very small particles (< 100 μm). An overview of techniques that can be used for pig feeds is provided below including dry sieving, wet sieving, microscopy, laser diffraction, static and dynamic image analysis is presented in Table 2.1.

2.3.1 Dry sieving

At present, dry sieving which discriminates particles into size classes and converts these to mass, is the most commonly used method to investigate particle size in animal nutrition. According to the recommended procedure of ASABE (2008), the feed ingredient mash was sieved into different size classes, and PSD can be presented with mass percentage in each class. Then the particle size can be calculated and expressed with geometric mean diameter (GMD) and geometric standard deviation (GSD). This standard procedure has made it possible to compare particle size of ingredients or diets between studies. Dry sieving is widely employed because it is a low-cost method that requires little training. However, this method has a number of drawbacks. For example, very small particles may stick to the sieves because of electrostatic forces. In addition, ingredients with a high fat content (like ground soybeans) tend to clog the sieves and make it difficult to obtain accurate data although using a dispersing agent can help prevent clogging while sieving (ASABE, 2008). In the study of Stark and Chewning (2012), it was shown that GMD decreased when a dispersing agent was added.

Table 2.1 Comparison of different particle size measurements.

Items	Principle	Range	Situation	Pros	Cons	Property measured
Dry sieving	-Mass	-Depends on sieve size	-Standard in feed industry	-Easy operation and low cost -Widely used	-Electrostatic forces make small particles stick to the sieves -Clogging of sieves -Not accurate enough	-Length
Wet sieving	-Mass	-Depends on sieve size	-Pellets -Mash	-Fines may be removed -Prevent blockage -More representative PSD than dry sieving with respect to animal performance	-Take long time -Not practical -Not suitable for soluble samples	-Length
Laser diffraction	-Volume	0.1-1000 µm	-Wet and dry samples	- Time saving -Small particle -Accurate -Easy operation	-Expensive -Geometry of particles is not considered -Lost small amounts of oversize and undersize particles	-Volume
Static image analysis	-Number	-Depends on the equipment	-Pastes -Sticky particles -Suspensions -When shape information is needed	-Distinguish individual particle -Predict the distribution of mixture	-Images can be affected by light and focus -Unrepresentative sample may be chosen -Limited mages/particles	-Shape -projected Area -Length -Width
Dynamic image analysis	-Volume -Number	20 µm~30 mm	-Powders -Granulates -Pellets -Extrudates -Glass beads	-Time saving -Accurate -Highly automated -Closely match sieve results -Collect shape information -Broad size distribution	-Expensive	-Shape -projected Area -Length -Width
Microscopy	-Number	-Depends on the equipment	-Simple analysis -When shape information is needed	-Measure size and shape of particles - Easy operation	-Time consuming -Not accurate enough -Cannot examine individually	-Shape -projected Area

2.3.2 Wet sieving

Wet sieving is commonly applied in soil science but can also be employed in the pig feed industry. Wet sieving is often used to determine the PSD of feed pellets. The pellet sample (50 g) is soaked in 1000 ml water for 1 h, and the feed-water-suspension is then poured onto a sieve tower (Wolf et al., 2010; Millet et al., 2012). Unlike dry sieving, flow of water should be provided from the top to the bottom when sieving to separate samples into different grades. After the sample is washed, the material that is left on the sieve is recovered, dried and weighed. Then particle size can be calculated and the characteristics of PSD (median, span etc.) can be reported.

Dirkzwager et al. (1998) measured the PSD of medium diets (composed by mixing 50% of a fine and 50% of a coarse diet) before and after pelleting with the dry and wet sieving method (see Fig. 2.3). A significant increase in the percentage of small particles (< 0.1 mm) can be observed after the meal was wet sieved. This might be due to the moving water bringing small particles to the lower sieves. Using water in a sieving procedure is considered to be more accurate because it prevents the clogging of particles; it should be noted that, however, wet sieving is more complicated and takes much more time than dry sieving.

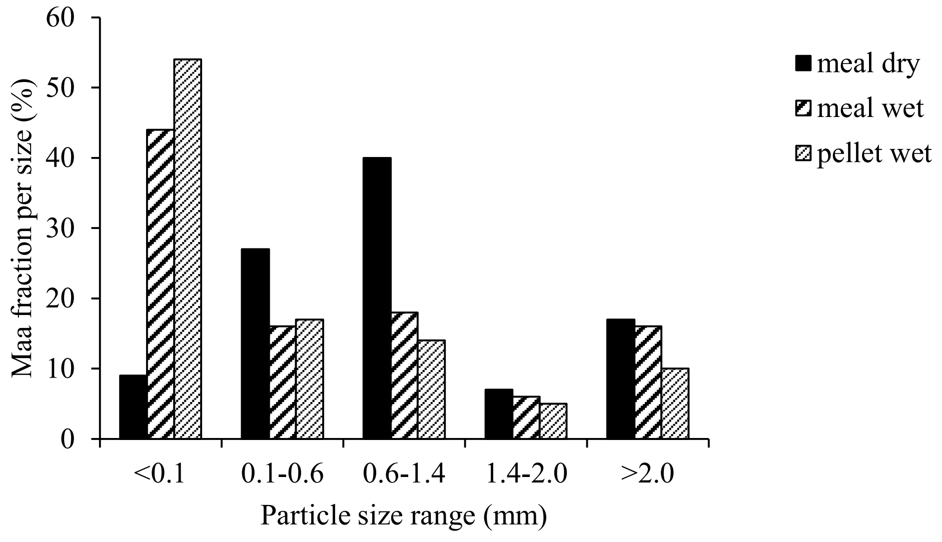

Fig.2.3 Particle size distribution of medium ground meal diet and pellet diet for starter pigs using dry sieving and wet sieving (Dirkzwager et al., 1998).

Wet sieving makes it possible to examine PSD in pellets and to investigate the influence of pelleting and expander treatment on PSD (Nielsen and Ingvartsen, 2000). Wolf et al. (2010) also indicated that measuring particle sizes in pellets with wet sieving made it possible to compare results from different studies. Wet sieving resembles the process of moisturizing in the gut, which could be a more specific and digestion-related representation of PSD than dry sieving (Engberg et al., 2002).

2.3.3 Other measurements

Optical microscopy can also be used to determine particle size and provide information on the shape of particles. Despite that, the use of microscopy in particle size determination can be influenced by the limited freedom in the orientation of the particles during measurement (Foqué et al., 2017). The drawback of using microscopy to measure PSD is that it is time-consuming and is a tedious measurement for analyzing a large number of particles: it is impractical to obtain a large enough set of measurements to obtain meaningful summarizing parameters such as mean, median and span (Ulusoy and Yekeler, 2014).

Laser diffraction measures angular variation in intensity of light scattered as a laser beam passes through a dispersed particulate sample to evaluate PSD. When the laser hits the flowing particles, it will provide a geometric size of the particles by measuring scatter light angle size and intensity (Dodds et al., 2013). Laser diffraction enables to determine particle size of samples within a large range, from 0.1 to 3000 μm. Additionally, the software used in conjunction with the laser diffraction techniques makes already an instant calculation of particle size from volume cumulative PSD, referred to as D_v10, D_v50, D_v90, particle uniformity and equivalent volume diameter like $D_{4,3}$, $D_{3,2}$ (see 4.1 for explanation). In addition, dry and wet samples are able to be measured by the laser diffraction technique. The high accuracy of measuring a large number of samples with low costs make laser diffraction a widely used technology (Fedotov et al., 2007).

Besides the size of particles, particle shape is considered another important characteristic. Particles sharing the same size in cross section but with different shapes may have different properties and behaviours (e.g. the flow ability of materials). Sedwell et al. (2017) indicated that pellets with low sphericity surfaces had a higher drug dissolve percentage compared to more spherical pellets (see Figures 2.4 and 2.5). As such, the shape of particles may also influence the nutrient release of feed in the gut of the pig.

Compared to traditional manual particle measuring methods, image analysis combined with computer programs has been widely employed due to the quicker and more objective measurement (Rodriguez et al., 2013). Static image analysis combines microscopy and a digital camera with computer software providing a full morphology of particles (2 dimensional) in a projected area. Extended subsequent statistical analysis is needed when using static image analysis. In addition, a good preparation of samples is required, so that computer can distinguish between the individual particle instead of analyzing agglomerates as one particle.

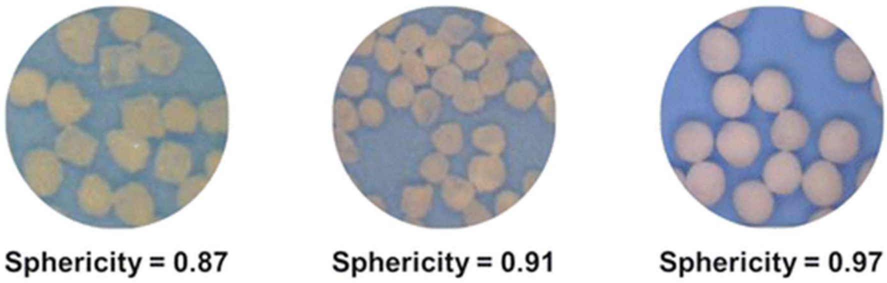

Fig. 2.4 Images of sugar spheres with different sphericity values (Sedwell et al., 2017).

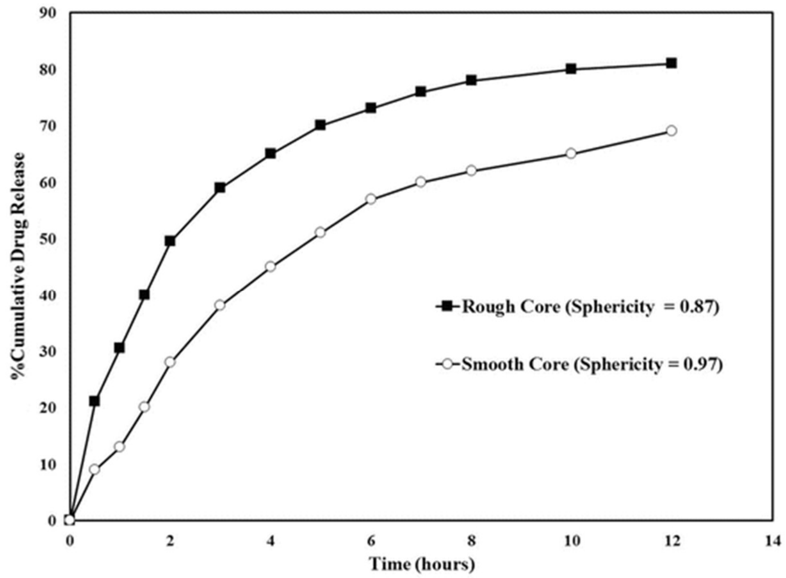

Fig. 2.5 Effect of particle shape as measured by sphericity of starting sugar spheres on release profile of chlorpheniramine maleate drug loaded on sugar spheres (Sedwell et al., 2017).

Dynamic image analysis allows materials to flow free over one or more cameras allowing a large number of particles to be projected in a random orientation (3D) during the measuring procedure. Consequently, a large number of images (several 10^3) can be obtained and both particle size and shape can be analyzed (Shang et al., 2017). This approach has the advantage over static image analysis, which can only do analysis on a limited number of images resulting in a small quantity of particles analyzed. Being sensitive to the different particle size and shape characteristics, dynamic image analysis provides more accurate results compared with sieving especially in determining particles of 38 μm and below (Ulusoy and Igathinathane, 2015). Technologies like sieving merely determine the equivalent spherical diameter (see 4.2 for explanation), while image analysis is able to detect various dimensions of irregularly shaped particles, such as length, width and area (Ulusoy and Yekeler, 2014).

The techniques mentioned above define a particle size in different ways. As such, the analytical facilities used to determine the particle size can yield different summarizing statistics of identical samples. In fact, no analysis method provides the true particle size except for spherical particles (Iacocca et al., 2010; Foqué et al., 2017) or other well-defined geometric shapes.

2.4 Particle size expression

Curves or plots are a very common tool to visualize the analysis of PSD measurements, which are very suitable for quality control. However, PSD are not always easily viewed using graphs, especially when a large quantity of PSD measurements needs to be compared or different size classes were used. Using numerical values to describe PSD makes a rapid comparison possible among different ingredients and analysis methods. In addition to calculated mean particle size and parameters derived from PSD, an equivalent particle size (EPS) can be determined. This EPS provides the possibility to describe irregular-shaped particles with one single parameter and may be an alternative way to express particle size by using other properties of particles, like volume, surface area or ratio of surface area to volume.

2.4.1 Description of particle size

According to the ASABE standard (2008), after sieving and weighing, both fractional and cumulative PSD can be obtained (see Fig. 2.6), which represents a visual mean of the distribution of particles in a range of particle size classes. In addition, the particle size can be calculated using a logarithmic approximation calculation, and reporting values as GMD and GSD, referring to the mean diameter of individual particles of a feed or simply the fineness of ground feeds (Eq. 1) and the width of distribution (Eqs. 2 and 3), respectively.

$$d_{gw} = log^{-1}[\frac{\sum_{i=1}^{n}(W_i \times \log(d_i \times d_{i+1})^{1/2})}{\sum_{i=1}^{n} W_i}] \quad (1)$$

$$S_{log} = \left[\frac{\sum_{i=1}^{n} W_i(\log(d_i \times d_{i+1})^{1/2} - \log d_{gw})^2}{\sum_{i=1}^{n} W_i}\right]^{1/2} \quad (2)$$

$$S_{gw} \approx \frac{1}{2} d_{gw}\left[log^{-1}S_{log} - \left(log^{-1}S_{log}\right)^{-1}\right] \quad (3)$$

where:

d_{gw}= geometric mean diameter or median size of particles by mass, mm

d_i = nominal sieve aperture size of the i^{th} sieve, mm

d_{i+1} = nominal sieve aperture size in next larger than i^{th} sieve (just above in a set), mm

W_i = mass on i^{th} sieve, g

n = number of sieves +1 (pan)

S_{gw} = geometric standard deviation of log-normal distribution by mass, dimensionless

S_{log} = geometric standard deviation of log-normal distribution by mass in ten-based logarithm, dimensionless

gw = geometric weight

Mean, median and mode are parameters that can be derived from absolute PSD values. The median is obtained by sorting all the sizes, which is not affected by the maximum and minimum extreme values and choosing the value where half of the data falls below and half above this number. The influence of extreme data values has no effect on the median. The mean is calculated based on all sizes of particles in the sample, so it is sensitive to each data change. The mode is the most frequently occurring particle size class in the sample, reflecting the concentration of a set of particles in the sample.

The cumulative distribution curve (see Fig. 2.6) is another commonly used way to provide a visualized description of the PSD which makes it possible to have a quick view of PSD. In addition, the particle size can also be described by parameters derived from the cumulative mass based PSD referred to as D_{50} (known as median), D_{10} and D_{90}, which indicate the 10%, 50% and 90% of the population below the respective size. This also enables the comparison of particle sizes among different samples.

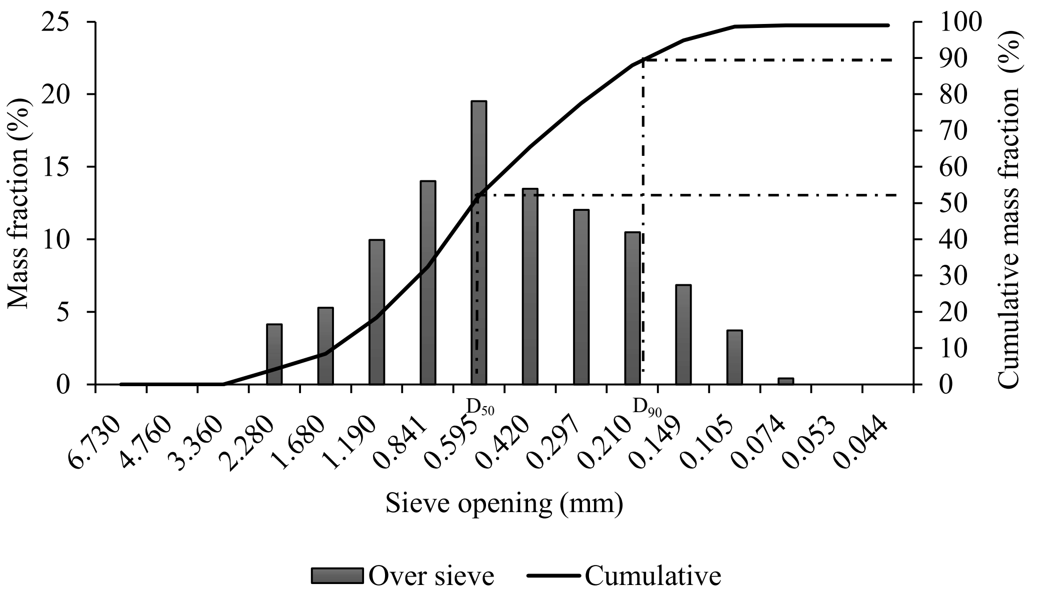

Fig. 2.6 Expression of fractional distribution (bars) and the undersize cumulative distribution (line) of particle size, based on mass (ASABE, 2008).

Besides GMD, GSD, mean, median and mode, the 'Sauter mean' diameter (surface weighted mean diameter) can also be used to estimate the mean particle size of a given particle distribution (see equation 4). It is defined as the diameter of a sphere that has the same volume-surface area ratio as the particle of interest (Filippa et al., 2012). Decreasing particle size will increase the surface area, thus increasing the surface-volume ratio. Sauter mean diameter is used in applications where specific area is important as in dissolution of material or reactivity of components (Merkus, 2009). For example, it would be a reasonable parameter to reflect the digestive processes where enzymes attack the surface of particles.

$$D_{3,2} = \frac{\sum_{i=1}^{n} n_i D_i^3}{\sum_{i=1}^{n} n_i D_i^2} \qquad (4)$$

where: n_i = the (number-based) frequency of occurrence of particles in size class i; D_i = the mean diameter of size class i (for linearly spaced size classes, the arithmetic mean size of the classes is taken, for logarithmically spaced classes, D_i can be taken as the geometric mean size).

Another weighted mean diameter is 'de Brouckere mean' diameter (volume weighted mean diameter). It can be applied where coarse particles that make up the bulk of volume is the determining factor. The number of particles is not required in this formula, and the laser diffraction detects the volume of the particles, not the quantity, so the results of the laser diffraction are presented with ($D_{4,3}$) (Anonymous 2016).

$$D_{4,3} = \frac{\sum_{i=1}^{n} n_i D_i^4}{\sum_{i=1}^{n} n_i D_i^3} \qquad (5)$$

where: n_i = the (number-based) frequency of occurrence of particles in size class i; D_i= the mean diameter of size class i.

The span of PSD is an additional parameter to the average particle size, which provides the user with additional information on the PSD (Anonymous, 2016). Span indicates the size range of the population of particles, and can be calculated from D_{10}, D_{50} and D_{90} values (see equation 6).

$$Span = \frac{D_{90} - D_{10}}{D_{50}} \qquad (6)$$

The type of parameter that should be used depends on the objective for which it may be used. For example, D_{10}, D_{50} and D_{90} are usually applied in comparative tests of quality among laboratories. When surface area is the factor of interest, the Sauter mean diameter - that relates to both particle quantity (volume) and weighting (by area) - is applicable (Merkus, 2009). Therefore, parameters to describe particle size should be chosen wisely, since different expression methods may produce quite different results for the same ground material.

2.4.2 Equivalent particle size

The main idea of expressing the particle size of a ground material is to obtain one descriptive parameter to allow comparison of values between ground materials and for quality control. However, it is difficult to describe a three dimensional (3D) and irregular-shaped particle with only one parameter. Spheres can be described by only one parameter (diameter); this directs us to describe these irregular shaped particles also with one parameter by equalling them to a sphere but on a different basis (see Fig. 2.7).

Equivalent particle size is a virtual diameter of a sphere to represent the size of a given irregular particle, calculated based on the same volume, surface or weight etc. So, an equivalent sphere has the same characteristics as the observed particle in relation to a given measurement principle, whether volume, projected perimeter, surface area, etc. This means that a given irregular particle can be represented with several equivalent spheres depending on the property considered. For example, consider a cube with a side length of 1 cm, then the volume of this cube is 1.0 cm^3. Then many equivalent sphere diameters can be obtained: an equivalent volume diameter of 1.24 cm, an equivalent area diameter of 1.38 cm and an equivalent projected perimeter of 1.27 cm. In practice, different instruments will give different EPS, for instance, laser diffraction gives results regarding the particle volume.

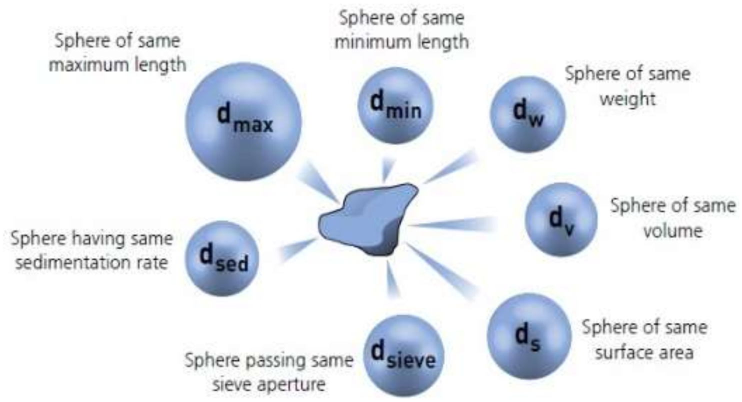

Fig. 2.7 Virtual diameters of sphere representing an irregular shaped particle (Rawle, 2003)

Estimates of certain EPS's can be derived when GMD and GSD are known (Lachman et al., 1987). The formulas were first described by Hatch and Choate (1929) and are used e.g. to describe pharmaceuticals. These formulas, equations (7) - (10), can calculate the EPS for arithmetic, surface, volume and volume-surface which provide more information on the particle size with respect to its intended use. Especially these calculations assumed that the PSD is log-normally distributed, which is the same as the ASABE standard being used to calculate the GMD. This makes it very suitable for recalculating the EPSs of animal feeds.

$$\log d_{ave} = \log d_{gw} - 5.7560 \times \log^2 S_{gw} \quad (7)$$

$$\log d_s = \log d_{gw} - 4.6052 \times \log^2 S_{gw} \quad (8)$$

$$\log d_v = \log d_{gw} - 3.4539 \times \log^2 S_{gw} \quad (9)$$

$$\log d_{vs} = \log d_{gw} - 1.1513 \times \log^2 S_{gw} \quad (10)$$

where: d_{ave} = Arithmetic mean; d_s = Surface mean; d_v = Volume mean; d_{vs} = Volume-to-surface mean (or Sauter mean); d_{gw} = geometric mean diameter; S_{gw} = geometric standard deviation of particle diameter by mass.

These four EPSs are used in very different ways for measurements, in pharmaceuticals, food or mining. According to Lachman et al. (1987), the arithmetic mean can be used for measurements of the evaporation of liquids; surface mean can be used in processes such as dissolution and absorption; as for volume mean, it can be used for the packing or flow of powders or granulation; and the volume-surface mean can be used for efficiency or chemical reactions (e.g. catalysis or combustion).

2.4.3 Equivalent particle size relating to pig performance

We use equations (7) - (10) to transform GMD and GSD to EPS. To explore the relationships between EPS and pig performance, studies in the scientific literature were collected which reported both GMD and indicators for pig performance, then EPS was calculated (Table 2.2). Data from Ohh et al. (1983; see Fig 2. 8) and Al-Rabadi et al. (2017; see Fig 2.9) were used as an example of analysis, and FCR was taken as the indicator of pig performance. We observed that FCR increased ($P < 0.01$) when particle size of corn or sorghum increased with all manners of expressing particle size (see Fig. 2.8). All EPS expressions showed a better fit to FCR in a linear model than GMD, with r^2 values from 0.80 to 0.85 against 0.70 for GMD. This indicates that the mean volume diameter and arithmetic mean diameter are a better predictor of pig performance when it comes to particle size.

Table 2.2 Effect of grain/complete diet particle size and recalculated equivalent particle size on pig performance (data collected from different studies).

Main dietary grain	Feed form	Weight class	GMD/ µm	GSD/ µm	AM/ µm	MSD/ µm	MVD/ µm	MVSD/ µm	ADG/ kg	ADFI/ kg	FCR	Reference
Barley	Meal	Fattening	1030	2.08	269.5	352.3	460.7	787.7	0.80	1.62	2.04	Al-Rabadi et
Barley	Meal	Fattening	700	1.88	258.5	315.5	385.0	573.5	0.86	1.60	1.88	
Sorghum	Meal	Fattening	830	2.38	126.7	184.5	268.7	569.9	0.85	1.84	2.20	
Sorghum	Meal	Fattening	540	1.90	192.8	236.9	291.1	439.5	0.87	1.72	1.98	
Barley	Pellet	Fattening	1030	2.08	269.5	352.3	460.7	787.7	0.41	1.66	1.96	
Barley	Pellet	Fattening	700	1.88	258.5	315.5	385.0	573.5	0.85	1.62	1.90	
Sorghum	Pellet	Fattening	830	2.38	126.7	184.5	268.7	569.9	0.80	1.60	2.02	
Sorghum	Pellet	Fattening	540	1.90	192.8	236.9	291.1	439.5	0.81	1.59	1.92	
Corn	Pellet	Finishing	555	3.14	21.0	40.5	77.9	288.4	1.08	2.90	2.69	Paulk et al., 2015
Sorghum	Pellet	Finishing	724	2.46	95.5	143.2	214.7	482.8	1.13	3.10	2.75	
Sorghum	Pellet	Finishing	573	2.31	99.3	141.0	200.2	403.6	1.10	2.99	2.71	
Sorghum	Pellet	Finishing	319	2.52	37.7	57.8	88.6	208.1	1.10	2.91	2.65	
Barley	Meal	Grower	390	2.20	82.4	112.5	153.5	285.8	0.79	1.74	2.19	Morel and
Barley	Meal	Grower	716	2.30	126.4	178.8	252.9	506.1	0.88	1.76	2.00	
Barley	Meal	Grower	1026	2.30	181.1	256.2	362.4	725.3	0.80	1.66	2.07	
Barley	Meal	Grower	880	2.60	89.8	141.7	223.7	557.5	0.92	1.83	1.99	
Barley	Meal	Finisher	479	1.70	236.9	272.8	314.0	416.1	1.15	2.81	2.46	
Barley	Meal	Finisher	854	2.10	215.7	284.0	374.0	648.5	1.09	2.62	2.44	
Barley	Meal	Finisher	1175	2.10	296.8	390.8	514.6	892.3	1.10	2.67	2.45	
Barley	Meal	Finisher	698	2.30	123.2	174.3	246.6	493.4	1.11	2.76	2.53	
Corn	Meal	--	610	2.17	136.1	183.7	247.9	451.9	0.90	2.41	2.68	Nemechek et al.,
Corn	Pellet	--	610	2.17	136.1	183.7	247.9	451.9	0.94	2.35	2.50	
Corn	Meal	--	464	2.33	77.6	110.9	158.6	324.5	0.86	2.26	2.63	
Corn	Pellet	--	464	2.33	77.6	110.9	158.6	324.5	0.92	2.35	2.55	

Main dietary grain	Feed form	Weight class	GMD/ µm	GSD/ µm	AM/ µm	MSD/ µm	MVD/ µm	M*VS*D/ µm	ADG/ kg	ADFI/ kg	FCR	Reference
Corn	Meal	--	502	2.25	97.0	134.7	187.2	361.3	0.89	2.37	2.66	
Corn	Pellet	--	502	2.25	97.0	134.7	187.2	361.3	0.93	2.37	2.55	
Corn	--	Fattening	624	2.26	118.4	165.1	230.2	447.5	0.46	0.78	1.70	Ohh et al., 1983
Corn	--	Fattening	877	2.25	169.5	235.4	327.0	631.2	0.45	0.80	1.78	
Corn	--	Fattening	822	2.04	230.7	297.4	383.5	637.5	0.46	0.84	1.81	
Corn	--	Fattening	1147	1.99	351.1	444.9	563.7	905.2	0.47	0.91	1.92	
Sorghum	--	Fattening	539	2.10	136.1	179.2	236.0	409.3	0.44	0.78	1.78	
Sorghum	--	Fattening	722	2.07	192.3	250.5	326.4	554.1	0.45	0.81	1.79	
Sorghum	--	Fattening	885	1.81	367.1	437.7	521.9	742.2	0.45	0.87	1.92	
Sorghum	--	Fattening	1217	1.74	565.2	658.9	768.1	1043.9	0.43	0.83	1.94	

dgw: geometric mean diameter; sgw: geometric standard deviation of particle diameter by mass; AM: Arithmetic mean; MSD: Mean surface diameter; MVD: Mean volume diameter; M*VS*D: Mean volume-surface diameter; ADFI: average daily feed intake; FCR: feed conversion ratio, = feed (g) / gain (g).

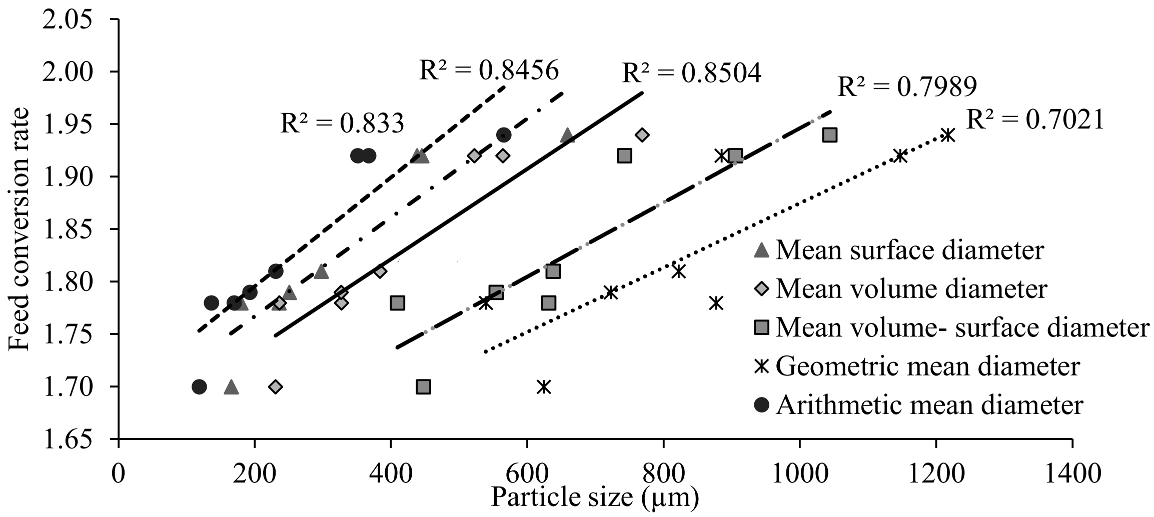

Fig. 2.8 Effect of differently expressed feed particle size on the feed conversion ratio of weaned pigs and fitted linear equations (data from Ohh et al., 1983).

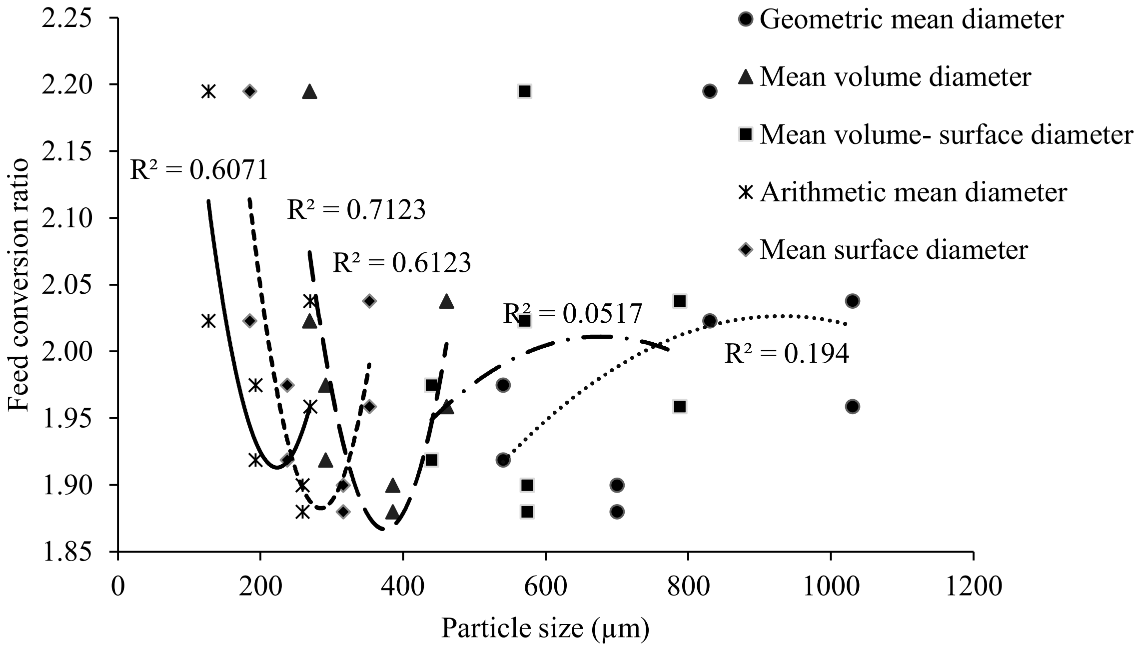

Fig. 2.9 Effect of differently expressed feed particle sizes on the feed conversion ratio of fattening pigs and fitted polynomial regression equations (data from Al-Rabadi et al., 2017).

However, similar results for EPS were not obtained from the data of Al-Rabadi et al. (2017). When data of Al-Rabadi et al. (2017) and data of Ohh et al. (1983) are compared, relevance between particle size and FCR cannot be observed with the expression of GMD or arithmetic mean, mean volume diameter and mean volume surface diameter ($P > 0.05$). However, when particle size was expressed as mean surface diameter, particle size had a significant influence on FCR ($P < 0.05$). Expressions of particle size with arithmetic mean, mean surface diameter and mean volume diameter fit the FCR better than GMD in a 2nd degree polynomial regression model with the r^2 values ranging from 0.61 to 0.71. The trend of the fitted lines is different: when particle size was expressed with arithmetic mean, mean surface diameter and mean volume diameter, FCR decreased and then increased with increasing particle size. However, when particle size was expressed with GMD and mean volume-surface diameter, FCR increased to a plateau with increasing particle size. This indicates that different methods of expressing particle size may lead to different conclusions.

These two examples indicate that some of these EPS expressions might be superior in explaining pig performance to GMD, but not in all cases. The variation of different study results should be accounted for in studies relating pig performance to particle size. The variation of results based on the different plots led to the unsuccessful construction of a mathematical model. It becomes clear that different expressions of particle size differ when related to pig performance, and directly measured EPS data contrary to recalculated data have a preference in further research.

2.5 Prediction of output PSD in milling

Grinding theory is supposed to provide design concepts and equations that can help engineers to improve milling systems, so that the required PSD can be obtained efficiently (Austin, 1971). A better understanding of breaking behaviour of ingredients in different milling systems is quite important in developing grinding theories. Breakage functions are mathematical formulas that describe the breakage of material and can be included in particle size prediction models. As a result, suggestions on milling operations can be given , which may further optimize the grinding process leading to a lower energy consumption and higher yield of particles in the desired size class.

2.5.1 Principle calculation of breakage functions

Breakage functions are mathematical functions for describing particles breakage behaviour during the comminution process, which can provide information on milling system design and grinding efficiency (Singh et al., 2016). Breakage functions can be defined as B (y, x), which is the mass fraction of breakage products from size x that fall below size y after grinding during a certain time ($x \geq y$). There have been many different forms of breakage equations developed at different milling conditions as shown in Table 2.3. Although different symbolism and mathematical techniques were used, the basic concept is generally the same: during the comminution process, the mass formula may be written as:

'Retained mass in a size interval = [original mass] - [mass broken into downsize interval] + [mass created from size interval above]'.

For example, consider a batch of material to be ground, which can be divided into 5 classes, named x_1, x_2, x_3, x_4 and x_5, then the cumulative PSD of the material can be illustrated as shown in Figure 2.10. Each class has input of material coming from the class of larger sized particles and output of material into smaller sized classes during the grinding process (except the largest particle size class without input and final smallest size class without output). After grinding for some time, some material in class x_1 is selected and ground into x_2, x_3, x_4 and x_5. At the same time, a fraction of particles in class x_2 is also broken into class x_3, x_4 and x_5, along with some material is added into class x_2. The same logic can be applied to the materials of size x_3, x_4 and x_5.

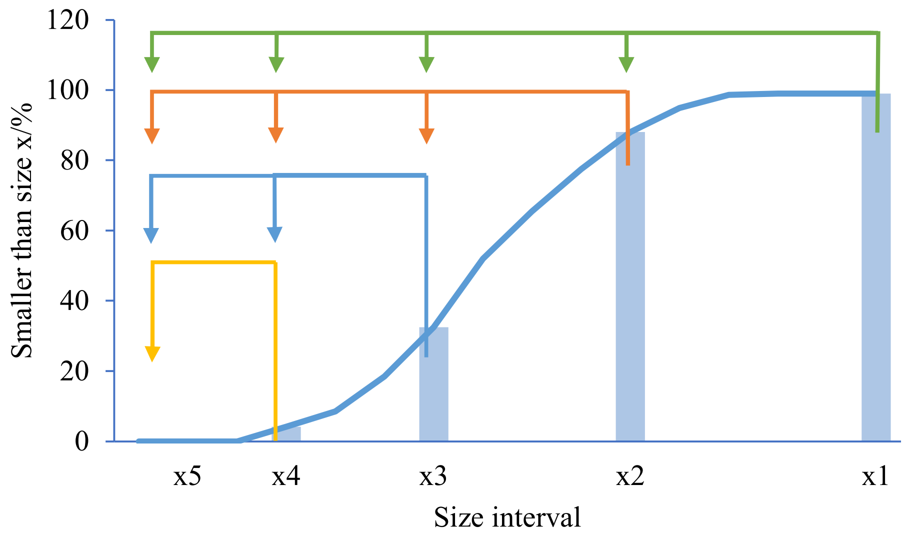

Fig. 2.10 Schematic diagram of illustrating the first-class breakage process.

Table 2.3 Forms of breakage functions and prediction models under different grinding conditions.

Conditions and forms	Property	Formulas	Parameters	References
Cumulative form. Assume that particles of every size break in the same way in every process.	Breakage function	$B_{x,y} = \frac{1-e^{-\frac{x}{y}}}{1-e^{-1}}$	$\frac{x}{y}$ = the reduction ratio.	a,b,c
Fitting breakage function. Material characteristics were included, like hardness, moisture level, kernel shape and kernel size.	Breakage function	$B\left(x,\frac{G}{D}\right) = a_0 + b_0x + c_0x^2 + d_0x^3 + (a_1 + b_1x + c_1x^2 + d_1x^3)\left(\frac{D}{G}\right) + (a_2 + b_2 + c_2x^2 + d_2x^3)\left(\frac{D}{G}\right)^2$	a_i, b_i, c_i and d_i = fitted coefficients, such as hardness, moisture level, kernel shape and kernel size; $\frac{D}{G}$ = milling ratio; G = roll gap; D = size of wheat kernel; x = particle size.	d
Cumulative form. Feed and product PSD change with time. For steady state continuous grinding in retention mills.	Prediction model	$\frac{\partial P(x,t)}{\partial t} = \int_{y=x}^{x_{max}} S(y)B(x,y)\rho_M(y,t)dy$	y = Input particle size; x = output particle size; $S(y)$ = selection function; $B(x,y)$ = breakage function; $\rho_M(y,t)$ = PSD in the mill at time t.	e
Cumulative form. Feed and product PSD do not change with time. For steady state continuous grinding in retention mills.	Prediction model	$P(x) = F(x) + \tau \int_{y=x}^{x_{max}} S(y)B(x,y)\rho_M(y)dy$	$F(x)$ = fraction of feed material of size less than x; τ = the average residence time in the mill; $\rho_M(y)$ = PSD in the mill.	e
Cumulative form. For once-through grinder with no retention.	Prediction model	$P(x) = F(x) + \int_{y=x}^{x_{max}} s(y)B(x,y)\rho_1(y)dy$	$P(x)$ = the amount smaller than x in the feed before grinding; $\rho_1(y)$ = input PSD.	e
Matrix form. Discrete time and size interval.	Prediction model	$P = M \times F$ Where: $M = BS + I - S$	P = product vector; F = feed vector; B = breakage matrix; S = selection matrix; I = unit matrix; M = milling matrix.	e
Matrix form. Recycle is included by means of classifier.	Prediction model	$P2 = [M \times I - (I - C)]^{-1} \times F \times C$	P2 = product vector; C = classification matrix.	f

a, b,c,: Broadbent and Callcott (1956a, 1956b, 1957); d: Fang and Campbell 92003a); e: Austin (1971); f: Holdich (2002).

In grinding, a certain rate of the material is selected and engaged in breakage, which can be described by a (mathematical) selection function. Particles selected for grinding from a certain size class depend on raw material characteristics, grinding time, as well as properties and geometry of the mill. In the batch processing, the material is kept in the mill until everything is ground; for continuous grinding process, the material flows continuously, and therefore, grinding time and properties are related to the selected particles. This means for instance there is a difference between batch processing and continuous grinding. However, it is difficult to determine the selection functions in practical feed manufacturing. Usually in practice the selection function is assumed to be equal to one and is combined with the breakage functions.

When the ground material can be distributed into *n* grades, and when we take b_{ij} as mass fraction of material entering into grade *j* from grade *i* ($i > j$), then the mass fraction retained on all grades originating from size x_i can be expressed as:

$$b_{ij} = B_{(x_i, x_j)} - B_{(x_{i+1}, x_j)} \tag{11}$$

It should be noticed that:

$$\sum_{i=j}^{n} b_{ij} = 1 \tag{12}$$

If the ground product is taken as *p*, and mill feed (material to be ground) as *f*, then the mass flows entering all the other sized fraction into grade *j* will be:

$$p_i = b_{ij} f_i \tag{13}$$

Then it is possible to present these equations in matrix form (take 5 classes as example):

$$\begin{bmatrix} p_1 \\ p_2 \\ p_3 \\ p_4 \\ p_5 \end{bmatrix} = \begin{bmatrix} b_{11} & 0 & 0 & 0 & 0 \\ b_{21} & b_{22} & 0 & 0 & 0 \\ b_{31} & b_{32} & b_{33} & 0 & 0 \\ b_{41} & b_{42} & b_{43} & b_{44} & 0 \\ b_{51} & b_{52} & b_{53} & b_{45} & b_{55} \end{bmatrix} \times \begin{bmatrix} f_1 \\ f_2 \\ f_3 \\ f_4 \\ f_5 \end{bmatrix} \tag{14}$$

The matrix (14) can be expressed in short as:

$$P = B \times F \tag{15}$$

where: P= product vector; B= breakage matrix; F= feed vector.

This matrix is referred to as the basic breakage matrix, which is considered sufficient for milling application (Campbell and Webb, 2001). It was built based on assumption that the selection function equals one, which means all the material is considered involving into comminution process. However, when the selection function is taken into account, the ground product vector may be illustrated with following formula:

'Product = [selected breakage material] + [non selected breakage material]'

Where the ground product vector (P) can be written as:

$$P = [B \times S \times F] + [(I - S) \times F] \tag{16}$$

in which: S= selection matrix; I=unit matrix.

Selection matrix (equation 17) is shown below: it's units depend on the layout of the process and can be expressed in (fractional) rate of degradation per second (s^{-1}) or as a function of some machine parameter (e.g. the number of revolutions of a mill).

$$\begin{bmatrix} s_{11} & 0 & 0 & 0 & 0 \\ 0 & s_{22} & 0 & 0 & 0 \\ 0 & 0 & s_{33} & 0 & 0 \\ 0 & 0 & 0 & s_{44} & 0 \\ 0 & 0 & 0 & 0 & s_{55} \end{bmatrix} \tag{17}$$

The basic breakage matrix (equations 14 and 15) is describing a once-through grinding process ('open circuit') without classification, like roller-milling or hammer-milling in the feed milling operation. While in practice, feed materials are hardly broken into the desired sizes within a single pass which, therefore means a classifier is needed to bring the oversized material back to the mill and remove undersized material. This process ('closed circuit grinding') is illustrated in Figure 2.11 (Holdich, 2002): fresh feed material (F) is milled into product $P1$, after classification, the desired sized product $P2$ comes out, while the oversized particles ($P1$-$P2$) are recycled into feeder, and together with the fresh feed material (which is k) are ground again, until desired products are obtained.

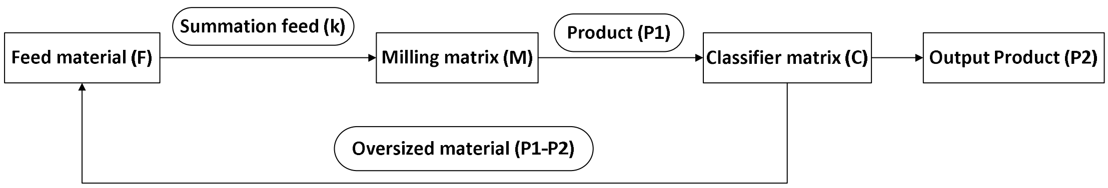

Fig. 2.11 Flow chart of the grinding process including classifier and recycled streams.

The classifier matrix is shown below:

$$C = \begin{bmatrix} c_{11} & 0 & 0 & 0 & 0 \\ 0 & c_{22} & 0 & 0 & 0 \\ 0 & 0 & c_{33} & 0 & 0 \\ 0 & 0 & 0 & c_{44} & 0 \\ 0 & 0 & 0 & 0 & c_{55} \end{bmatrix} \tag{18}$$

From Fig. 2.11 referred to the various flows, we know that the output product *P2* is:

$$P2 = P1 \times C \tag{19}$$

The milling product *P1* is:

$$P1 = M \times k \tag{20}$$

And the material to recycled is:

$$P1 - P2 = (I - C) \times P1 \tag{21}$$

Then the mass (k) flows into the mill which is the summation of feed material and recycled material:

$$k = F + (I - C) \times P1 = F + (I - C) \times M \times k \quad (22)$$

Therefore, k can be presented as:

$$k = [I - (I - C) \times M]^{-1} \times F \quad (23)$$

Then combine equation (19) - (23), it is possible to provide the recycled product:

$$P2 = [I - (I - C) \times M]^{-1} \times F \times C \quad (24)$$

The above equations can be used for all grinding processes, however, its accuracy will differ between the type of machines, since materials will show a different breaking behaviour in different machines. For example, in the roller mill, particles are ground independently, while in the hammer mill, there are interactions among particles during grinding. Therefore, selection of particles for grinding within the mill as well as the total grinding time must be considered in order to optimize the grinding efficacy of the milling operation. Developing the breakage equations and turning them into breakage matrices greatly improves understanding of certain milling systems and the raw materials that are comminuted by those systems. However, having determined the breakage matrix of a product and machine, this matrix can only be used for these products/machine combinations specifically. This makes this method very inflexible. A breakage matrix determined for one set of grinding conditions cannot be instantly used for another set of conditions. Since diet ingredients in animal feeds may differ in their nutrient level, size, moisture content, hardness etc., this is not desirable. Breakage functions have been widely discussed in the mining and coal industry comminution process. However, in feed manufacturing practices, to the best knowledge of authors, the breakage function was only developed for the combination wheat/roller mill by the research group of Campbell (Campbell and Webb, 2001; Campbell et al., 2001; 2007; 2012; Fang and Campbell, 2003a; 2003b; Mateos-Salvador et al., 2011) and Fistes and Tanovic (2006).

2.5.2 Development of breakage functions in milling wheat

Campbell and Webb (2001) investigated the discretized form of breakage equation in the roller mill with wheat and applied the equations to predict the output PSD that covered different size ranges. The results showed a high agreement between predicted and experimental data. In addition, the research group of Campbell also explored the factors that may influence the breakage pattern of individual particles. They showed that the breakage pattern for wheat depends heavily on chemical, structural and physical characteristics of the grain, such as moisture level (Fang and Campbell, 2003b), endosperm hardness and shape (Campbell et al.,

2007) and density (Galindez Najera, 2014). Fistes and Tanovic (2006) found that breakage equation can be used not only to predict output PSD, but also to predict the ash and protein content of wheat over various particle sizes. Furthermore, technical mill parameters affect the breakage pattern, thus the effects of roll gap (Campbell et al., 2001), roll disposition (Fang and Campbell 2003a), roll velocity, roll surface, and ratio of roll gap to kernel size were investigated (Campbell et al., 2001).

As Campbell and Webb described in their study, a single setting cannot be used all year round to mill a certain product. Therefore, it is needed to develop a breakage function which considers the characteristics of the whole and milled product and the traits of the milling machine. Consequently, some conditions in the breakage equation were considered, which include the concept of normalization, selection and first-order law of breakage (Austin, 1971; Campbell and Webb, 2001). This led to the involvement of the cumulative breakage function in the breakage equation, ensuring that the different circumstances of input material and machine are covered.

2.5.3 Development of cumulative breakage function in milling wheat

Cumulative breakage functions are currently investigated to be employed in the breakage equations of wheat (Galindez-Najera et al., 2016). Fang and Campbell et al. (2003a) fitted many different characteristics of wheat (e.g. hardness, moisture level, kernel shape and kernel size) into cumulative breakage function (see Table 2.3). The incorporation of wheat kernel characteristics and milling process parameters in the breakage function made it flexible in predicting output PSD, but this function required inordinate experimental data. This major drawback led to the development of the Normalised Kumaraswamy Breakage Function (NKBF), which is a more practical function (Mateo-Salvador et al., 2011). This method involves less factors to be determined, however, less accurate results were obtained, but such decrease in accuracy was considered to be acceptable (Campbell et al., 2012). Therefore, NKBF is thought to be a quicker method in predicting PSD output (Mateos-Salvador et al., 2011). Further development led to the Double Normalised Kumaraswamy Breakage Function (DNKBF), which describes two types of breaking behaviour). Type 1 describes a narrow range of medium sized particles, whilst the type 2 describes more the extremes of the PSD: the larger and smaller particles in the distribution (Campbell et al., 2012). Eventually the DNKBF is used to give the composition of the broken particles into four major wheat components: pericarp, aleurone, endosperm and germ (Galindez-Najera, 2014) and the predictive power for wheat is good (Galindez-Najera et al., 2016). Both types of breakage equations - compositional breakage equations - make it possible to mill anatomical parts of cereals to a desired size, which is helpful in exploring the relations between particle size and nutrient composition of a feed ingredient. A complementary benefit from developing the two breakage equations is that it gives insight in the variation between milling conditions and product.

2.5.4 Breakage functions for other raw materials and mills

As illustrated above, many researches were done with respect to the breakage of wheat using a roller mill. Jindal and Austin (1976) also investigated breakage functions for hammer mills. This research was executed by using an experimental mill, which differed from a commercial hammer mill, but this kind of experimental trials may help to identify factors that are of influence on the kinetics of hammer-milling. Specific development of breakage functions, such as done for wheat with roller milling, should be done for other feed materials in both roller mills, hammers mills or a multicracker system, with many characteristics (e.g. moisture contents, hardness, shape) of different ingredients being considered.

2.6 Particle size reduction in downstream processing

As shown above, reducing particle size of diet ingredients can bring many benefits. In order to further increase the availability and digestibility of nutrients, thermal treatments (pelleting, extrusion or expander treatment) are often involved as feed processing technologies. Therefore, the effects of particle size reduction in the downstream feed manufacturing processes should also be considered.

There are some researches indicating that the pelleting process can even out the PSD of feed (Amerah et al., 2007; Abdollahi et al., 2011). In Fig. 2.12, particle size of fine and coarse diet were determined before and after pelleting (Engberg et al., 2002). It can be observed that in the fine diet, the percentage of small particles (< 0.5 mm) increased, and the fraction of large particles (> 0.5 mm) decreased after pelleting. In the coarse diet, a similar trend was observed. The gap between rolls and die in a pellet press was considered to lead to further comminution of feed particles (Svihus et al., 2004), and the reduction of particle size was stronger when the diet was conditioned at a lower temperature (Abdollahi et al., 2011). This might be because a high temperature brings more moisture from steam which can decrease the frictional force in the die. In addition, the combination of more moisture and a higher temperature moves the amorphic polymeric materials through the glass-transition, turning the physical characteristics of the materials from brittle and hard into rubber and leathery (Roos and Karel, 1991). Dirkzwager et al. (1998) determined the PSD of mash feed and pelleted feed by wet sieving and observed that the fraction of fine particles (< 0.6 mm) increased while the fraction of coarse particles (> 0.6 mm) was reduced. Grosse Liesner et al. (2009) also found that PSD changed after pelleting especially the fraction of particles smaller than 0.4 mm, which increased by 27% and 37% for finely and coarsely ground diet, respectively. This was in line with the research of Vukmirović et al. (2016), who found that the fraction of small particles (< 0.125 mm) was increased significantly after pelleting, while the number of large particles (> 2.5 mm) was decreased.

Particle size reduction during pelleting also gave some reasonable explanation about differences in animal performance. According to the research results of Engberg et al. (2002), the influence of feed form on broilers growth performance was larger than that of grinding the feed, which may have been due to the particle size reduction as a result of pelleting. Nemechek et al. (2016) found that feed efficiency for finishing pig was increased as particle size of corn was decreased in mash diet, while this trend was not observed in feeding pigs a pelleted diet. The authors concluded that this was because particle size reduction occurred during the pelleting process due to the force and pressure from the pellet mill die.

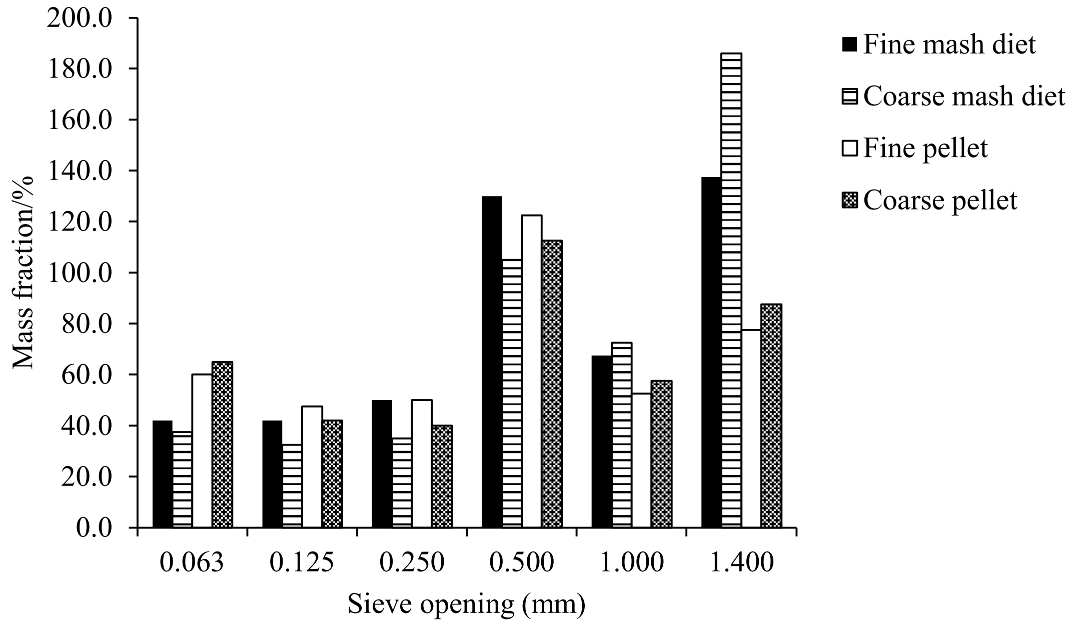

Fig. 2.12 Particle size distribution of mash diet and pellet diet with fine and coarse grinding (Engberg et al., 2002).

Extrusion and expander technology can also be involved in the feed processing for pigs starter diets to increase the palatability and nutrient availability. High temperature steam is then added in this process to reach a higher moisture content (20~30%) and temperature (up to 130°C) of diets which cannot be realized in pelleting (van der Poel et al., 2018). High friction and shear forces, heat and water in the expander contribute to a more rapid gelatinisation of starch from the combination of shear between screw and barrel, which can be considered as a secondary grinding; as a result this leads to particle size reduction. If a pellet form is required, then the grinding intensity can be lower (Vukmirović et al., 2017). That is also why a pellet mill die usually can be 50% thinner if the material is conditioned in an expander prior to pelleting (Lucht, 2007).

2.7 Conclusions and perspectives

Reducing the energy consumption during grinding, whilst obtaining an optimal PSD for pigs is one of the key objectives in a feed milling operation. Roller mill, hammer mill and the multicracker all have different advantages and drawbacks and have been reviewed in this article. In addition, new configurations and systems can be used, like multi-stage grinding. However, only limited research data is available for these two technologies, and further research in comparing these configurations and systems in terms of PSD and energy utilization should be carried out.

Dry sieving is the most used method in pig feed research regarding particle size determination. Wet sieving, laser diffraction, microscopy or statistic/dynamic image analysis are further methods for determining particle size to explore additional characteristics of particles like volume, surface area. As a result, better suggestions about the efficacy of milling operations may be provided. However, no research has been done to address such possibilities. For example, the use of EPS along with span of PSD, D_{v50}, and Sauter mean may be alternative ways to indicate size characteristics. Yet, it is not clear how these expressions relate to pig performance, neither it is conclusive if the equivalent expressions are a better predictor of technical performance in comparison to the GMD. For further investigation, it is suggested to obtain original data and relate these data to young animals, because more significant effects of particle size on young pig growth performance are expected compared to those of adult livestock. As reported by some papers, surface area can be an interesting parameter to be considered in the further research.

Breakage functions are applicable in predicting the output PSD. The development of breakage equations for the first milling of wheat with a roller mill has been done, and many factors of milling operation and material characteristics then can be considered. This type of research can be extended to predict the output PSD using hammer mill to grind ingredients for pig diets. Factors that may affect the milling of feed ingredients such as grinding time, feeder capacity and material characteristics will lead to a better understanding of breaking behaviour of specific feed ingredients in the hammer mill. Therefore, a better-defined PSD of feed can be realized with a higher accuracy: the development of breakage functions is necessary to increase the accuracy of prediction and to predict output PSD of different feed materials under several different grinding conditions in different types of mills. Whether the breakage function can be used to predict defined PSD for animal performance is a further issue that can be examined in the future. To ensure a certain PSD in animal feeds, the use of breakage functions shows a promising application.

After grinding and mixing, material is processed by pelleting, expander treatment or extrusion. Particle size reduction also takes place in these downstream processes, which is considered a possible reason for pigs performance differences in this kind of studies. Pelleting can decrease the large particle size fraction, so in order to achieve a defined PSD for pigs, particle size changes as a result of the pelleting process should not be ignored. However, there are few researches being done which show how expander treatment and extrusion affect the PSD of diets. This can be a direction for further research into the particle size distribution in animal feed processing technology.

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Chapter 3 **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 circularity ($R^2 = 0.608$) 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.

Keywords: particle size; nutrient content; *in vitro* digestibility; maize; soybean meal; pig

3.1 Introduction

Feed and feed ingredients particle size are important in both feed manufacturing and the nutrition of pigs (Laurinen et al., 2000; Fastinger and Mahan, 2003; Huang et al., 2019; 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 characteristics. 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 (Sunderberg et al., 1995a,b;

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.

3.2 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.

3.2.1 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 1,500 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 particle-size 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.

3.2.2 Additionally grinding 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., 2018). For this reason, unsieved maize and SBM as well as the samples with a GMD larger than 1.0 mm, were additionally ground 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.

3.2.3 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):

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

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

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

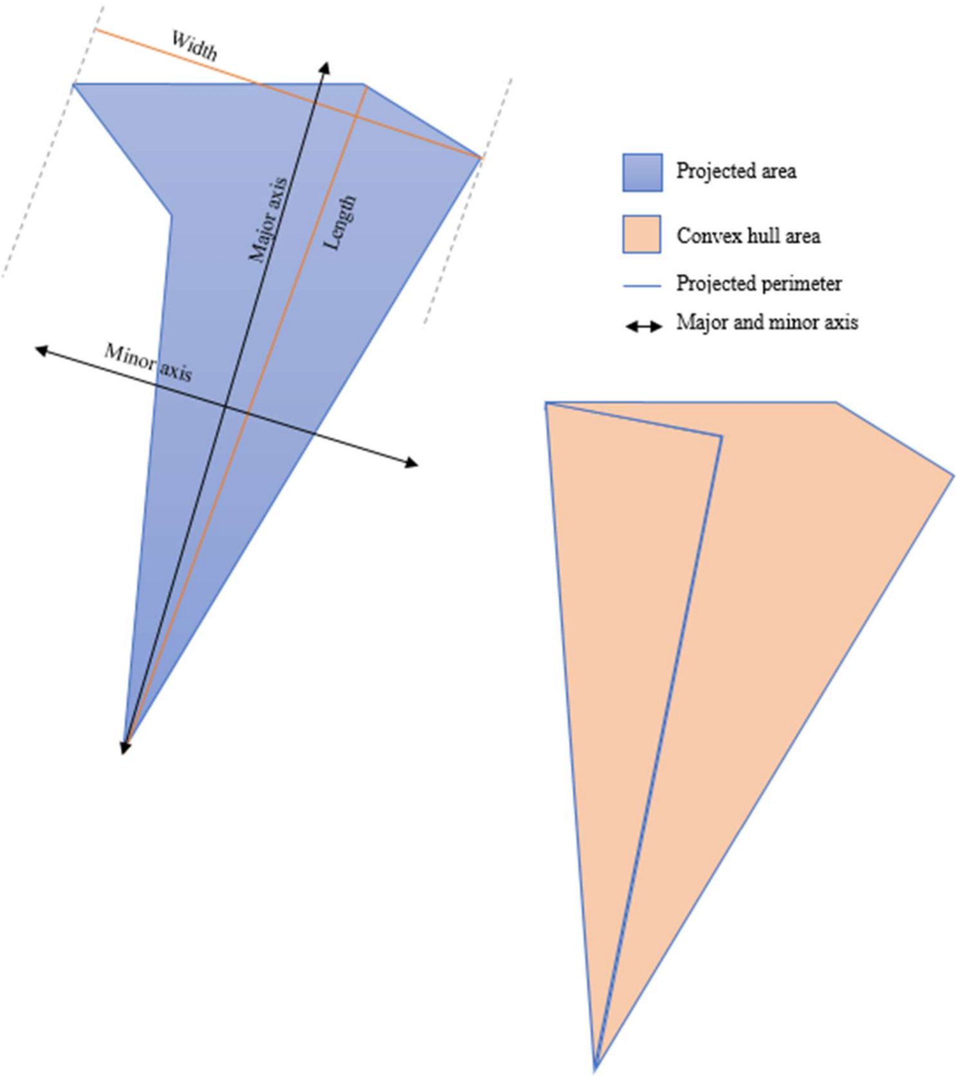
$$d_{v-s} = \frac{\sum_{i=1}^{n}\left(\overline{d_i}^3 \times w_i\right)}{\sum_{i=1}^{n}\left(\overline{d_i}^2 \times w_i\right)} \tag{5}$$

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

where w_i = mass on i^{th} sieve in g, n = number of sieves +1 (pan), d_{ari} = arithmetic mean diameter in mm, d_s = surface mean diameter in mm, d_v = volume mean diameter in mm, $d_{v\text{-}s}$ = volume-to-surface mean diameter in mm, and d_W = weighted mean diameter in mm. The calculated mean values as given by equations 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 ImageJ (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 particle sizes, 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 (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. 3.1.



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 = $\frac{projected\ area}{convex\ area}$

Fig. 3.1 Illustration of particle physical characteristics.

3.2.4 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 to 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).

3.2.5 *In vitro* digestibility

The *in vitro* digestion of OM (in both maize and SBM), CP (in SBM) and starch (in maize) were 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 °C 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 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.

3.2.6 Statistical analysis

R (3.6.1) was used to analyze the data (R core team, 2019). Nutrient content of 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-milling run 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 of 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).

3.3 Results

3.3.1 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. 3.2) 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 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 3.1). 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 3.1).

3.3.2 Physical characteristics of different size fractions

The various measurements of the shape of particles using image analysis (Table 3.2) 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, 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 fractions (0.972 *vs* 0.908).

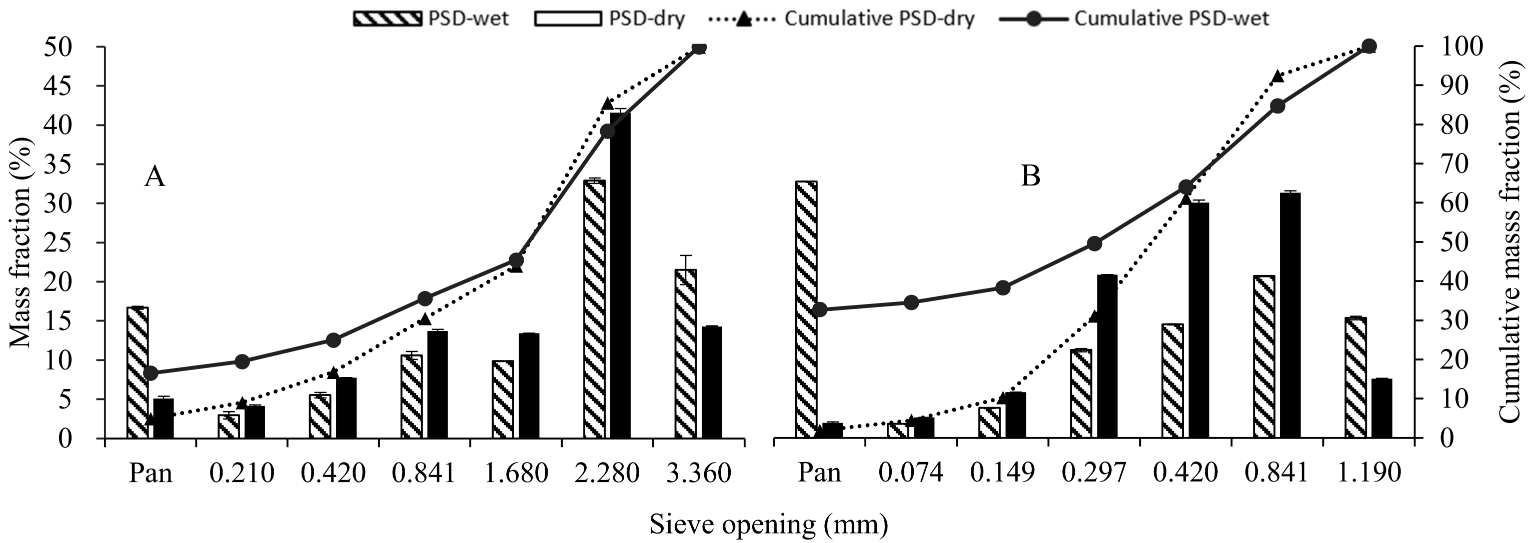


Fig. 3.2 Mass and cumulative mass distribution (dry base) of the dry and wet sieving of hammer milled maize (A) and soybean meal (B). Error bars represent standard deviations.

Table 3.1 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

* Significantly different ($P < 0.05$) to corresponding wet sieving value.

SEM: standard error of the mean.

Table 3.2 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 (µm2)	Projected perimeter (µm)	Circularity	Aspect ratio	Roundness	Solidity
Maize	0.0*	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*	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 of particles were analysed by Morphology 4 and remaining fractions were analysed with microscope and imageJ software.

3.3.3 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 3.3). 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.

Table 3.3 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.
† 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.
- not determined.

Table 3.4 Relationships between *in vitro* digestibility coefficient of organic matter and starch in maize and of organic matter and crude protein in soybean meal with the nutrient composition (g/kg DM) 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

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

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 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 3.4. 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 ($P < 0.05$).

3.4 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.

3.4.1 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 swell in water and 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. (1995), 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.

3.4.2 Characteristics of fractionated particles

Breaking behaviour 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).

3.4.3 Nutrient composition in different particle size fractions

The nutrient composition among particle size fractions has been previously reported for barley (Sunderberg et al., 1995a,b), sorghum (Al-Rabadi et al., 2009; 2012), rice grains (De la Hera et al., 2013) and peas (Maaroufi et al., 2000) but no data exist 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., 1995a,b; Lawrence et al., 2003; Ball et al., 2015; Huang et al., 2015; Rojas and Stein, 2015; Shi et al., 2017). The sieving of 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. Sunderberg et al. (1995a,b) 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. De la Hera et al. (2013) hammer milled (200 µm screen size) two types of rice grains and found the CP and amylose/amylopectin ratio to be affected by the particle size heterogeneity. The lowest CP content was observed in the finest particle size fraction, which agrees with the present study for maize and SBM, although Maaroufi et al. (2000) found that the finest fraction of hammer milled peas contained the highest CP content. 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 3.3) 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 behaviour 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 3.3).

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 3.3). 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) and 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) assay, are provided. For hammer milled maize, Table 3.5 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. 3.2). 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 3.4).

Table 3.5 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.

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., 1995). 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 3.6) 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. 3.2). 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).

Table 3.6 *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 + lab milled
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

3.5 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.

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Chapter 4 **Particle size distribution, energy consumption, nutrient composition and *in vitro* digestion characteristics of hammer milled maize and soybean meal as affected by moisture content**

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Abstract

Grinding is an important feed processing technology, determining physical and nutritional characteristics of ground materials, which affects nutrient digestion in animals and their growth performance. This study aimed to clarify if differences in moisture content (MC) lead to differences in nutrient composition over various particle size fractions after grinding that have potential to affect feed manufacturing characteristics or animal performance. Maize and soybean meal (SBM) with targeted MC of 120, 140 and 160 g/kg (adding no (0), 30 and 60 g/kg of tap water, respectively) were hammer milled and the physical and chemical characteristics as well as *in vitro* digestibility of particle size fractions were determined. The mill was fitted with a 6-mm (maize) or 2-mm (SBM) sized screen, with milled material subsequently separated by dry sieving (size ranging from < 0.075 to > 3.36 mm) and each fraction was analyzed for its nutrient composition, morphology characteristics and *in vitro* organic matter (OM) and crude protein (CP) digestibility. For the latter assay, specific particle size fractions were additionally ground using a laboratory mill (1 mm screen). The results showed that geometric mean particle size and energy consumption increased with increasing MC. Chemical composition, physical characteristics and *in vitro* digestibility of particle size fractions were significantly different ($P < 0.001$). Overall, MC had a limited effect with the interaction between particle size fraction and MC significantly affecting all nutritional parameters and projected area, projected perimeter, solidity for both ingredients and circularity for maize. Physical characteristics of particles, especially particle size affected *in vitro* digestibility of OM and CP most. Additional grinding of samples before determination of *in vitro* digestibility increased the OM digestibility by up to 0.684 in maize ($P < 0.001$). Additional grinding of particles larger than 595 µm increased *in vitro* digestibility of OM and CP. In summary, increasing MC has limited effect on the breakage behaviour of maize and SBM, but increased energy consumption during hammer-milling. *In vitro* digestibility measurement of fractionated particles by the Boisen and Fernández (1995; 1997) appears to require material should be ground to pass a 0.595 mm sieve rather than the prescribed 1 mm.

Keywords: grinding behaviour; particle size; nutrient content; *in vitro* digestion; maize; soybean meal

4.1 Introduction

Grinding performance is important in feed processing as it not only significantly contributes to the feed processing costs (energy consumption), but more importantly determines the physical and nutritional characteristics of the final ground material (Jagtap et al., 2008), including gastro-intestinal digestibility and kinetics of nutrient absorption. There are many factors affecting grinding performance including those intrinsic to the material to be ground (e.g. initial particle size, moisture content (MC), material properties) and those related to the equipment used in grinding (e.g. sieve opening, (tip) speed of hammers, feed rate settings, geometry of the grinding chamber with respect to position and number of breaker plates) (Chen et al., 1999; Mani et al., 2004; El-Shal et al., 2010; Yancey et al., 2013; Gil and Arauzo, 2014; Guo et al., 2016; Mugabi et al., 2017). Moisture content plays an important role and can affect various intrinsic properties of ingredients such as strength, stiffness, elasticity and plasticity (Jung et al., 2018) which, in turn, influences breaking behaviour and energy consumption (Jagtap et al., 2008) during grinding of feed raw materials. In general, a low MC of ingredients results in a lower energy consumption and a smaller mean particle size after grinding (Velu et al., 2006; Doblado-Maldonado et al., 2013; Lee et al., 2013) as these ingredients are usually more brittle and easier to grind (Grochowicz and Andrejko, 2006; Dabbour et al., 2015). In this respect, investigation into particle size distribution (PSD) besides the single estimate of a mean particle size of a ground ingredient is important, as differences in uniformity of PSD could lead to differences in animal growth performance even though the mean particle size after grinding is similar for ingredients (Wondra, 1995; Lyu et al., 2020). Within PSD, materials belonging to different size classes showed differences in nutrient content and physical characteristics after grinding for peas, barley, sorghum, maize and soybean meal (SBM) (Sundberg et al., 1995a,b; Maaroufi et al., 2000; Al-Rabadi, 2013; Lyu et. al, 2021). Larger particles tend to have a higher crude protein (CP) content and lower circularity in hammer milled maize and SBM (Lyu et al., 2021), while for peas, larger particles contain a lower starch content (Maaroufi et al., 2000). When samples are milled and subsequently fractionated (by sieving or air classification), the *in vitro* digestibility of nutrients in pea (Maaroufi et al., 2009), maize and SBM (Lyu et al., 2021), and digestion rate of starch in barley and sorghum (Al-Rabadi et al., 2009) are affected, with smaller particles show a higher digestibility and starch digestion rate.

Moisture content has been shown (Moon and Yoon, 2018) to affect PSD. The latter authors found that coarser particles make up a smaller proportion of the ground material when the initial MC is low. Lee et al. (2013) also found that size fractionated SBM powders with different MC showed different grinding patterns at a given grinding time. These observations have potential for designing grinding facilities or developing grinding strategies to produce higher-digestibility feeds and improve animal growth performance utilizing tailor-made selection of fractions. The influence of MC on physical characteristics (e.g. particle shape information) and nutrient content of different maize and SBM particle size fractions have not been investigated. Such study could provide more information on the use of moisture as a control measure when developing novel feed mill and grinding strategies. In addition, further understanding of the breaking behaviour of ingredients could be obtained from both physical characteristics and chemical composition data.

The digestibility of ground ingredients and feeds is one of the primary criteria in animal nutrition to obtain an efficient and sustainable animal production. High digestibility values mean less waste and a higher nutrient utilization from ingredients/feed. *In vitro* digestion simulation assays are a cost and time saving technology and widely used to provide an indication of *in vivo* digestibility of nutrients of ingredients/feeds for a variety of animal species (Jobling and Sumpter, 1993; Theodorou et al., 1994; Boison and Fernandez 1995; 1997). Recently, Lyu et al. (2021) unexpectedly observed that the routinely used Boisen and Fernández (1997) assay to determine *in vitro* digestibility of organic matter (OM) was affected by the size of maize and SBM particles although the developers of the assay reported minor differences (-1.4 to -0.4 % units) between ingredients ground over a 1- and 3-mm sieve.

In the current study, the effect of MC on the physical characteristics and nutritional composition as well as *in vitro* digestibility of hammer milled and sieve-fractionated maize and SBM particles was investigated. Moisture-treated and -untreated maize and SBM were hammer milled and energy consumption, PSD, geometric mean diameter (GMD) of particles and geometric standard deviation (GSD) measured while after sieving, the nutrient composition and physical characteristics of particles from different size fractions were determined. In addition, in order to corroborate or contradict the preliminary observation by Lyu et al. (2021) that the *in vitro* OM and CP digestibility assay of Boisen and Fernández (1995; 1997) is affected by PSD, the effect of additional grinding of size-fractionated particles on *in vitro* digestibility of OM and CP was investigated.

4.2 Material and methods

4.2.1 Sample preparation

4.2.1.1 Material moisture adjustment

Whole maize kernels (France) and Brazilian SBM (Research Diet Service B.V., Wijk bij Duurstede, The Netherlands) originating from a single batch were divided over six, 20 kg plastic lined paper bags per ingredient. The MC of four bags per ingredient was increased by gradually adding either 0.6 (+30 g/kg, M3) or 1.2 kg (+60 g/kg, M6) tap water while the ingredient was stirred in a paddle mixer (Forberg, type F60, Larvik-Norway) for 120 s. The other two bags per ingredient were also individually stirred in the mixer for 120 s but no water was added (M0). All six bags (M0, M3 and M6, duplicates) per ingredient were individually sealed in air-tight plastic bags and kept at 4° C for at least 48 h to ensure uniform water distribution throughout the material. At least 12 h before hammer-milling, each sealed bag was acclimatized to room temperature.

4.2.1.2 Hammer-milling

Twenty kg of maize or SBM (from the same original batch) was first hammer milled (Engl hammer mill, Dongen, The Netherlands, type 30, 7.5 kW motor) to warm up the machine and to ensure all batches were ground at the same technological conditions for each ingredient. After the warming-up run, the order of milling was M0, M3 and M6 with duplicate batches following each other and thorough cleaning of the hammer mill with a vacuum cleaner between runs. For each of the three MC levels (M0, M3 and M6), the two 20 kg bags of maize and SBM were first poured into a feeding hopper of which the adjustable inlet was opened to the 80% position and fixed when the hammer mill running speed reached 1500 rpm. All batches were hammer milled at a fixed running speed of 1500 rpm, with a 6- and 2-mm screen-sized plate sieve used for maize and SBM, respectively. The choice for the sieve openings was based on the study of Lyu et al. (2021) which ensured balanced mass fractions across size classes. A data-logger (Hiflex, OPT-2-2USB485-OBUS, The Netherlands) connected to the controller was used to record various parameters (e.g. motor current, motor voltage, grinding time) every second during grinding from which effective energy consumption was calculated by subtracting idle load from the total load (kJ/kg).

4.2.1.3 Sampling and sieving

After hammer-milling, approximately 5 kg of a representative subsample was collected from each 20 kg run using the quartering and coning method (Campos-M and Campos-C, 2017) from which ~1.25 kg of subsample was collected using a multi-slot divider (Mooij-Argo, Hegelsom, the Netherlands) to determine PSD (in duplicate) using the 15-sieve method (ASABE, 2008). In this method, 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 shaking time of 6 s. Two rubber balls with a diameter of 20 mm were used as sieving aid on each sieve where the sieve opening was smaller than 300 μm. Geometric mean diameter and GSD were calculated based on the PSD according to ASABE (2008) and reported as the mean value of the duplicate samples. The remainder of the hammer milled material (~3.75 kg) was used to obtain six particle size fractions for each of the batches by additional sieving. For this purpose, five sieves were selected from the PSD determination data to yield fractions with sufficient mass for subsequent chemical and physical analysis. For maize these sieves included 3.360, 2.380, 1.680, 0.841 and 0.420 mm (+pan), and for SBM 1.190, 0.841, 0.595, 0.420 and 0.210 mm (+pan). Multiple sieving (at least 10 times) was performed to obtain sufficient material for each fraction (> 70 g) for physical/chemical analyses and *in vitro* digestion. The material on each sieve layer (+pan) from the multiple sieving was collected, pooled per treatment, thoroughly mixed and kept at -20° C until further analysis.

4.2.1.4 Additionally grinding of samples

To determine the effect of particle size on the *in vitro* OM and CP digestibility, material retained on the five sieves and collected in the pan were additionally ground at 12,000 rpm in a centrifugal mill (ZM200, Retsch GmbH, Hann, Germany) fitted with a 1.0 mm screen, as prescribed for the *in vitro* digestion analysis protocol of Boisen and Fernández (1995).

4.2.2 Physical characteristics and chemical composition analysis

Physical characteristics of hammer milled particles retained on the five sizes and collected in the pan were measured using the image analysis methods as described in Lyu et al. (2021). Briefly, multiple images were taken by a laboratory microscope combined with a digital camera (Bresser, microcam 3.0, megapixel, software version 7.2.1.7), and analysed with ImageJ (software version 1.51f) software. For the finest particles, < 420 μm (maize) or < 210 μm (SBM) microscopical resolution was insufficient to obtain clear images and for these fractions additional image analyses were conducted using a Morphologi 4 (Malvern Panalytical Ltd, Almelo, The Netherlands). The analyzed physical characteristics included projected area, projected perimeter, circularity, aspect ratio (AR), roundness and solidity. The calculations were based on the illustration as provided in Fig.3.1 of Lyu et al. (2021).

For chemical analyses, the various samples were dried in an air circulation oven at 103° C for 4 h before determination of residual dry matter (DM) content (ISO 6496, 1999) and calculation of DM. Ash content was determined after dried samples were combusted at 550° C for 3 h in a muffle furnace (ISO 5984, 2002). Neutral detergent fibre (NDF) was determined with heat-stable amylase (thermamyl) and alcalase, using the standard procedure of van Soest et al. (1991). Nitrogen (N) content was determined by the DUMAS technique (ISO 16634-1, 2008), and CP was calculated by multiplying the N content by 6.25. Starch content of maize samples was determined using an enzymic method (ISO 15914, 2004).

4.2.3 *In vitro* digestibility

The *in vitro* digestibility of both hammer milled and additionally ground samples was determined as described by Lyu et al. (2021) which is based on the method published by Boisen and Fernández (1995). Briefly, 10 g of sample was mixed with 250 m 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° C 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. Digestibility was calculated according to the difference in nutrient content before and after digestion.

4.2.4 Statistical analysis

Data on physical characteristics and nutrient composition of particle size fractions for the different MC treatments were analyzed by two-way analysis of variance using the general linear model in R 3.6.1 (R Core Team, 2019). The statistical model used was:

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

where γ_{ijk} = response variable (response variables) (k = 1 or 2, the number of measurements), μ_0 = overall mean, α_i = effect of moisture content i (i = 1..3), β_j= effect of fraction j (j = 1..6), $(\alpha \times \beta)_{ij}$ = interaction of moisture content i and fraction j and ε_{ijk} = residual error with a mean of 0 and variance σ^2. α_i and $\beta_j$were fixed effects and the minimum significance threshold was set at 0.05.

4.3 Results

4.3.1 Grinding performance

The recovery of the hammer milled maize and SBM during the determination of the PSD (14 sieves + pan) across treatments (M0, M3 and M6) ranged from 98.8 ± 0.35 to 99.9 ± 0.05%. After the addition of 30 and 60 g/kg water, the MC of maize increased from 122.7 to 144.2 and 161.8 g/kg, respectively, with the MC of SBM increasing from 117.1 to 142.7 and 164.4 g/kg (Table 4.1), respectively. Moisture content showed a significant effect on the GMD of maize with the M3 and M6 treatments (2.01 and 2.07 mm, respectively) having higher values than the M0 treatment (1.71 mm). There was no effect of MC treatment on GSD. Figure 4.1 shows the mass and cumulative mass distribution of the hammer milled maize and SBM as affected by MC. The PSD of ground maize and SBM with different MC showed different patterns, and significance was mainly observed in the middle (0.297 to 0.841 mm) and largest (1.680 SBM and 3.360 maize) particle size fractions. The cumulative mass fraction reached 50% at an approximate sieve opening of 1.68 mm for maize and 0.595 mm for SBM.

Table 4.1 Geometric mean diameter (GMD) and standard deviation (GSD) of hammer milled[1] maize and soybean with different moisture contents.

Ingredient	Moisture addition (g/kg)	Moisture content (g/kg)	Feed rate (kg/s)	GMD (mm)	GSD (mm)
	0	122.7	0.348	1.71^{b}	1.79
Maize	30	144.2 (+21.5)[2]	0.322	2.01^{ab}	1.85
	60	161.8 (+39.1)	0.320	2.07^{a}	1.77
SEM			0.007	0.077	0.019
P-value			0.260	0.043	0.253
	0	117.1	0.231	0.67^{b}	0.44
Soybean meal	30	142.7 (+25.6)	0.212	0.72^{a}	0.46
	60	164.4 (+47.3)	0.135	0.70^{ab}	0.43
SEM			0.021	0.007	0.005
P-value			0.082	0.020	0.066

[a,b] Values with different superscripts within column per ingredient are different ($P < 0.05$).
SEM: Standard error of the mean.
[1] Screen size used for maize 6 mm and soybean meal 2 mm.
[2] Values between brackets are the increase in moisture content compared to 0 moisture addition per ingredient.

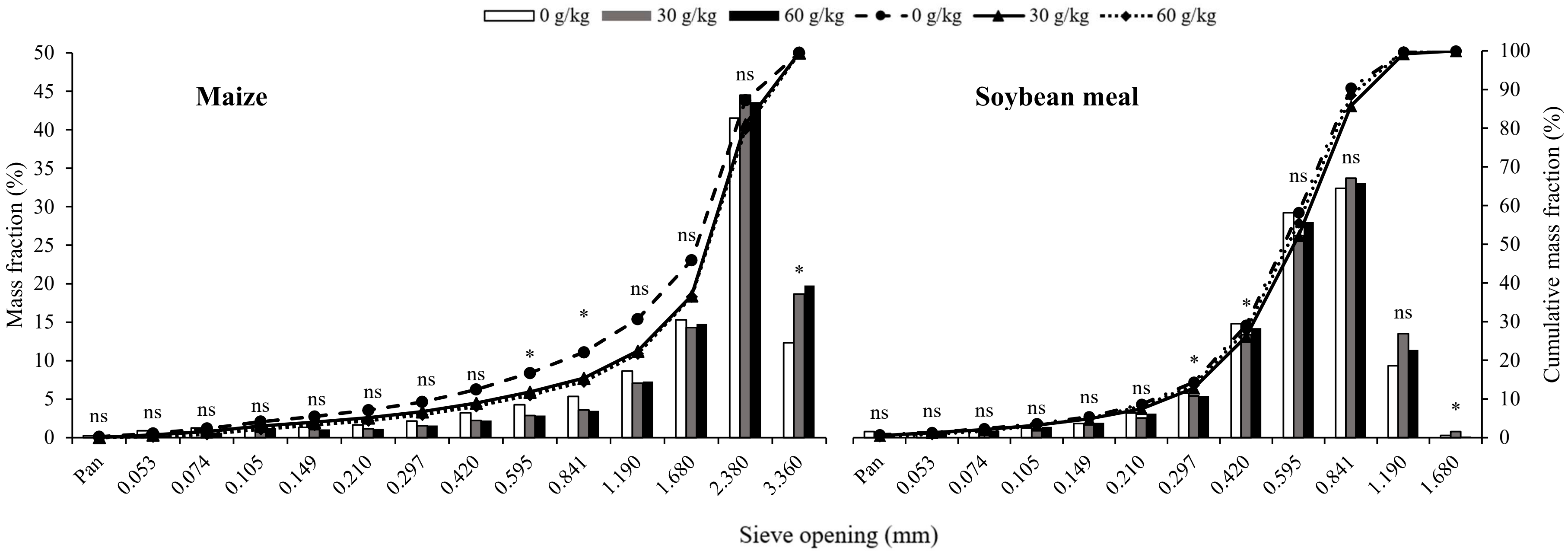


Fig. 4.1 Mass (bar) and cumulative mass (line) distribution of hammer milled maize and soybean meal as affected by no (0 g/kg), 30 and 60 g/kg moisture addition to the ingredient. *Significant (P < 0.05) different mass fractions within sieve opening, ns = not significant (P > 0.05).

Figure 4.2 reports the net energy consumption required for hammer-milling maize and SBM wuth different MC. Maize showed a linear increase in net energy consumption with all three treatments being significantly different from each other while for SBM, the M6 treatment requiring significantly more energy for milling the same mass material compared to milling M0 and M3 SBM. No significant difference was observed between M0 and M3 treatments for SBM.

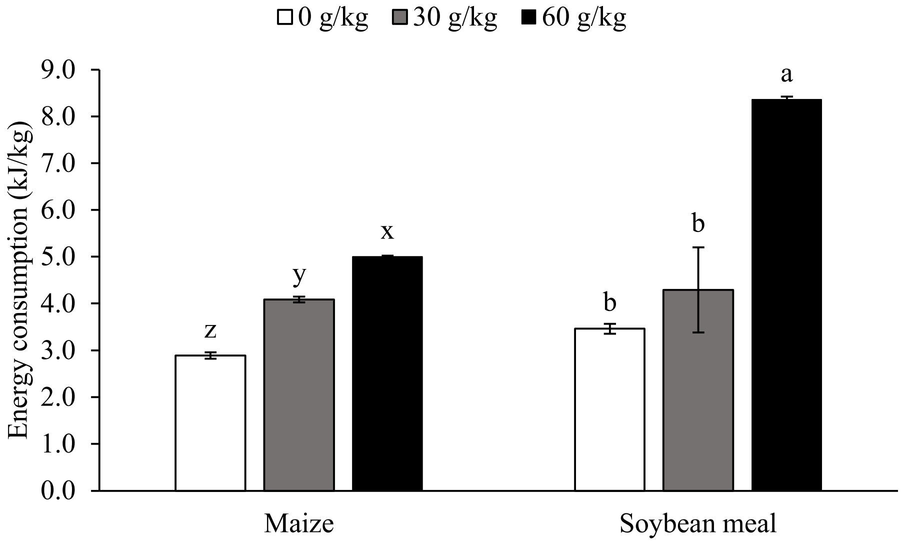


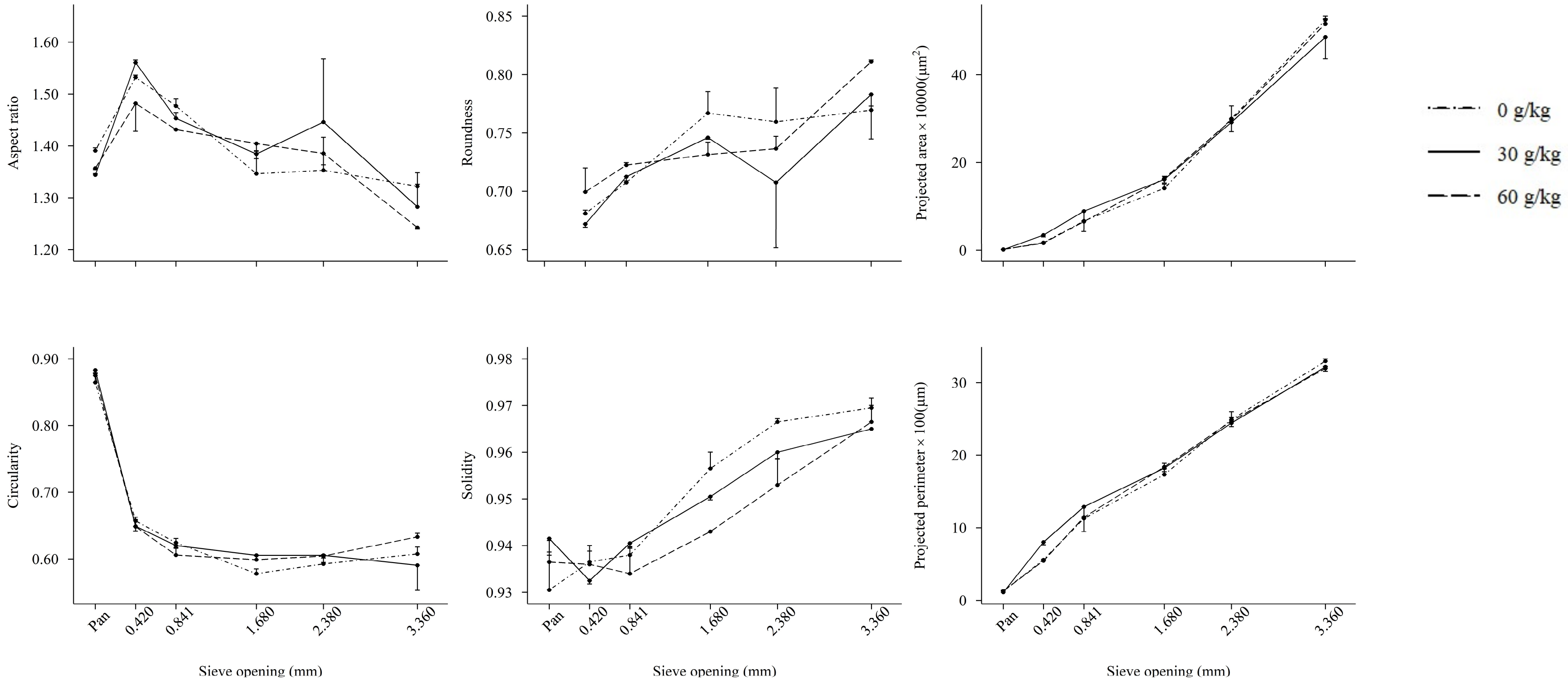
Fig. 4.2 Net energy consumption of hammer milled maize and soybean meal as affected by no (0 g/kg), 30 and 60 g/kg moisture addition to the ingredient. Error bas represent standard deviations. Values with different superscripts within ingredient are significantly different (P < 0.05).

4.3.2 Physical characteristics of particles

Physical characteristics of particles from the different size fractions for the three MC treatments of maize and SBM are presented in Fig. 4.3 and Fig. 4.4, respectively. Projected area and perimeter increased from 1,647 to 508,982 μm^2, and 123 to 3,236 μm, respectively in maize and from 1,629 to 69,348 μm^2 and from 120 to 1,121 μm in SBM. The circularity of particles in the various fractions was approximately 0.60 - 0.70 except for the particles in the pan fraction, which were in the range of 0.85 - 0.90. In general, the solidity of maize particles increased with particles > 0.841 mm. The range in solidity values of SBM particles among the different MC treatments was rather small with a maximum value of 0.942 and minimum of 0.956 (Fig. 4.3). In both maize and SBM, the effect of fraction was significant (P < 0.001) for all measured physical parameters (projected area, projected perimeter, circularity, AR, roundness, and solidity). In maize, interaction effects between MC and fraction were found for circularity (P < 0.05) and solidity (P < 0.001). In SBM, projected area (P < 0.05), circularity (P = 0.005) and solidity ($P < 0.05$) were affected by MC, and the interactions between size fractions and MC were also observed in projected area and solidity.

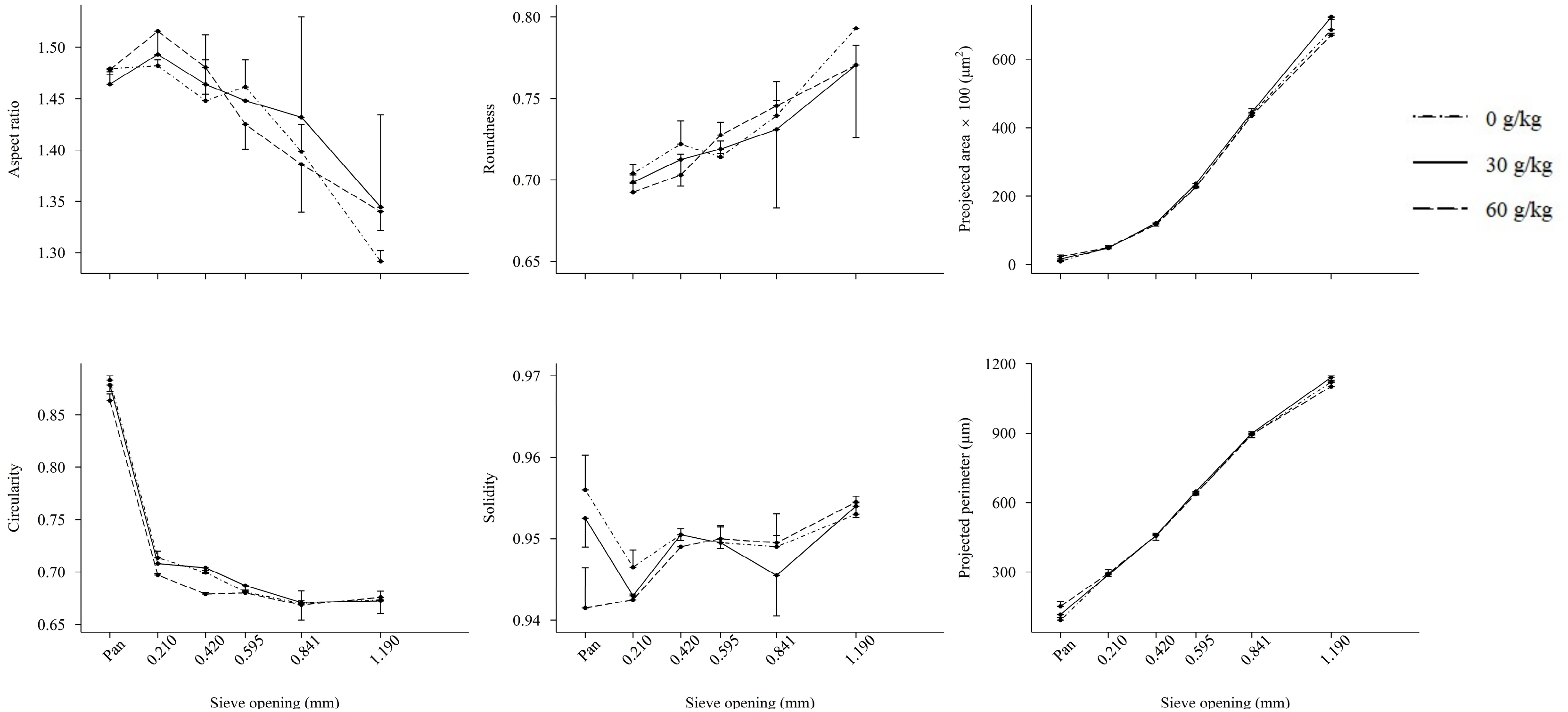
4.3.3 Nutrient content

Fractionation showed marked differences in the nutrient content among particle size fractions in both maize and SBM (Fig. 4.5 and Fig. 4.6, respectively). Moisture content did not significantly influence the nutrient content between particle fractions, except for the DM content in both ingredients and ash content in SBM. For SBM, the pan fraction contained the largest ash content (84 g/kg), while those of other fractions were relatively similar and close to 72 g/kg. The larger the particle size, the higher the CP content (~500 to 570 g/kg) in the case of SBM (Fig. 4.6). The CP content of maize was low in the pan fraction and increased in the fractions collected on the 0.420- and 0.841-mm sieves, then decreased on the 1.680 mm fraction. With increasing particle size, the DM content first decreased and then increased for fractions collected on the 0.841 (M0 and M3) and 1.680 mm (M6) sieve in maize. A similar pattern was observed for SBM, where the minimum DM content was observed in the fraction of 1.680 mm (M0) and 0.841 mm (M3 and M6). Neutral detergent fibre in maize showed a similar pattern to CP, first increasing and then decreasing when particle size increased, with particles > 0.841 mm containing more NDF compare with the other fractions when MC was increased. In maize, the ash content of particles on the 1.680 mm sieve at each moisture level was the lowest among all fractions. With higher MC, particles in the largest size fraction contained less ash and CP, but more starch. However, lower ash and CP contents were observed when particles were < 2.380 mm.



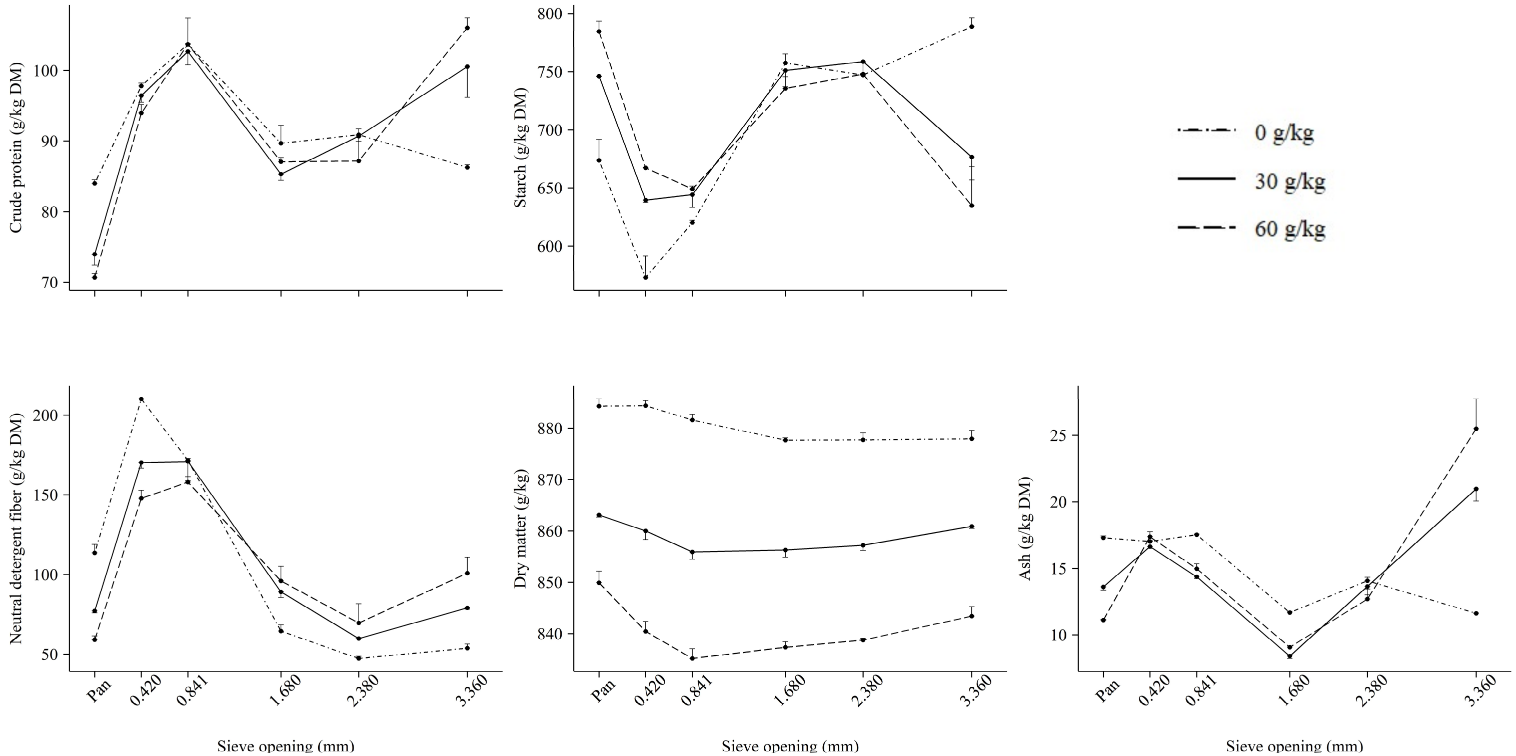
Variable	Projected area	Projected perimeter	Circularity	Aspect ratio	Roundness	Solidity
Moisture	0.963	0.228	0.341	0.250	0.225	0.016
Fraction	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Moisture × Fraction	0.513	0.290	0.045	0.224	0.219	0.019

Fig. 4.3 Physical characteristics of particles retained on different sieves (+pan) as affected by no (0 g/kg), 30 and 60 g/kg moisture addition before hammer-milling maize. Error bars represent the standard deviation of duplicate measurements. Probability values of effects are provided in the table.



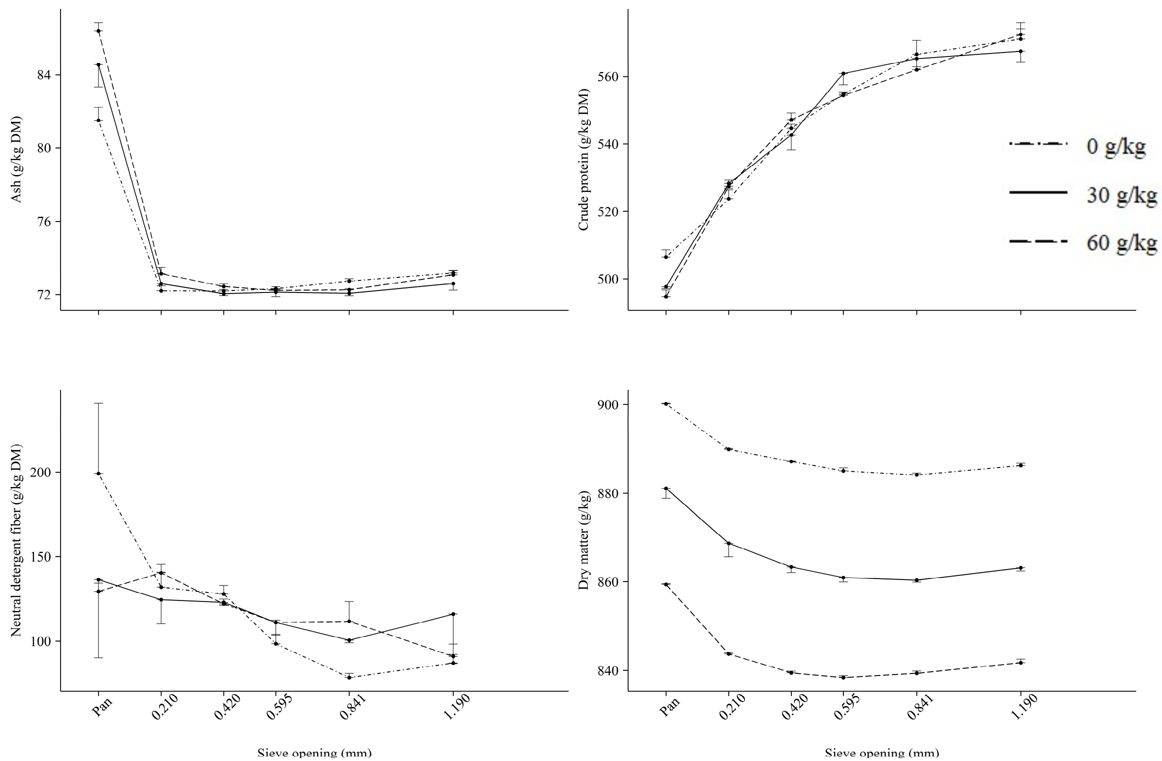
Variable	Projected area	Projected perimeter	Circularity	Aspect ratio	Roundness	Solidity
Moisture	0.018	0.270	0.005	0.658	0.617	0.033
Fraction	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Moisture × Fraction	0.024	0.055	0.192	0.838	0.911	0.009

Fig. 4.4 Physical characteristics of particles retained on different sieves (+pan) as affected by no (0 g/kg), 30 and 60 g/kg moisture addition before hammer-milling soybean meal. Error bars represent the standard deviation of duplicate measurements. Probability values of effects are provided in the table.



Variable	Ash	Crude protein	Starch	Neutral detergent fibre	Dry matter
Moisture	0.170	0.715	0.145	0.136	<0.001
Fraction	<0.001	<0.001	<0.001	<0.001	<0.001
Moisture × Fraction	<0.001	<0.001	<0.001	<0.001	<0.001

Fig. 4.5 Nutrient content of particles retained on different sized sieves (+pan) as affected by no (0 g/kg), 30 and 60 g/kg moisture addition before hammer-milling maize. Error bars represent the standard deviation of duplicate measurements. Probability values of effects are provided in the table.

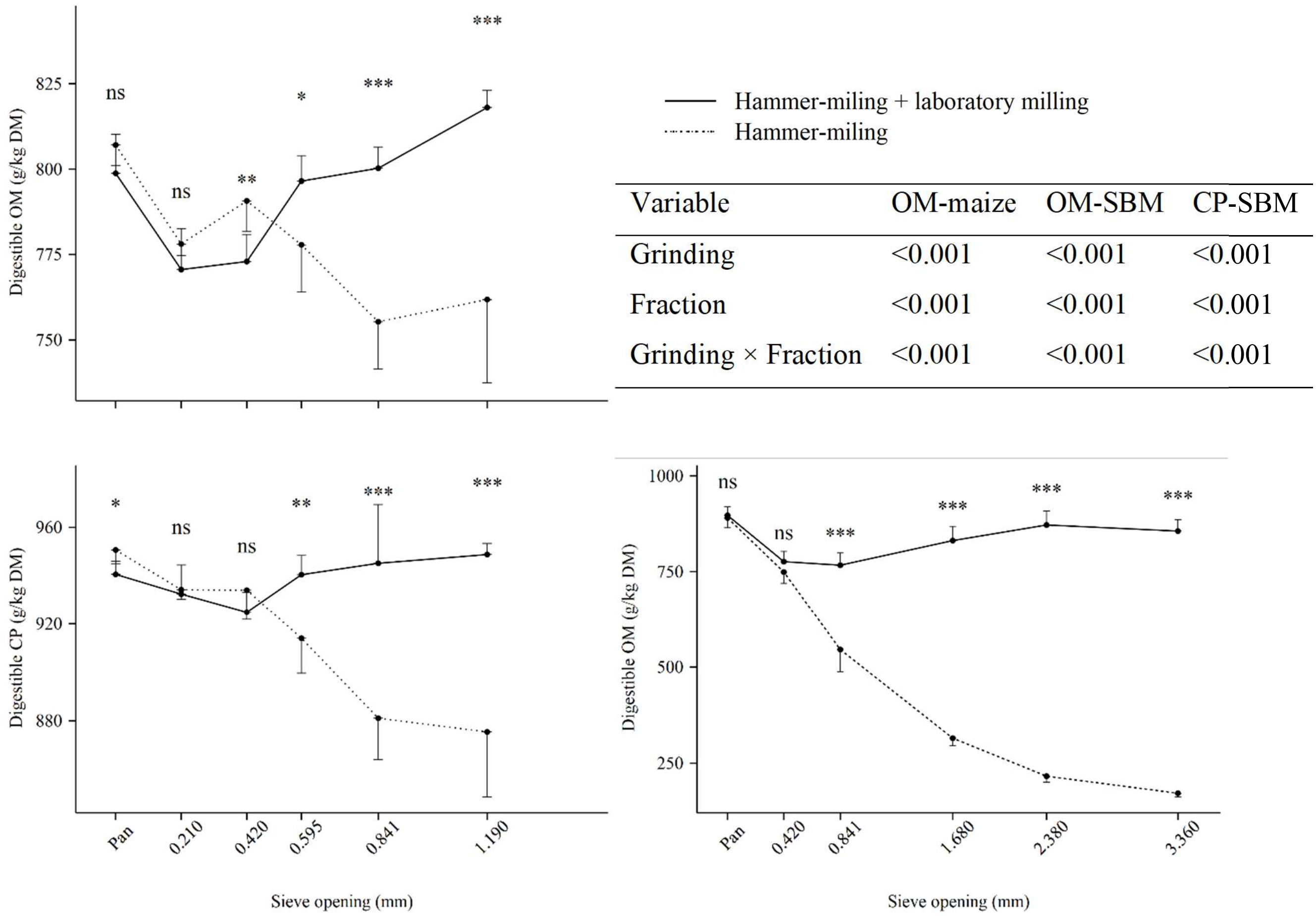


Variable	Ash	Crude protein	Neutral detergent fibre	Dry matter
Moisture	<0.001	0.390	0.919	<0.001
Fraction	<0.001	<0.001	<0.001	<0.001
Moisture × Fraction	<0.001	0.005	0.028	0.008

Fig. 4.6 Nutrient content of particles retained on different sized sieves (+pan) as affected by no (0 g/kg), 30 and 60 g/kg moisture addition before hammer-milling soybean meal. Error bars represent the standard deviation of duplicate measurements. Probability values of effects are provided in the table.

4.3.4 *In vitro* digestibility

The moisture adjustment of maize and SBM did not affect ($P > 0.05$) the *in vitro* digestibility of OM and CP among the fractions and the data were, therefore, combined per MC treatment. Figure 4.7 shows the *in vitro* digestibility of OM (maize and SBM) and CP (SBM) of particle size fractions originating from the hammer milled as well as the additional grinding (1 mm). A significant effect of fraction and additional grinding as well as an interaction between the two was found on *in vitro* OM and CP digestibility for both ingredients. For the hammer milled maize, the *in vitro* OM digestibility increased with decreasing particle size from 0.172 in the 3.360 mm sieve to 0.890 in the material collected in the pan. Unlike the only hammer milled fractions, the *in vitro* OM digestibility values of the additionally ground particles were relatively similar, ranging from 0.767 to 0.897. Additional grinding increased ($P < 0.001$) the *in vitro* OM digestibility for maize fractions ≥ 0.841 mm compared to the hammer-milling only. In SBM, additionally grinding particles ≥ 595 µm increased the *in vitro* OM and CP digestibility values with a higher value ($P < 0.01$) found for the material collected on the 0.420 mm sieve. For CP, a higher *in vitro* digestibility value was found for the material collected in the pan ($P < 0.05$) that was only hammer milled.



Variable	OM-maize	OM-SBM	CP-SBM
Grinding	<0.001	<0.001	<0.001
Fraction	<0.001	<0.001	<0.001
Grinding × Fraction	<0.001	<0.001	<0.001

Fig. 4.7 *In vitro* digestibility coefficient of organic matter (OM) and crude protein (CP) of hammer milled maize and soybean meal (SBM) fractionated by sieving (···) and the same fractions additionally ground over a 1.0 mm sieve (—) as per assay requirements. Error bars represent the standard deviations. Probability values of effects are provided in the table. Significance levels within sieve opening: ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant.

4.4 Discussion

The present study encompasses three main parts: (1) grinding performance of hammer-milling maize and SBM with different MC; (2) physical and chemical characteristics of fractionated particles of moisture-treated maize and SBM; (3) effect of additional grinding of fractionated particles of hammer milled maize and SBM on *in vitro* OM and CP digestibility. Although, 30 and 60 g/kg water was added to the two ingredients during mixing, the MC increased between 22-26 and 39-47 g/kg (Table 4.2), respectively with the MC of SBM being increased more than maize. It is likely that the water added was not completely absorbed by the ingredients with some moisture lost during mixing and storage. The SBM was already ground and compared to the intact maize kernels, the water could have been more easily distributed and absorbed. Moisture loss during hammer-milling as a result of heat generation is unlikely. Under the assumption that all energy in grinding (Fig. 4.2) was converted to heat and using a heat capacity of 1.8 kJ/kg/K, the maximum temperature rise is 4.7 K for the SBM M6 treatment. For the other treatments, the temperature rise would be lower. This is only a slight temperature increase, insufficient to cause moisture loss due to grinding. Probst et al. (2013) found that there was significant loss of moisture after hammer-milling maize containing 162 and 196 g/kg moisture but not for lower MC (104 g/kg) maize, supporting the observations in the present study.

4.4.1 Grinding performance

Generally, increasing the MC of whole maize kernels led to more energy consumption during hammer-milling, more coarse particles and a larger GMD, which is in line with results of Dziki (2008) and Lee et al. (2014). Increasing MC could increase the plasticity of material, which makes it more difficult to grind (Mabille et al., 2001; Dziki et al., 2012; Deng and Manthey, 2017; Hassoon and Dziki, 2018). To test the latter, an additional analysis was conducted. Moisture-treated and hammer milled maize and SBM were compressed using a plunger in a barrel (Ø 60 mm × 65 mm hight) using an Instron 3366 series with a constant force of 4.5 kN with the total percentage displacement relative to the initial height recorded after 6.5 h. Results showed that measurement of plastic deformation of both maize and SBM increased with an increase in MC, with a percentage of total displacement of M0, M3 and M6 of 22.0, 26.5 and 31.0 % for maize and 54.9, 65.3 and 75.6 % for SBM, respectively. Due to increased plastic deformation of the material, more of the grinding energy is absorbed and transferred to heat. As a result, less energy is being transferred to create new surfaces (Dziki and Laskowski, 2005; Probst et al., 2013; Hassoon and Dziki, 2018). Moisture addition increased particle size (Table 4.1), which agrees with the results of Braun et al. (2019). As for the increase in coarse particles with increased MC in maize, this could be attributed to the decrease in secant modulus of elasticity (Babic et al., 2013). Therefore, the destruction of the kernel most likely took place with the appearance and spread of large cracks resulting in large fragments (Haddad et al., 1999; Babic et al., 2013). The GMD of the M3 and M6 maize did not differ significantly. This can possibly be attributed to the role of the seed coat as it is important in determining grinding efficiency (Lee et al., 2013). Since high MC makes seed coats less affected by rupture force (Jung et al., 2018), the M3 and M6 maize may have had similar conditioned seed coats, resulting in similar GMD. The non-significant differences in GMD may also be due to the relatively small range of MC. Dziki (2008) found similar results in grinding wheat at MC of 120, 140 and 160 g/kg. In SBM, the GMD of the M6 was not different from the M3 treatment. However, the net energy consumption was more than double in the M6 treatment. Material with higher MC has a lower flowability (Seifi and Alimardani, 2010) which results in a lower feed rate at a fixed adjustable inlet (Table 4.1): it took a longer time to grind the same amount of material. This results in a difference in degree of fill or active volume in the mill which may account for the observed differences or, as indicated, differences in plasticity of the material by the creep test may account for the large effect on net energy consumption in the M6 SBM treatment.

4.4.2 Physical and chemical characteristics

Nutritional characteristics but not physical characteristics of fractionated material were previously investigated for barley (Sundberg et al., 1995a,b), sorghum (Al-Rabadi et al., 2009; 2012) and rice grains (De La Hera et al., 2013). Physical characteristics of ground peas fractionated by sieving were studied by Maaroufi et al. (2000), although different parameters were investigated: median diameter, a dispersion parameter (logarithmic standard deviation), specific surface area, total porous volume, bulk density and apparent density. In the present study, fractionated material of maize and SBM showed marked differences in physical and chemical characteristics (Fig. 4.3 to 4.6). The data are in good agreement with results obtained by Lyu et al. (2021). Information on the effects of adding moisture before hammer-milling and possible changes in chemical and physical characteristics of fractionated materials are, however, scarce.

Studies investigating nutrient distribution over fractionated materials can potentially be used to remove specific components from raw materials in food industries (Maaroufi et al., 2000). On the other hand, the differences in nutrient levels could also be considered as indicators for breakage behaviour of materials after grinding (Lyu et al., 2020), because such differences can be due to a separation of different botanical constituents of the seed when impacted upon in the hammer mill chamber. For example, NDF content was mainly present in maize particles collected on the 0.420- and 0.841-mm sieves (Fig. 4.5). Since the maize kernel hull contains 51% of the total grain fibre (Watson, 1987), it can be inferred that in the grinding of maize kernels, the hull is probably ground to particles that can pass a 1.680 but not a 0.420 mm sieve, which could also be observed from the images (not provided) taken of fractionated maize.

Moisture content is considered to influence the hardness of materials (Lee et al., 2013) and the strength of the seed coat (Jung et al., 2018), which is also likely, therefore, to influence the grinding behaviour of materials. According to the results of the current study, MC in maize only showed a significant impact on solidity. The latter is a measure of the overall concavity of a particle, a lower solidity is an indication of a more fragmented particle and can show an increase in brittleness (Olson, 2011). According to Barbosa-Cánovas et al. (2005) and Jung et al. (2018), a low MC material is more likely to be broken by shattering and tends to produce more irregular-shaped particles which increases solidity values. Soybean meal as a by-product obtained after the oil extraction of soybeans, was already in a ground form before hammer-milling in the current study. As such, the physical factors related to grinding might be different from those of whole grain kernels. In SBM, MC showed a significant impact on projected area and solidity, as well as ash content. Interactions between MC and fraction were also observed.

Generally, the nutrient composition and morphology characteristics of maize and SBM fractions is influenced to a minor extent by MC before hammer-milling. The physical properties (e.g. plasticity) of maize and SBM were affected by moisture addition. The Instron measurements showed changes in plastic deformation for both maize and SBM but this influence appears to have limited impact on the final physical and chemical characteristics of fractionated material after hammer-milling, although the MC levels studied in the current study are relevant to the feed industry.

4.4.3 *In vitro* digestibility

The *in vitro* digestibility of OM and CP were different ($P < 0.001$) among various size fractions for both maize and SBM (Fig. 4.7), which agrees with the data of Lyu et al. (2021). The latter authors also found that hammer-milling maize and SBM resulted in different OM and CP *in vitro* digestibility values for different size fractions. *In vitro* OM and CP digestibility data indicate that MC in the observed range, besides having a minor impact on physical and chemical characteristics of particles, does not affect the OM and CP digestibility, at least *in vitro*.

The *in vitro* digestion was performed based on the method described by Boisen and Fernández (1995), in which the material is prescribed to be ground over a 1-mm sieve. Lyu et al. (2021) reported preliminary results that the additional grinding of hammer milled maize and SBM fractions with a GMD larger than 1 mm, significantly affected the *in vitro* OM digestibility (CP was not measured). For hammer milled maize particles with a GMD of 3.999 mm, additional grinding increased *in vitro* OM digestibility from 0.161 to 0.907 (Lyu et al., 2021). In order to corroborate the results of the latter authors and extend the measurement to also include CP, all fractions collected in the present study were additionally ground in a standard laboratory mill to pass a 1 mm sieve size, as the assay specifications of Boisen and Fernández (1995) before determination of *in vitro* OM and CP digestibility. The additional grinding was found to have a major effect on *in vitro* digestibility values especially for OM in maize (Fig. 4.7). This indicates that the *in vitro* digestibility values were highly dependent on the particle size in contrast to observations of Boisen and Fernández (1997) who showed that the *in vitro* total tract OM digestibility of maize and SBM was only reduced by 1.4 and 0.4% units, respectively when ingredients were ground at 3 instead of 1 mm. An explanation could be that Boisen and Fernández (1997) investigated the whole ingredient while the present study focused on fractionated materials, which contain different levels of nutrients. In the study of Boisen and Fernández (1997), results for samples with a particle size larger than 1 mm were shown to be less reproducible. According to the results in the present study (Fig. 4.7), particles collected on sieves smaller than 0.592 mm, the effect of additional grinding was largely non-significant. Further studies should be conducted to ascertain the importance of particle size on the *in vitro* digestibility assay as published by Boisen and Fernández (1995; 1997).

4.5 Conclusion

Increasing the moisture content of maize and soybean meal can increase the plasticity of the material which results in increased energy consumption during and an increased geometric mean diameter of particles after hammer-milling. Size fractionated particles differ in terms of nutrient content and morphological characteristics after the hammer-milling of maize and soybean meal which may be indicators for breaking behaviour. The effects of moisture addition on the breakage behaviour of maize and soybean meal during hammer-milling, however, may be limited. The *in vitro* digestibility of OM and CP appears to be highly dependent on particle size when it comes to fractionated ingredients with particles ≥ 595 μm.

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Chapter 5 Breaking behaviour and interactions of maize and soybean meal while grinding of a hammer mill

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Abstract

Grinding and mixing are fundamental procedures in feed processing technology. Many studies have investigated the grinding characteristics of single feed ingredients. However, research into grinding of mixtures of feed ingredients is limited, although in practical feed production, dosing and mixing the ingredients in specific proportions before grinding is practiced in many feed factories. 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 ground with a hammer mill using a 2-mm screen, while, energy consumption was recorded. The particle size distribution of ground material was determined with 14 sieves and a pan. The geometric mean diameter (GMD), geometric standard deviation (GSD) and equivalent particle size (EPS) of hammer milled material were calculated. The obtained flour was separated into seven fractions using sieves with size ranges from 0.149 to 1.190 mm. Fractionated particles were characterized for nutrient content and *in vitro* coefficient digestibility of organic matter (OM) and crude protein (CP). Results show that energy consumption of the ground mixtures increased from 3.8 to 48.4 kJ/kg with the maize proportion increasing from zero to 100% ($P < 0.001$). Mixing proportion of maize and SBM showed significant effects on nutrient content of fractionated material ($P < 0.001$). For hammer milled material larger than 595 µm, the *in vitro* digestibility of CP and 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. The GSD, other EPSs and OM *in vitro* digestibility of hammer milled material increased while GMD and CP *in vitro* digestibility decreased with increasing maize proportion. Equivalent particle size and GSD related to OM and CP *in vitro* digestibility of hammer milled maize and SBM and their mixtures correlated better than GMD 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.

Keywords: mixing ratio; grinding behaviour; particle size; nutrient content; *in vitro* digestion; maize; soybean meal

5.1 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. (2004) 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 (Kim et al., 2005; Lahaye et al., 2008; Ball et al., 2015; Huang et al., 2015; Nemechek et al., 2016; Rojas and Stein, 2016). Coarse particles can help reduce gastric ulcers in pigs and maintain gastrointestinal health (Healy et al., 1994; Wondra et al., 1995a; Grosse Liesner et al., 2009; Flis et al., 2014; Ulens et al., 2015; Lyu et al., 2020). Because of the impact 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 (Chen et al., 1999; El Shal et al., 2010; Yancey et al., 2013; Guo et al., 2016; Mugabi et al., 2017). 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 (Mani et al., 2004; Velu et al., 2006; Doblado-Maldonado et al., 2013; Lee et al., 2013; Gil and Arauzo, 2014).

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 nutrients/components or digestion rate of starch (Sundberg et al., 1995a,b; Maaroufi et al., 2000, 2009; Al-Rabadi et al., 2009; Lyu et al., 2021a). The latter investigations 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. Research into breaking behaviour of ingredient mixtures 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 using a 2 mm screen. Energy consumption during grinding was recorded, and particle size distribution (PSD), nutrient content and *in vitro* digestibility of OM and CP of ground material was determined. After the material was sieved into different size fractions, the fractionated materials were analyzed for nutrient content and *in vitro* OM and CP digestibility.

5.2 Material and method

5.2.1 Sample preparation

5.2.1.1 Material mixture

Whole maize (France) (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.

5.2.1.2 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 7.5kW 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 (Fellows, 2009):

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

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.

5.2.1.3 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 (Campos-M and Campos-C, 2017). 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 (ASABE, 2008). 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., 1987). 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 595 µm 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 ℃ until further analysis.

5.2.1.4 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), 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.

5.2.2 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 ℃ for 4 h (ISO 6496, 1999), with ash content determined after combustion at 550 ℃ for 3 h in a muffle furnace (ISO 5984, 2002). Neutral detergent fibre (NDF) was determined with heat-stable amylase (thermamyl) and alcalase, using the standard procedure of Van Soest et al. (1991). Nitrogen (N) content was determined by the DUMAS technique (ISO 16634-1, 2008), and CP was calculated by multiplying the N content by 6.25. The starch content of maize was determined using the enzymic method (ISO 15914, 2004).

5.2.3 *In vitro* digestibility analysis

The *in vitro* digestibility was determined as per the method described by Lyu et al. (2020) which is based on the method described by Boisen and Fernández (1995). 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.

5.2.4 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 (R Core Team, 2019), followed by Tukey's multiple comparisons using 'HSD.test' function in 'agricolae' package (Mendiburu and Muhammad, 2020). 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), and the statistical model cannot account for this additional grinding effect.

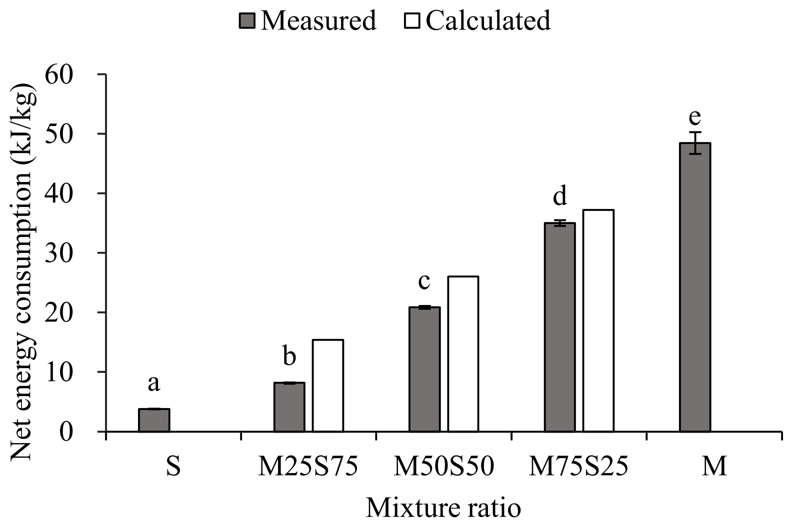
5.3 Results

5.3.1 Grinding performance

The energy consumption (as measured) during the hammer-milling of maize and SBM mixtures is presented in Fig. 5.1. 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 5.1. Geometric mean diameter, GSD and EPS were significantly different (P < 0.05) among mixtures. The significant difference of GMD was only observed between the single SBM of 658.0 µm and the other three mixtures M25S75, M50S50, 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 5.1). 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.



Material	Soybean meal	M25S75	M50S50	M75S25	Maize
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. 5.1 Net energy consumption of hammer milled maize (M) and soybean meal (S) and three mixtures (% M:S; 25:75, 50:50, 75:25) of the two, 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.

On average, the recovery of the hammer milled material on the various sieves + pan to determine the PSD was in excess of 99.9%. The PSD and cumulative PSD as affected by the mixing ratio are presented in Fig. 5.2. 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.

Table 5.1 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]	587.7[b]	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	

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

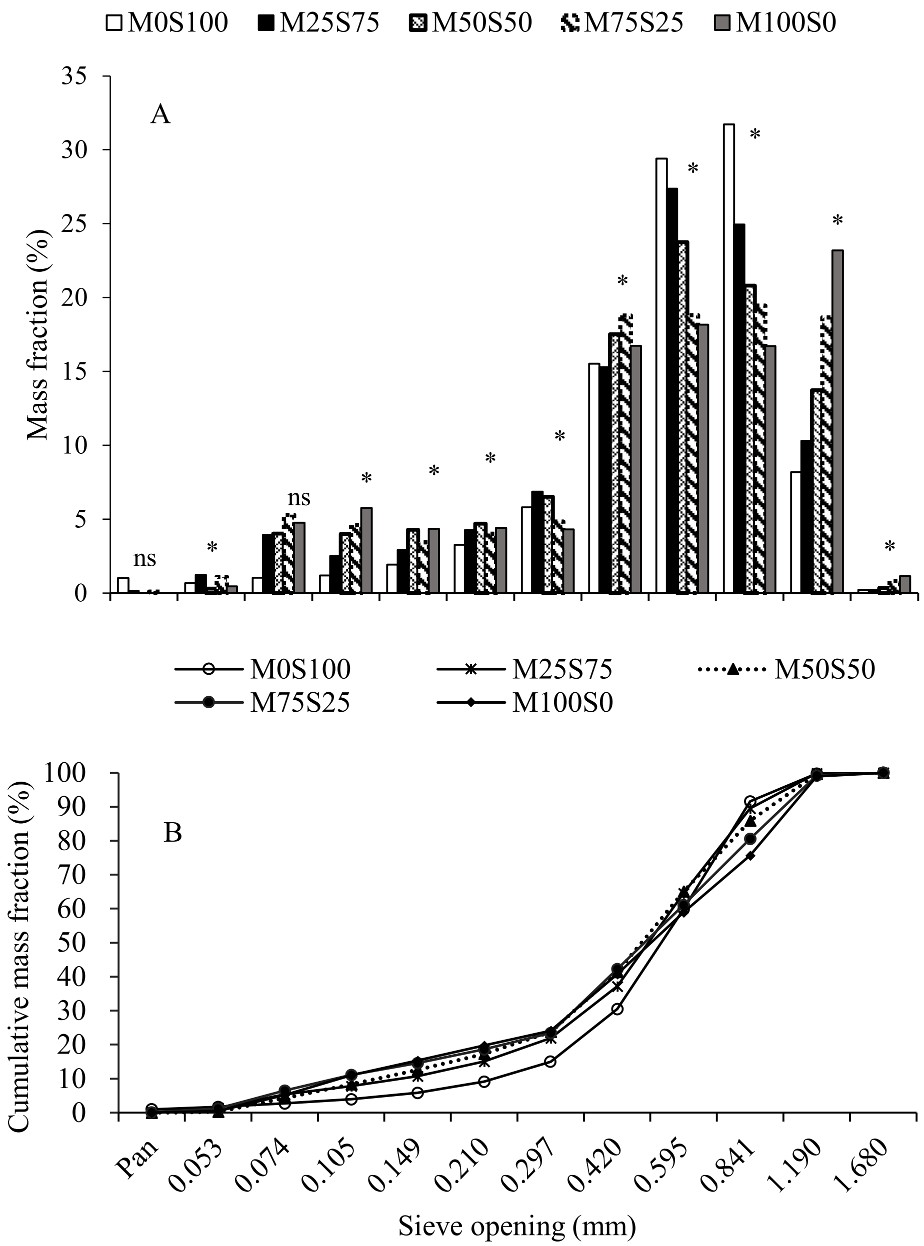
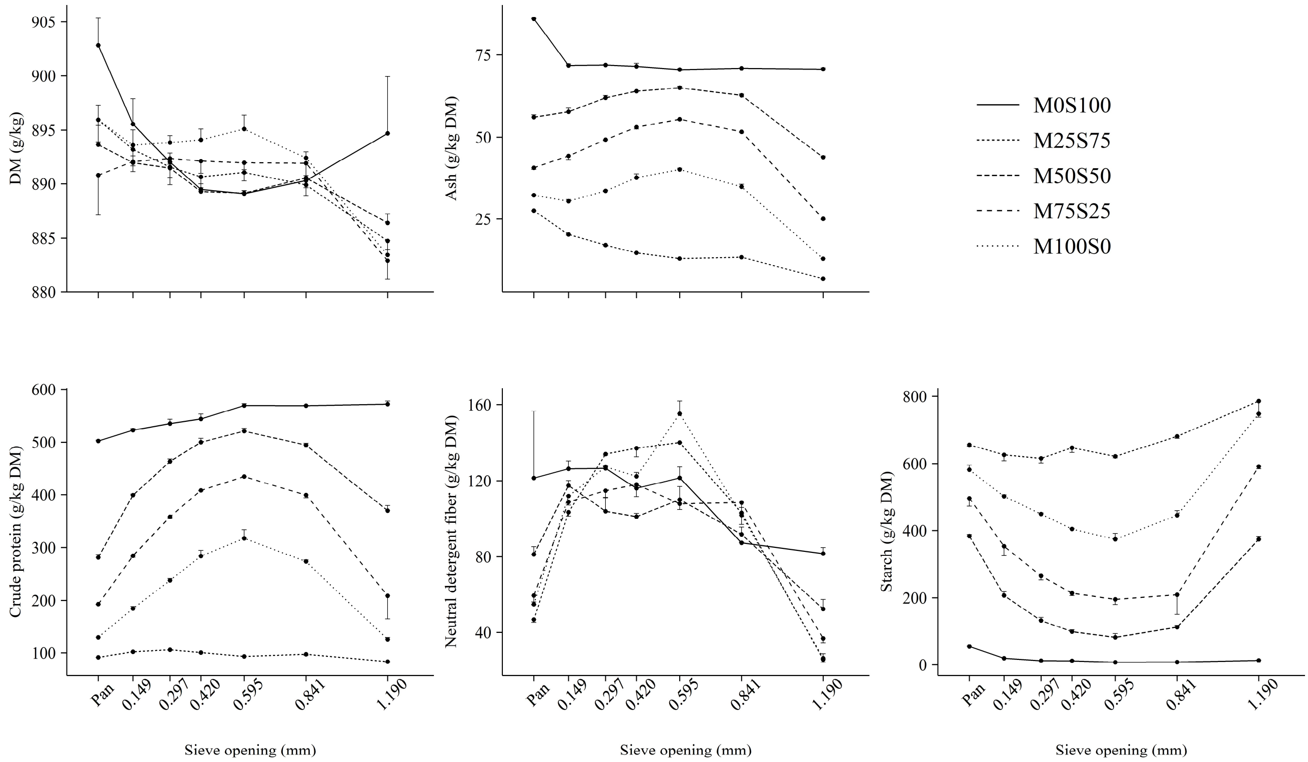


Fig. 5.2 Mass (A) and cumulative mass (B) distribution of hammer milled maize (M) and soybean meal (S) and three mixtures (% M:S; 25:75, 50:50, 75:25) of the two. Significant differences (P < 0.05) between mixtures within each sieve are indicated by *. ns = not significant (P > 0.05).

5.3.2 Nutrient content

The recovery of nutrient content (ash, CP, starch, NDF) 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. 5.3). In SBM (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 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.

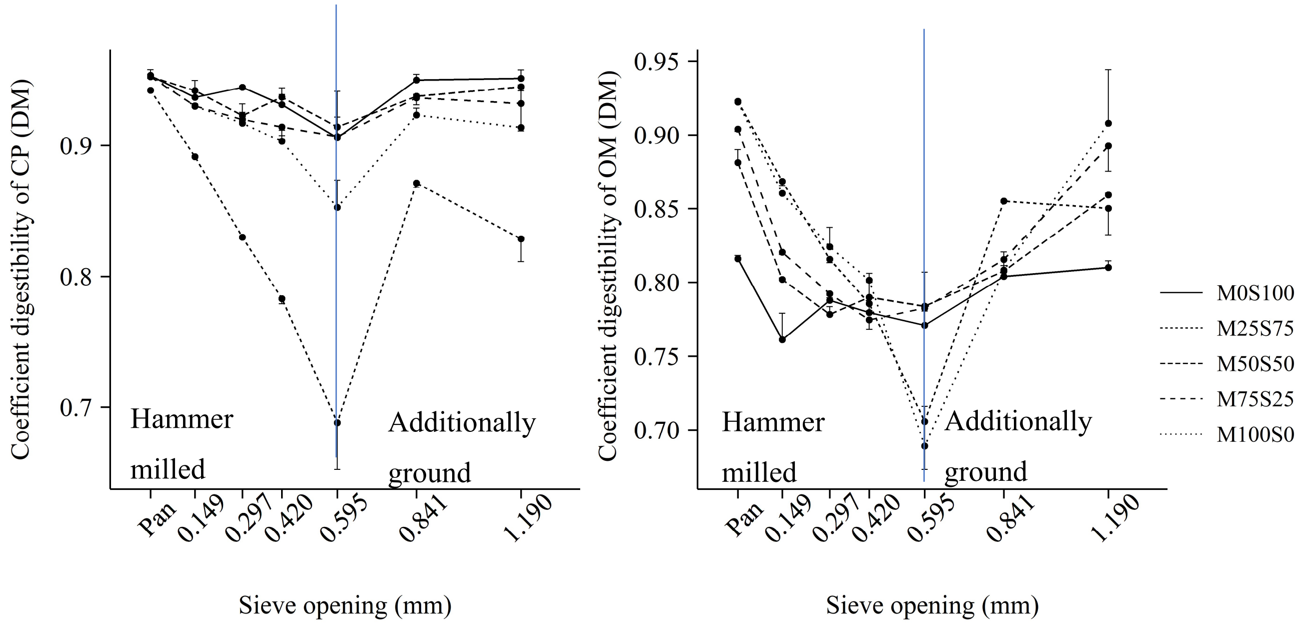


Variable	Ash	Crude protein	Neutral detergent fibre	Dry matter
Mixture	<0.001	<0.001	<0.001	<0.001
Fraction	<0.001	<0.001	<0.001	<0.001
Mixture × Fraction	<0.001	<0.001	<0.001	<0.001

Fig. 5.3 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.

5.3.3 *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. 5.4). 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.



Variable	Crude protein	Organic matter
Mixture	<0.001	<0.001
Fraction	<0.001	<0.001
Mixture × Fraction	<0.001	<0.001

Fig. 5.4 *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.

5.4 Discussion

5.4.1 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 mixture, 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 (Lyu et al., 2021b), which makes it more difficult to be ground and pass through a 2-mm screen resulting in a longer residence time in the hammer mill chamber. Goodband et al. (2006) 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 (Goodband et al., 2006), 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. 5.1). 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 the difference between (K_m) and (K_k) values being less pronounced in the two remaining mixtures. 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. 5.3). This is in agreement with studies Sundberg et al. (1995a,b), Maaroufi et al. (2000, 2009), Al-Rabadi et al. (2009; 2012), Al-Rabadi (2013) and Lyu et al. (2021a,b), which focused on the nutrient content of single ingredients, such as peas, 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. 5.3). This could be due to the moisture content being distributed unevenly in the maize kernel. Zhang et al. (2013) 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. (2021b) 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 (Watson, 1987).

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.

5.4.2 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 (Healy et al., 1994; Livesey et al., 1995; Wondra et al., 1995a; Blasel et al., 2006). 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 (1995). 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 (Lyu et al., 2021a,b). 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 maize in the mixture reduces the digestibility of OM and CP more significantly.

5.4.3 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 (Mavromichalis et al., 2000; Kim et al., 2009; Rojas and Stein, 2015; Bao et al., 2016; Acosta et al., 2019). 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 5.1). 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 5.1 presented, the EPSs and GSD fit the OM and CP *in vitro* digestibility better than GMD. The metanalysis done by Lyu et al. (2020) 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 5.1). Wondra et al. (1995b) 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 were 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.

5.5 Conclusion

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.

Acknowledgments

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Chapter 6 The importance of particle size on organic matter and crude protein *in vitro* digestibility of maize and soybean meal

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Abstract

Particle size plays an important role in the digestibility of nutrients by animals. Methodologies developed to simulate the digestive system to determine digestibility values require the grinding of samples to pass a specific sieve size (e.g. 1 mm) before *in vitro* digestion. The objective of this study was to evaluate if particle size affects *in vitro* (ileal) digestibility values of organic matter (OM) and crude protein (CP) in maize and soybean meal (SBM). Both ingredients were ground in a laboratory mill over four screens (1.50, 1.00, 0.75 and 0.50 mm) with trapezoidal holes in a sequential manner from large to fine. Particle size distribution, nutrient content and *in vitro* digestibility were determined of the various samples. With decreasing screen size, geometric mean diameter of particles significantly decreased from 351.4 to 203.2 μm for maize, and from 239.1 to 99.1 μm for SBM. Ash, CP, and starch content were not affected whereas the neutral detergent fibre in maize and dry matter content in SBM differed after grinding with various sized screens ($P < 0.05$). The *in vitro* digestibility of OM and CP of maize and CP of SBM ground over the four different screens did not differ ($P > 0.185$). The OM *in vitro* digestibility differed ($P < 0.05$) with values for SBM ground over the 1.50 mm screen being lower ($P < 0.05$) than the smaller screen sizes: 0.814 (1.50 mm) *vs* 0.833 (1.00 mm), 0.829 (0.75 mm) and 0.834 (0.50 mm), respectively. Based on the current work and literature data, particles ≥ 595 μm of comminuted maize and SBM affect OM and CP *in vitro* digestibility. Analysis of three feeds and 10 feed ingredients ground over a 1.0 mm sieve showed that the fraction ≥ 595 μm can make up to 32.1% of the mass. It is recommended that before *in vitro* digestibility determination, particle size distribution is assessed, especially the mass fraction of particles ≥ 595 μm and further grinding is conducted to ensure that particles are < 595 μm for maize and SBM. The cut off size where particles size affects OM and CP *in vitro* (ileal) digestibility of other feeds or feed ingredients than maize and SBM should be determined.

Keywords: screen size; particle size distribution; *in vitro* digestibility; maize; soybean meal

6.1 Introduction

Particle size of diets and ingredients is important in animal nutrition. In pigs, it was found that reducing particle size can increase the surface area of particles for digestive enzymes to interact, resulting in a higher digestibility of nutrients as measured in both *in vivo* and *in vitro* trials (Healy et al., 1994; Livesey et al., 1995; Wondra et al., 1995a; Blasel et al., 2006; Lyu et al., 2021a,b).

In vitro digestion methods have been widely used over many years to estimate *in vivo* nutrient digestibility, as they are relatively inexpensive and rapid. The most commonly used *in vitro* models simulate the physiological conditions in the gastrointestinal tract of animals and humans by adjusting the pH, use of buffers, addition of enzymes and bile salts (Butts et al., 2012) and mimicking absorption. The *in vitro* protocol developed by Boisen and Fernández (1995; 1997) in which it is prescribed that materials should be ground to pass a 1 mm sieve before digestion simulation has been, and still is, widely used in animal nutrition. Furuya et al. (1979) reported that grinding over a 1.0 mm screen of three out of four pig diets increased the dry matter (DM) and crude protein (CP) *in vitro* digestibility, with lower digestibility values showing larger effects of grinding material over a 1.0 mm before *in vitro* digestion. Although Löwgren et al. (1989) reported that an increased particle size requires a longer incubation time, these authors found no significant effect of incubations of ground (5.0, 2.0, 1.0 and 0.5 mm screens) barley with duodenal, ileal and faecal inocula on final digestibility values if incubations lasted >45 h. Boisen and Fernández (1997) showed that the organic matter (OM) *in vitro* total tract digestibility of maize and soybean meal (SBM) decreased by only 1.4 and 0.4% units, respectively when ground over a 3 instead of 1 mm sieve. Recently, Lyu et al. (2021a,b), when studying fractionated hammer milled maize and SBM, found that the grinding of fractions over a 1 mm sieve significantly increased the *in vitro* digestibility coefficient of OM up to 75% (from 0.16 to 0.91) for maize and for CP up to 7% in the case of SBM (from 0.88 to 0.95). The effect of grinding on *in vitro* digestibility was general seen for fractions collected on sieves ≥ 595 μm (Lyu et al., 2021a,b). There is a relative dearth of information in the literature whether particle size distribution (PSD) may explain the results of Lyu et al. (2021a,b). Further investigations are warranted to determine the influence of particle size on the values of OM and CP *in vitro* digestibility generated using the Boisen and Fernández (1995; 1997) assay.

The objective of this experiment was to evaluate the effect of mill screen size on the estimation of OM and CP *in vitro* digestibility of maize and SBM by the Boisen and Fernández (1995; 1997) assay.

6.2 Material and Methods

6.2.1 Grinding

Two, 20 kg bags of French maize kernels and two, 20 kg bags of Brazilian SBM (both Research Diet Service B.V., Wijk Bij Duurstede, The Netherlands) both originating from one batch were used. For each ingredient, 4 kg/bag were randomly collected. Maize kernels were first ground using a cut mill with screen size of 4 mm (SM300, Retsch, Germany) to avoid excessive heat generation during subsequent milling at 1.5 mm. An ultra-centrifugal mill (ZM 200, Retsch, Germany) was used to grind maize and SBM using screens with trapezoidal holes of 1.50, 1.00, 0.75 and 0.50 mm at a running speed of 12000 rpm. The 4 kg of ground maize and SBM were ground over the 1.50 mm screen before 1 kg was collected using a multi-slot divider (Mooij-Argo, Hegelsom, the Netherlands). The remaining material was ground again with the ultra-centrifugal mill using the 1.00 mm screen where after another 1 kg sample was collected using the multi-slot divider. This process was repeated with the 0.75 mm screen and the final material was ground over the 0.50 mm screen and collected. Great care was taken to ensure that the mill temperature remained similar to the initial starting temperature during grinding by slow feeding the material into the ultra-centrifugal mill and maintaining the running speed of 12000 rpm. After grinding and directly after collection, ground material was stored individually in airtight plastic bags and kept at 4 °C for further analysis.

6.2.2 Particle size analysis

The PSD of the various ground maize and SBM samples were determined (in duplicate) using the 15-sieve method (ASABE, 2008). In this method, 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 shaking time of 6 s. Two rubber balls with a diameter of 20 mm were used as sieving aid on each sieve where the sieve opening was smaller than 300 μm while sieving SBM. During the sieving procedure of maize, it was observed that very fine particles agglomerated on the sieves, which may be due to the molecular interactions like Van der Waals and electrostatic interactions. To reduce this agglomeration, four rubber balls were used for the sieves with an opening of ≤ 595 μm. Geometric mean diameter (GMD) and geometric standard deviation (GSD) were calculated based on the PSD according to ASABE (2008) and reported as the mean value of the duplicate samples.

6.2.3 Nutrient content and *in vitro* digestibility

The nutrient content of ground maize and SBM including DM, ash, neutral detergent fibre (NDF), CP and starch were analysed as previously described by Lyu et al. (2021a). The OM and CP *in vitro* digestibility coefficients of ground maize and SBM were determined as described by Lyu et al. (2021a,b) which is based on the method published by Boisen and Fernández (1995). Briefly, 10 g samples were first digested in a solution containing pepsin and pancreatin, where after the undigested residues were collected by filtration through nylon gaze with a pore size of 40 µm and porosity of 0.30 (PA 40/30, Nybolt, Switzerland) and then dried at 70 ℃ overnight. The DM, ash and CP content of residue was determined, and the digestibility coefficients calculated based on DM according to the difference in OM and CP content before and after digestion.

6.2.4 Statistical analysis

Data on nutrient content and *in vitro* digestibility of OM and CP for each ingredient were analysed by one-way analysis of variance using a general linear model in R 3.6.1 (R Core Team, 2019). The main factor was the screen size of the ultra-centrifugal mill containing four levels (1.50, 1.00, 0.75 and 0.50 mm). For each screen size, two bags of maize as well as SBM were used in duplicate, yielding 16 samples which each were analysed once. Multiple comparison of means was performed by Tukey's multiple comparisons using 'HSD.test' function in the 'agricolae' package (Mendiburu and Muhammad, 2020). The minimum significance threshold was set at 0.05.

6.3 Results

The recovery of maize and SBM ground over the four screen sizes (1.50, 1.00, 0.75 and 0.50 mm) during the determination of the PSD ranged from 98.86 ± 0.09% to 99.80 ± 0.20%. Screen size showed a significant effect (Table 6.1) on GMD and GSD of maize and SBM ($P < 0.001$). With a decreasing screen size, the GMD decreased from 351.4 to 203.2 µm for maize, and from 239.1 to 99.1 µm for SBM, respectively. The GSD decreased from 256.7 to 86.3 µm for maize, and from 213.4 to 68.6 µm for SBM, respectively.

Table 6.1 Geometric mean diameter and geometric standard deviation of comminuted maize and soybean meal as affected by mill screen size.

Ingredient	Mill screen size (mm)	Geometric mean diameter (μm)	Geometric standard deviation (μm)
Maize[1]	0.50	203.2^{d}	86.3^{d}
	0.75	219.0^{c}	111.4^{c}
	1.00	260.3^{b}	148.2^{b}
	1.50	351.4^{a}	256.7^{a}
P-value		<0.001	<0.001
SEM		21.77	24.61
Soybean meal	0.50	99.1^{a}	68.6^{a}
	0.75	133.3^{b}	104.8^{b}
	1.00	186.2^{c}	163.6^{c}
	1.50	239.1^{d}	213.4^{d}
P-value		<0.001	<0.001
SEM		20.09	20.92

[1] Ground first using cut mill with 4 mm screen size.
[a, b, c, d] Values with different superscripts within a column per ingredient are significantly different ($P < 0.05$).
SEM, standard error of the mean.

As shown in Fig.6.1, maize particles were mainly retained on the middle size sieves (from 74 μm to 420 μm). For the two smallest fractions (pan, and 53 μm sieve), a very small mass percentage was collected (< 0.4%). The 841 and 1190 μm sieves collected less material if maize was ground over the 0.50, 0.75 and 1.0 mm screen (< 0.3%), compared to milling over the 1.5 mm screen where these sieves contained 1.6 and 6.8% of the maize. On the 595 μm sieve, maize particles ground over the 1.5 mm screen collected the largest percentage (17.2%), followed by the maize ground over the 1.00, 0.75 and 0.50 mm screen (4.4, 0.5 and 0.3%, respectively). For SBM, the mass collected on the pan and small sieves (53, 74, 105 and 149 μm) increased with a decreasing screen size during grinding, and a decreased mass was collected on the medium size sieves (297, 420, 595 μm) with increasing sieve size during grinding. Similar to the maize, little of the SBM material was found on the larger sized sieves (595, 841 and 1190 μm), especially for the SBM that was milled over the 1.00, 0.75 and 0.50 mm screens, which were less than 2.1%. The median particle size (D_{50}) was visually taken from cumulative PSD of maize and SBM (Fig. 6.1), and increased with increasing milling screen size: <149, >149, <210 and <297 μm (maize), and <74, >105, >149 and >210 μm (SBM) for screen size of 0.50, 0.75, 1.00 and 1.50 mm, respectively.

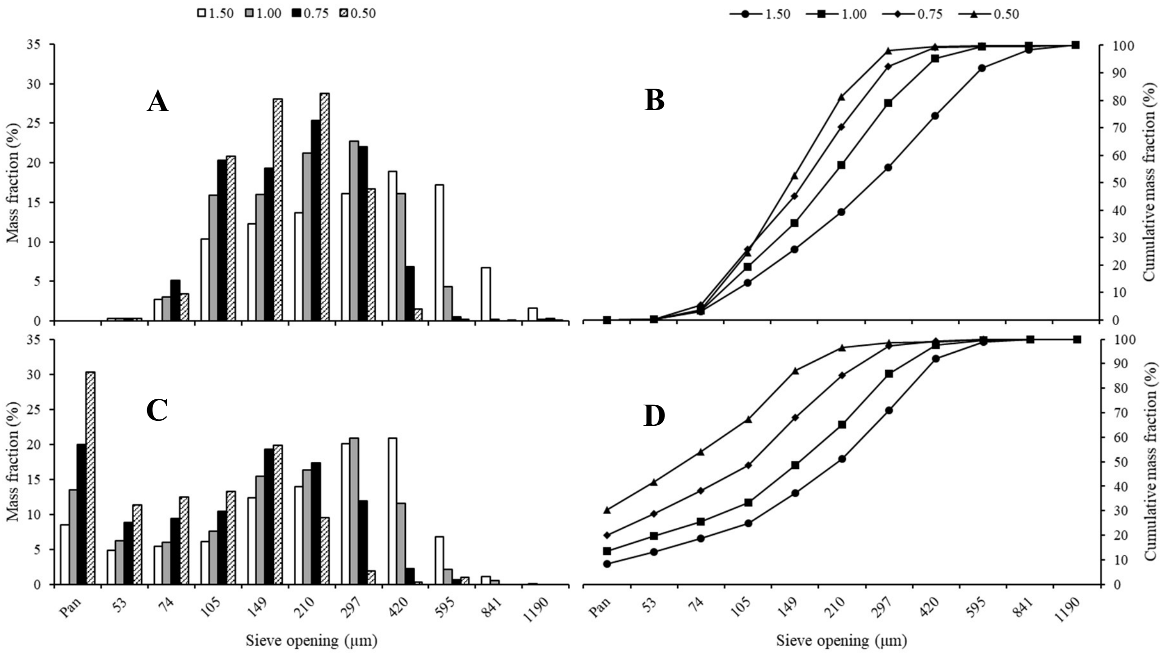

Fig. 6.1 Mass and cumulative mass fraction distribution of comminuted maize (A, B, resp.) and soybean meal (C, D, resp.) using mill screen sizes of 1.50, 1.00, 0.75 and 0.50 mm. Maize was ground first using a cut mill with 4 mm screen size.

After grinding over the four screens, the nutrient content of comminuted maize and SBM was analysed (Table 6.2). There was no significant effect ($P > 0.05$) of mill screen size on the DM, ash, CP and starch content of maize, and no effect of mill screen size on CP, ash and NDF content of SBM. The NDF content of maize and DM content of SBM when ground over the different-sized screens were significantly different ($P < 0.05$). The maize that was ground over a 0.50 mm screen contained the lowest concentration of NDF (69.6 g/kg DM), and the maximum content of NDF (82.2 g/kg DM) was observed when maize was ground over a 1.00 mm screen. In SBM, the DM content decreased from 913 to 897.6 g/kg with increasing screen size.

The OM and CP *in vitro* digestibility of maize was not affected ($P > 0.05$) by screen size (Table 6.3). Similarly, the screen size of the mill did not show a significant effect on CP *in vitro* digestibility of comminuted SBM. However, the OM *in vitro* digestibility of comminuted SBM was significantly different ($P <0.05$) although the differences were small (0.02 between lowest and highest value). The lowest value (0.814) was recorded for screen size 1.50 mm which was not significantly different to the value (0.829) for screen size 0.75 mm.

Table 6.2 Nutrient content of comminuted maize and soybean meal as affected by mill screen size.

Ingredient	Mill screen size	Dry matter	Ash	Crude protein	Starch	Neutral detergent fibre	Remaining fraction[1]
	(mm)	(g/kg)	(g/kg dry matter)				
Maize[2]	0.50	902.6	14.5	90.4	643.9	69.6[d]	181.6
	0.75	899.9	14.2	90.2	699.2	75.4[c]	121.1
	1.00	897.1	14.6	89.6	707.4	82.2[a]	106.3
	1.50	897.3	14.4	89.3	687.3	78.4[b]	130.6
SEM		0.95	0.06	0.28	11.00	1.74	12.2
P-value		0.068	0.058	0.565	0.144	<0.001	0.089
Soybean meal	0.50	913.0[a]	73.5	551.1	-	146.8	228.6
	0.75	906.1[a]	72.7	549.6	-	89.7	288.0
	1.00	897.8[b]	72.9	549.6	-	93.2	284.2
	1.50	897.6[b]	73.1	548.3	-	97.7	280.9
SEM		2.63	0.16	0.48	-	10.68	10.9
P-value		0.041	0.473	0.237	-	0.174	0.146

[1] 1000-ash-crude protein-starch-neutral detergent fibre.
[2] Ground first using a cut mill with 4 mm screen size.
[a, b, c, d] Values with different superscripts within a column per ingredient are significantly different ($P < 0.05$).

Table 6.3 *In vitro* digestible organic matter and crude protein (g/g dry matter) of comminuted maize and soybean meal as affected by mill screen size.

Ingredient	Mill screen size (mm)	*In vitro* organic matter digestibility	*In vitro* crude protein digestibility
Maize[1]	0.50	0.888	0.898
	0.75	0.897	0.903
	1.00	0.884	0.881
	1.50	0.871	0.867
SEM		0.0045	0.0067
P-value		0.257	0.194
Soybean meal	0.50	0.834[a]	0.958
	0.75	0.829[ab]	0.957
	1.00	0.833[a]	0.958
	1.50	0.814[b]	0.953
SEM		0.0032	0.0009
P-value		0.028	0.185

[1] Ground first using a cut mill with 4 mm screen size.
[a, b] Values with different superscripts within a column per ingredient are significantly different ($P < 0.05$).

6.4 Discussion

Sequential grinding was used to obtain the 1.00, 0.75 and 0.50 mm comminuted maize and SBM. In addition, the maize kernels were first ground using a cut mill with screen size of 4 mm before grinding over the 1.50 mm screen. This scheme, the slow and careful grinding, the quantitative collection of all ground material, proper mixing and division of material were employed in order to not only avoid unwanted heat generation but also to obtain representative samples differing only in particle size as the aim was the determination of the influence of particle size on *in vitro* digestibility.

Grinding over a smaller-sized screen decreased the GMD and GSD in maize and SBM. Similar results of a decreased GMD and GSD with reducing diameter of screen holes (6/64″, 10/64″ and 16/64″) while grinding maize in a hammer mill were also reported by Saensukjaroenphon et al. (2017). Wondra et al. (1995b) reported that hammer-milling maize over a screen opening of 9.53 (3/8″) and 1.59 (1/16″) mm reduced the GMD of particles from 826 to 419 μm and the GSD from 2.5 to 1.7 μm, respectively.

The non-significant effect of milling screen size on CP and starch in the present study, is as expected. Grinding mainly changes the physical properties such as the particle size and/or particle shape instead of chemical composition. Although there was no clear direction of the effect, the NDF content between the four comminuted maize samples were significantly ($P < 0.001$) different. It is highly likely that this is the result of the analytical method by which NDF is determined. Unlike CP and starch, in the NDF analysis a filtration step is used where finer particles already present or generated due to treatment with laurate and amylase, are not retained. Although differences were small (0.4 g/kg between smallest and largest value), a trend in ash content was observed for maize. Possibly this may be related to the NDF effect. The NDF content of SBM ground over different sized screens did not differ due to the large standard error of the mean (10.7 g/kg DM). The DM content increased with increasing fineness of grind with significant differences observed in SBM samples and a trend for maize. This loss of moisture can likely be attributed to the additional grinding of the 1.00, 0.75 and 0.50 mm samples and may indicated that, although great care was taken, some additional heat was generated that increased moisture loss. Probst et al. (2013) reported that a significant amount of heat was generated in the grinding chamber due to particle-particle and particle-hammer friction when hammer-milling maize over a 1.6 mm screen. In addition, the increase in surface area of the broken particles can further facilitate moisture loss. Alternatively, the additional processing time required for material with a finer screen size (exposure to air) may have affected the DM content.

The present study was designed to determine the influence of mill screen size on the estimation of OM and CP *in vitro* digestibility of maize and SBM by the Boisen and Fernández (1995) assay as previously Lyu et al. (2021a,b) found a major effect of grinding of fractions on *in vitro* analysis. The effect of particle size of the material on the digestibility of nutrients is also observed in *in vivo* trials (Kim et al., 2005; Lahaye et al., 2008; Ball et al., 2015; Rojas and Stein, 2015) with smaller particles resulting in higher nutrient digestibility values in pigs. In the present study, particle size did not significantly affect OM and CP *in vitro* digestibility of maize and CP digestibility of SBM. These results are in line with data of Boisen and Fernández (1997) who showed that grinding of maize and SBM over a 3 instead of 1 mm sieve had a minor effect (-1.4 and -0.4% units, respectively) on OM *in vitro* total tract digestibility. Effects on OM *in vitro* digestibility may, however, be larger compared to total tract digestibility as the additional step of using a mixed multi-enzyme complex containing a wide range of microbial carboanhydrases including arabinase, cellulase, B-glucanase, hemicellulase, xylanase and pectinase, is omitted. Lyu et al. (2021b) found that the grinding of hammer milled and size fractionated maize and SBM over a 1 mm screen (as per specification of the assay) significantly increased the OM and CP *in vitro* digestibility coefficients: grinding maize particles > 3360 µm increased the OM *in vitro* digestibility value from 0.161 to 0.907 and grinding SBM particles > 1190 µm increased CP *in vitro* digestibility from 0.783 to 0.830. The authors also reported a significant interaction effect between grinding before *in vitro* digestion and sieve size indicating different effects depending on sieve size. In general, *in vitro* digestibility of both OM and CP were significantly lower for fractions collected on sieves ≥ 595 µm with smaller particles showing no significantly lower values (Lyu et al., 2021a,b). The non-significant results in the present study can be explained by the fact that most of the maize and SBM particles were < 595 µm (Fig. 6.1). The mass of particles ≥ 595 µm made up less than 5% of the material milled over the 0.50, 0.75 and 1.00 mm screens with maize ground over the 1 mm screen having a value of 2.6% and SBM 4.6%. As such the data of Lyu et al. (2021a,b) and those reported here appear to be in agreement. As the fraction of material collected on sieves ≥ 595 µm was small, the *in vitro* digestibility values were not significantly affected. The major effect observed in the studies of Lyu et al. (2021a,b) were due to the particles ≥ 595 µm making up approximately 88% for maize and 70% for SBM. Especially the grinding over a 1.0 mm screen of these larger fractions (≥ 595 µm) significantly increased *in vitro* digestibility.

From the above it is clear that it is important that before OM and CP *in vitro* digestibility determination according to the Boisen and Fernández (1995) method and likely also other methods, the PSD is determined, especially the mass fraction of particles ≥ 595 µm for at least maize and SBM. In order to provide first estimates, we determined this mass fraction (including use of additional rubber balls during sieving) for three compound feeds and 10 commonly used feed ingredients after direct grinding (not sequential) over a 1 mm screen with trapezoidal holes as would be practiced in the assay (Table 6.4). Soybean meal had the lowest percentage (8.0%) of mass fraction ≥ 595 µm. The three compound feeds, together with maize, wheat and rapeseed meal showed values between 10 and 15% followed by sunflower seed meal and barley with 17.3 and 19.4%. For rye, oats and wheat bran, more than 25% of the mass was collected on sieves with a size ≥ 595 µm with rye having the largest mass fraction of 32.1%. As expected, the percentages for maize (12.8 *vs* 2.6%) and SBM (8.0 *vs* 4.6%) were greater than the values obtained when the sequential grinding was used. It should be noted that the values in Table 6.4 were determined on one sample for each feed/ingredient and that variation within feed/ingredient may affect this percentage. Whether the cut off value of 595 µm also applies to these feeds/ingredients as is the case for maize and SBM is unknown. As feed/ingredient breaking characteristics, mill design, mill operation, screen design and other factors can affect PSD, the variation in PSD within and between feeds and ingredients and its effect on OM and CP *in vitro* digestibility should be further investigated. Particle size distribution next to composition of materials may be a significant contributor to the variation in OM and CP *in vitro* digestibility values.

Table 6.4 Mass fraction of particles larger than 595 µm of three compound feeds and 10 commonly used feed ingredients milled over a 1 mm sized screen.

Material[1]	Mass fraction (%)
Weaned piglets compound feed	13.4
Lacto sow compound feed	10.1
Poultry compound feed	11.9
Maize	12.8
Soybean meal	8.0
Barley	19.4
Rye	32.1
Peas	28.9
Oats	28.9
Wheat	12.3
Wheat bran	25.6
Rapeseed meal	14.8
Sunflower seed meal	17.3

[1] One feed or ingredient was analyzed.

6.5 Conclusions

Grinding maize and SBM over a ≤ 1 mm sieve did not affect the determination of OM and CP *in vitro* (ileal) digestibility. Particles of ground maize and SBM should not exceed 595 µm as this appears to be the cut off for the influence of particle size on *in vitro* digestibility of OM and CP for these two feed ingredients. The grinding of feeds and feed ingredients over a 1 mm sieve as specified in the *in vitro* digestibility assay of Boisen and Fernández (1995) shows a significant mass fraction (8.0 to 32.1%) exceeding 595 µm that may affect digestibility values. Breaking behaviour of feeds and feed ingredients should be investigated in more detail as well as the cut off where particle size affects *in vitro* (ileal and total tract) digestibility values of other ingredients than maize and SBM, and of feeds.

Acknowledgments

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Chapter 7 **General discussion**

The objective of the studies reported in this thesis was to gain insight into the breaking behaviour of maize and soybean meal (SBM) after hammer-milling, via the physical and chemical characteristics of size-fractionated particles, relating to the *in vitro* digestibility of nutrients. In addition, the *in vitro* digestibility of crude protein (CP) and organic matter (OM) of fractionated ingredients were analysed to provide a potential explanation for inconsistent results in e.g. pig growth performance (Chapter 2). In Chapter 3, it was found that the nutrient content and the physical properties of various size fractionated particles differed significantly. These results revealed possibilities of using nutrient content and particle physical properties to use as an indicator for the breaking behaviour of ingredients such as maize and SBM. Further research was conducted to study the effect of moisture content (MC) and mixing ratio of ingredients on breaking behaviour (Chapters 4 and 5). In Chapter 3, as the protocol describes, grinding samples to pass a 1mm sieve before the determination of *in vitro* nutrient digestibility showed unexpected effects on the digestibility results. The effect of particle size on the *in vitro* digestibility was further investigated in Chapter 4 and 6.

In this final chapter, the following three aspects will be discussed i) limitations of particle size determination methods, ii) breaking behaviour, and iii) effect of particle size on *in vitro* digestibility measurement. Finally, conclusions are drawn from the research described in this thesis and recommendations are provided.

7.1 Limitation in particle size determination methods

There are different ways to determine the size of particles produced via various grinding processes of the many ingredients used in the feed industry (reviewed in Chapter 2). Particle size are normally expressed with geometric mean diameter (GMD) that derived from particles size distribution (PSD), which may be limited in application and do not fully capture the information of particles. In this project, wet sieving and image analysis were used in addition to dry sieving to determine physical characteristics of particles (Chapter 3). Wet sieving involves soaking, washing and drying samples which takes approximately 2 h on average for one sample, compared to 15 min for dry sieving. The soaking and washing procedure make wet sieving more applicable for pelleted feeds, digesta or feces samples than dry sieving (Uden and Van Soest, 1982; Dixon and Milligan, 1985; Dirkzwager et al., 1998; Wolf et al., 2010). During wet sieving, particles swell and ultimately may lead to a different PSD compared to dry sieving. On the other hand, determination of PSD by wet sieving can be argued from first principles to be more related to the digestibility of nutrients than dry sieving because particles in the gut also reside in a moist/wet environment. For dry mash diets and ground ingredients, the less labour-intensive dry sieving is, however more convenient (Chapter 3) although it has its limitations in determining PSD of finely ground material. Agglomeration of finely ground material can occur during this sieving procedure (Chapter 6). In the current thesis, PSD was determined according to ASABE (2008) standard procedure as also described in Chapter 3. In this procedure, rubber balls are used to aid reduction of particle agglomeration on the sieve sizes < 300 μm. The PSD of maize and SBM sample sequentially ground to 0.50 mm in Chapter 6 were also determined using this method. For the maize, it was observed that large amounts of fine, agglomerated particles were retained in varying amounts on the 595 μm sieve (~ 40% of the mass for the 0.50 mm ground maize, Fig. 7.1 A). For SBM, this agglomeration was not observed indicating that the agglomeration was likely related to the starch particles of the material. To reduce agglomeration, two rubber balls were additionally added to each sieve smaller than 595 μm. Figure 7.1 B shows the effect of adding rubber balls to these sieves on PSD of maize. Agglomeration on the sieves with an opening of 420 (left) and 595 μm was no longer observed (see photograph Fig. 7.1 B) leading to a different PSD more closely representing the distribution of particles. On the edge of the smaller-sized sieves (149 and 210 μm, Fig. 7.2), however, small amounts of agglomerated material were still observed. This material collected on the tapered rim of the sieve where the rubber balls could not roll. Sieves with a different design (containing no (tapered) rims) are likely to be more accurate to determine PSD of very finely ground material, especially those containing high amounts of starch. Alternatively, other particle size determination methods can be employed, such as automatic image analysis or laser diffraction. The devices using these two methods includes sample dispersion units to reduce particle adhesion. Despite this, the image analysis and laser diffraction methods have their application restrictions in the field of animal feed because of the wide particle size ranges of the measured material, even though these methods have been widely used in biology, pharmacy, and food processing. As indicated in Chapter 3 and 4, the particle size of hammer milled maize and SBM ranged from > 3.36 to < 0.053 mm. The combination of a microscope and camera is not able to detect particles < 0.053 mm, as this approach has difficulties in the manual dispersion of particles. The Morphologi 4 (Malvern Panalytical company) can disperse the fine samples by high pressured air and analyse the particle size and shape automatically, which is time saving and accurate. However, this method can only handle materials with a particle size range < 1.3 mm. A similar situation applies to the laser diffraction device. Although Mastersizer 3000 (Malvern Panalytical company) claims it can analyse particles over a wide range of 0.0001-3.5 mm, there is a high risk of blockages in the dispersion unit when particles are relatively large or the PSD in the sample has a large range.

All these particle size determination methods have their own advantages (Chapter 2) and limitations, how to choose these methods is dependent on the study purpose, demand of the accuracy of the results, and the cost of money, time, and labour. And the results obtained from different methods should be treated carefully based on a good understanding of the advantages and drawbacks of these methods.

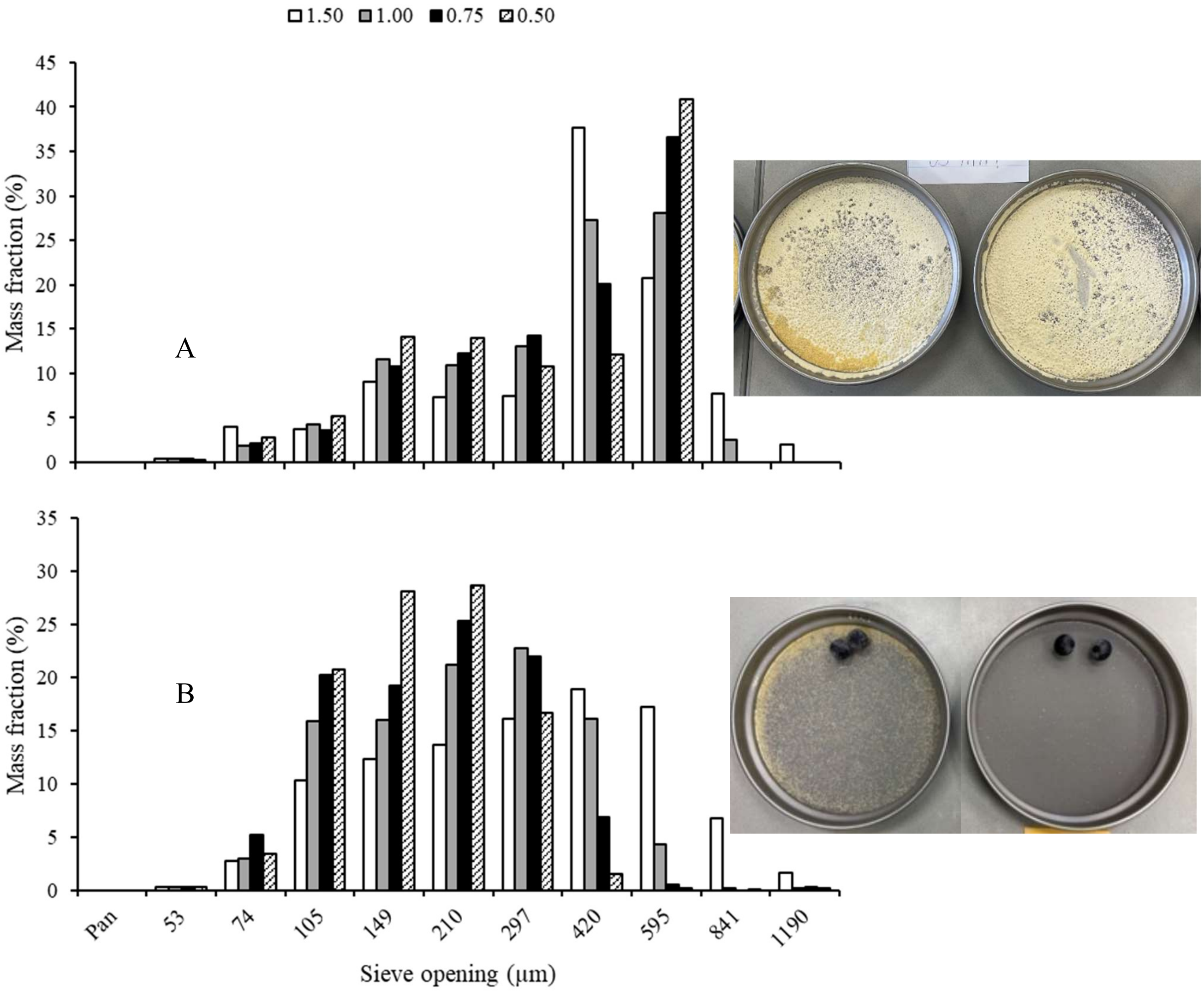


Fig. 7.1 Mass fraction distribution of comminuted maize using mill screen sizes of 1.50, 1.00, 0.75 and 0.50 mm with two rubber balls on each sieve opening ≤ 297 μm (A), and with two rubber balls on each sieve opening of 595, 420 and 297 μm, four rubber balls on each sieve opening < 297 μm (B). Maize was ground first using a cut mill with 4 mm screen size. Each photograph shows a sieve with an opening of 420 (left) and 595 (right) μm.

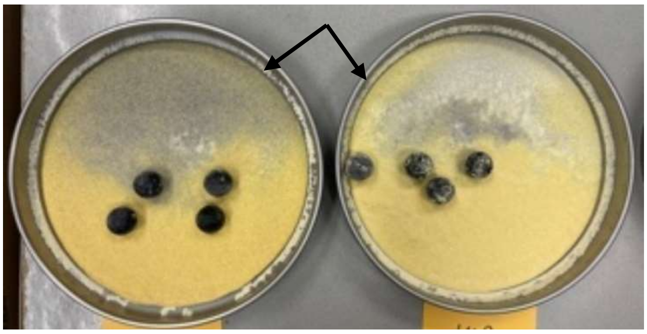


Fig. 7.2 Agglomerated ground maize material (arrows) on the 210 (left) and 149 (right) μm sieves.

7.2 Breaking behaviour

Grinding is an essential procedure in animal feed processing. Appropriate reduction of the particle size of feed ingredients is conducive for the mixing and conditioning, pelleting, and expansion of feeds, and can significantly increase the conversion rate of feeds, reduce animal excreta, and improve animal production. There have been numerous studies investigating the effect of particle size of feed ingredient or diet on animal performance. However, the observed results are not always consistent (Lawrence et al., 2003; Li et al., 2019) and the underlying reasons are not fully understood. This maybe because the current particle size determination and expression methods used are insufficient to capture key characteristics of the particles (e.g. circularity, surface area of particles) (Chapter 2) related to nutrition. As mentioned before, wet sieving which is less often used compared to dry sieving may be more representative of conditions in the gut. Considering that particle characteristics can be affected by many factors (e.g. MC of material, interactions between particles) during grinding, the reproducibility of hammer-milling ingredients and how this influences the properties of particles become important.

7.2.1 Reproducibility of hammer-milling maize and soybean meal

In Chapter 3, 4 and 5, the maize and SBM were hammer milled under similar conditions, which makes it possible to assess the reproducibility of hammer-milling such ingredients via physical, chemical, and *in vitro* digestibility characteristics of fractionated particles. In Chapter 3, maize and SBM were hammer milled over a 6- or 2-mm sized screen, respectively. In Chapter 4, non-moisture treated maize and SBM (with 0 g/kg moisture addition) were ground under identical grinding conditions (same machine, rpm, screen size, etc), although the adjustable inlet was opened to a different position to control the feeding speed during milling: 50% position in Chapter 3 and 80% position in Chapter 4. In Chapter 5, the 100% SBM sample was hammer milled under the same condition as in Chapter 4. In these three chapters, maize and

SBM were sieved into different fractions after hammer-milling. To collect sufficient material from each sieve (at least 70 g) for chemical and *in vitro* digestion analysis, the choice of sieve size was adjusted according to the PSD of maize and SBM. The sieve openings for classifying maize and SBM by size in Chapters 3, 4, and 5 are presented in Table 7.1.

Table 7.1 Sieve openings for sieving maize and soybean meal in Chapter 3, 4, and 5.

Chapter	Ingredient		Sieve openings (mm)						
3	Maize		3.360	2.380	1.680	0.841	0.420	0.210	Pan
4	Non-moisture treated maize		3.360	2.380	1.680	0.841	0.420		Pan
		Fraction*	F1	F2	F3	F4	F5	F6	F6
3	Soybean meal		1.190	0.841	0.595	0.297	0.149	0.074	Pan
4	Non-moisture treated soybean meal		1.190	0.841	0.595	0.420	0.210		Pan
5	Soybean meal		1.190	0.841	0.595	0.420	0.297	0.149	Pan
		Fraction	F1	F2	F3	F4	F4	F4	F4

*For fractions that the sieve openings are different throughout the chapters, the physical characteristics, nutrient amount and *in vitro* digested OM and CP of material on each sieve were combined into one fraction.

As reported in Textbox 7.1, the reproducibility of hammer-milling maize and SBM was assessed using PSD, physical characteristics, nutrient amount and *in vitro* digested OM and CP. It was found that the PSD, nutrient amount as well as the *in vitro* digested OM and CP of fractionated maize and SBM did not differ ($P > 0.05$) between chapters after hammer-milling. This means that the hammer-milling of the maize and SBM appear to be very reproducible, at least from the perspective of PSD, nutrient amount distribution and the *in vitro* digested OM and CP. However, the physical characteristics of the maize and SBM were less reproducible with significant ($P > 0.05$) differences being found between the chapters.

As stated in Chapter 3, the results of studies into the effect of particle size on pig performance are not consistent. The reproducibility in PSD and non-reproducibility in physical characteristics provides an indication for a new reason to explain this inconsistency. Hammer-milling ingredients can result in similar PSD and GMD, however, the physical properties of particles are different even though they were ground under identical condition. In Chapter 3 and 5, it is found that the physical characteristics were better related to *in vitro* digestibility of

OM and CP than GMD, however, they have not been considered in previous studies, which have focused on PSD and from which the GMD was calculated. It is possible that effects of particle size on the pig growth performance may be due to the other physical characteristics of particles, e.g. projected area, circularity, and aspect ratio rather than GMD.

Textbox 7.1. Reproducibility of hammer-milling maize and soybean meal

Aim

Determine whether hammer-milling maize and SBM is reproducible.

Material and methods

Data were used from Chapters 3, 4 and 5. Considering that some of the sieving openings used in these three chapters were different, for example, in Chapter 3 and 4, maize was sieved with/without 0.212-mm sized sieve, the material on the sieve of 0.212 mm and in the pan were considered as one fraction to compare physical characteristics and nutrient amounts in fractions (Table 7.1). The latter were calculated from the nutrient content and PSD data. Similarly, the amount of *in vitro* digested nutrients was calculated as digestibility coefficient × weight of material of each fraction. The physical characteristics were calculated into weighted average using the PSD as weight.

Data on the physical characteristics and the nutrient amount of fractions reported in Chapter 3, 4 and 5 were analysed by two-way analysis of variance using the general linear model in R 3.6.1 (R Core Team, 2019). The statistical model used was:

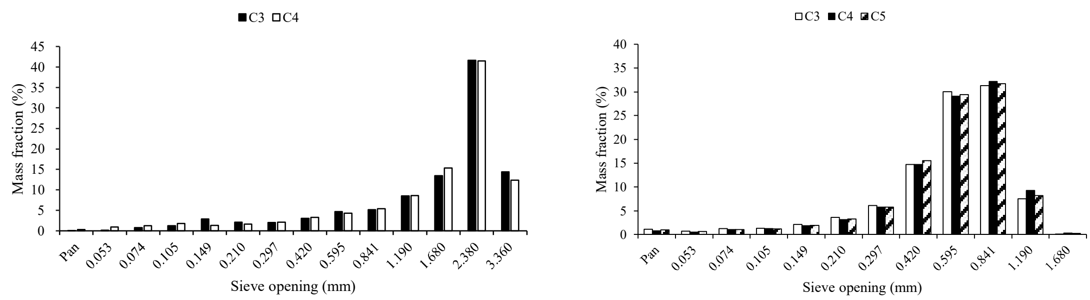
$$\gamma_{ijk} = \mu_0 + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ijk}$$

where γ_{ijk} = response variable (response variables) (k = 2, the number of measurements), μ_0 = overall mean, α_i = effect of chapter, i (i = 1..3), β_j= effect of fraction j (j = F1..F4 for SBM or j =F1...F6 for maize, Table 7.1), $(\alpha \times \beta)_{ij}$ = interaction of chapter i and fraction j and ε_{ijk} = residual error with a mean of 0 and variance σ^2. α_i and $\beta_j$were fixed effects and the minimum significance threshold was set at 0.05.

Results

As shown in Fig. 7.3, there was no significant difference between the PSD which was generated in different chapters. From the perspective of nutrient amount, hammer-milling maize and SBM are also reproducible with no significant difference being found in DM and CP for both ingredients, starch and ash for maize, and NDF for SBM. Also, the *in vitro* digested OM and CP of maize and SBM did not differ (P > 0.05). However, after hammer-milling, the physical characteristics of maize were different (P < 0.05) between the Chapter

3 and 4. Similarly for SBM, projected area, projected perimeter and solidity differed between the chapters, although the aspect ratio, circularity and roundness of hammer milled SBM particles did not differ ($P > 0.05$).



a b

Fig 7.3 Mass distribution of hammer milled maize (a) and soybean meal (b). Data was obtained from Chapter (C) 3, 4 and 5.

Conclusion

The PSD, nutrient amount and the *in vitro* digested OM and CP of fractions are reproducible for both maize and SBM. However, the physical characteristics of maize and SBM particles after hammer-milling were not reproducible between chapters. The unreproducible physical characteristics might be a lead to explain inconsistent results obtained in previous studies in the literature regarding the effect of particle size on pig growth performance.

7.2.2 Factors affecting breaking behaviour of hammer-milling maize and soybean meal

In Chapter 3, it was found that the nutrient content, physical characteristics of maize and SBM particles that were (sieve size) classified differed significantly ($P < 0.001$). This showed the possibilities of using nutrient distribution and physical characteristics to investigate the breaking behaviour of feed ingredients during grinding. For example, in maize, bonds between starch and protein are mainly presented in the endosperm, and after grinding the smallest sized maize fraction contains the highest starch content, which means that the soft endosperm is more likely to be milled into fine particles (Chapter 3). Moisture content of material is considered an important factor in grinding as it affects various intrinsic properties of ingredients such as strength, stiffness, elasticity and plasticity (Jung et al., 2018) which, in turn, may influence breaking behaviour and energy consumption (Jagtap et al., 2008). Therefore, in Chapter 4, the effect of MC on the breaking behaviour of maize and SBM was investigated. The MC of ingredients were adjusted to 14 and 16% by adding 30 and 60 g/kg water, respectively. Together with the original MC of maize and SBM (~12%), these MC levels were considered relevant to the feed industry. The results showed that the nutrient content and physical characteristics of fractionated particles were less affected by MC. This indicates that the MC has limited effects in hammer-milling on the breaking behaviour of maize and SBM in the range of investigated MC, although the compression and tension test results showed changes in the plastic deformation of the raw materials.

From the perspective of practical feed production, it is common practise in the feed factory to combine feed ingredients before grinding. For this reason, the effect of mixing ratio on the breaking behaviour of feed ingredients was investigated. Maize and SBM were mixed in percentages of 25:75, 50:50 and 75:25 and like the original ingredients hammer milled under identical conditions. The results showed that the nutrient content of fractionated particles and the PSD were significantly ($P < 0.001$) affected by the mixing ratio. In addition, SBM tended to end up in the middle-sized fractions and maize in the coarse and fine fractions when examining the PSD, starch and CP content: the mixture with more SBM made up a higher percentage of CP but showed a lower percentage of starch in fractions of sieving openings of 0.595 and 0.841 mm. Compared with grinding first and then mixing, mixing before grinding affects the breaking behaviour of maize and SBM with interactions between fraction and mixing ratios being observed ($P < 0.001$) and results in a lower energy consumption. If the maize and SBM were milled first and then mixed, then the nutrient content distribution can be considered as a linear relationship and can be calculated as:

$$P_i = x \cdot n_{im} + (1 - x) \cdot n_{is}$$

where: P is the predicted nutrient content (g/kg DM) on the i^{th} sieve; x is the maize to SBM ratio of the mixture (equals 0.25, 0.50 and 0.75); n_{im} is the nutrient content of 100% maize on the i^{th} sieve and n_{is} is the nutrient content 100% SBM on the i^{th} sieve;

The comparison between the calculated and the measured nutrient content distribution along with sieve size make it possible to visualize the interactions between maize and SBM while hammer-milling (Fig. 7.4).

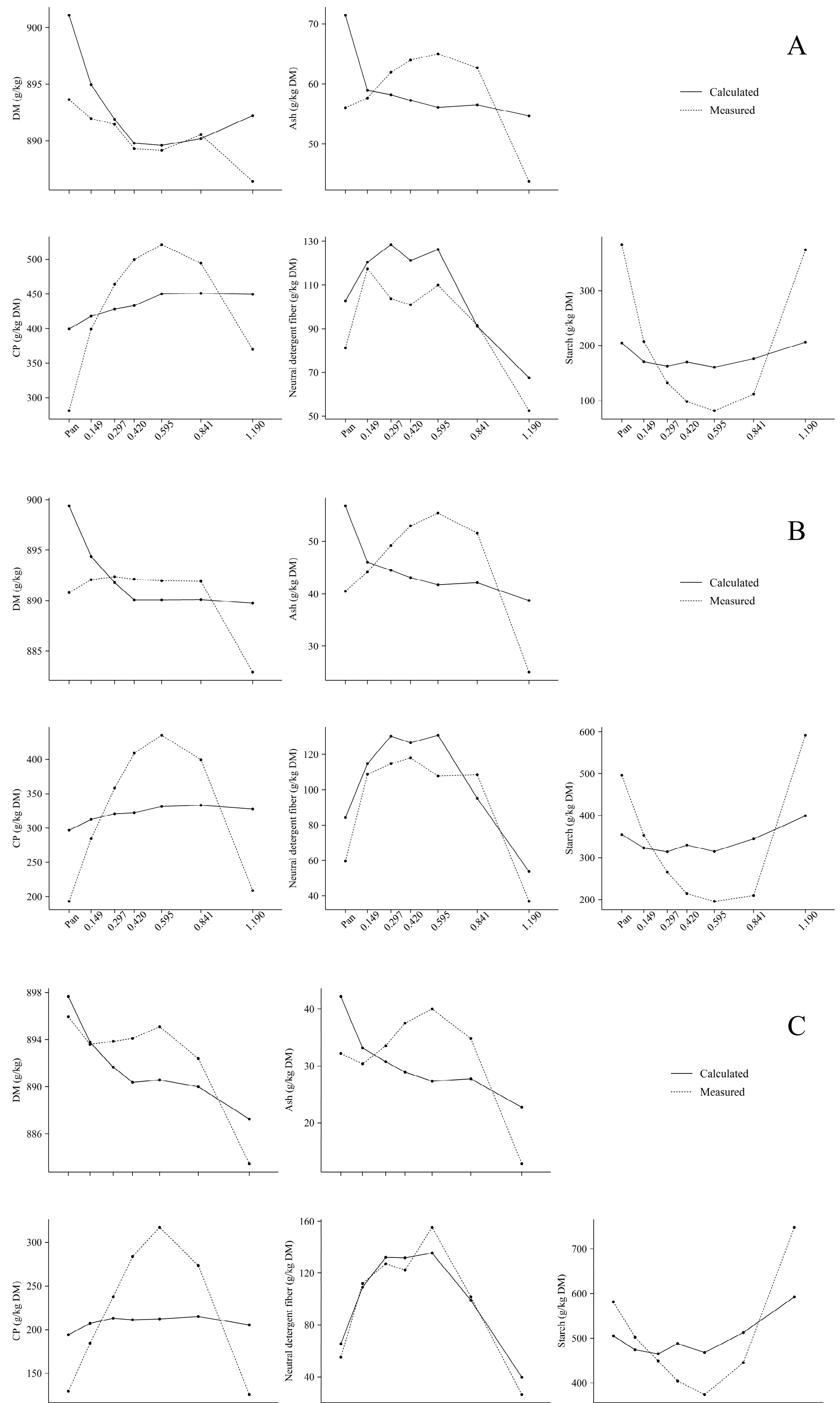


Fig.7.4 Interactions between maize (M) and soybean meal (S) while hammer-milling the mixtures (% M:S; 25:75 (A), 50:50 (B), 75:25 (C)) of the two ingredients as measured and calculated based on M and S.

7.2.3 Nutrient content *vs* nutrient amount

The nutrient content of ingredients has always been the focus of nutritionists over the decades. However, according to the results in this thesis, after grinding, the in vitro digestibility of nutrients of fractionated particles differed (Chapters 3, 4 and 5). Therefore, how much material was retained on each sieve layer and how much nutrients were delivered to animals per size class, becomes even more important than the nutrient content of fractionated particles. In the previous chapters, the nutrient content and *in vitro* digestibility of OM and CP coefficients were expressed based on the quantity of material retained on each sieve layer, instead of the whole ingredients (the total amount of material). As Fig. 7.5 shows, after hammer-milling, the amount of maize, SBM and their mixtures distributed differently, which led to a different nutrient amount distribution.

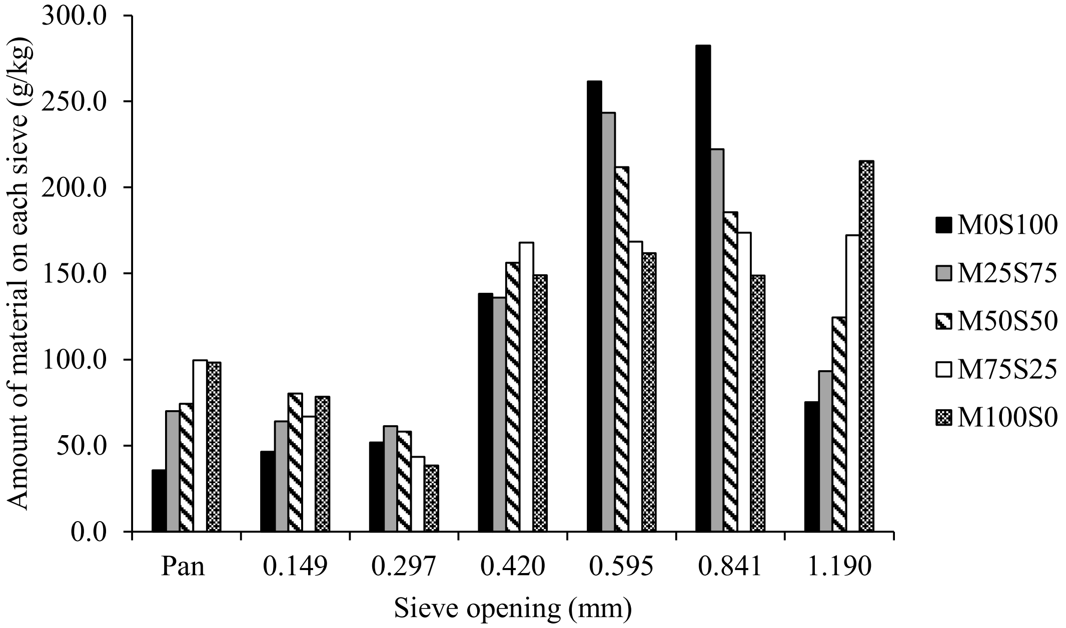


Fig. 7.5 Amount of material retained on different sized sieves of hammer milled maize (M), soybean meal (S) and three mixtures (%M:S; 25:75, 50:50, 75:25) of the two. Expressed based on dry matter.

Figure 7.6 presents the comparison of nutrient content and nutrient amount of hammer milled maize and SBM and their mixtures (%M:S; 25:75, 50:50, 75:25; data from Chapter 5). As can be seen in the mixture of M25S75, starch makes up a large fraction on each sieve ranging from 81.4 to 383.4 g/kg DM (Fig. 7.6 A). However, there is a small amount of starch in the mixture, which is from 8.1 to 34.8 g/kg DM (Fig. 7.6 B). Similar for the material of SBM (M0S100), the CP content on each sieve is similar ranging from 502.2 to 571.7 g/kg DM (Fig. 7.6 A), whereas the amount of CP on each sieve are quite different with the smallest amount of

17.9 g/kg DM and the largest amount of 160.7 g/kg DM (Fig. 7.6 B). This is related to the PSD of hammer milled SBM in which a large amount of material was retained on the sieves size of 0.595 and 0.841 mm compared to the other fractions.

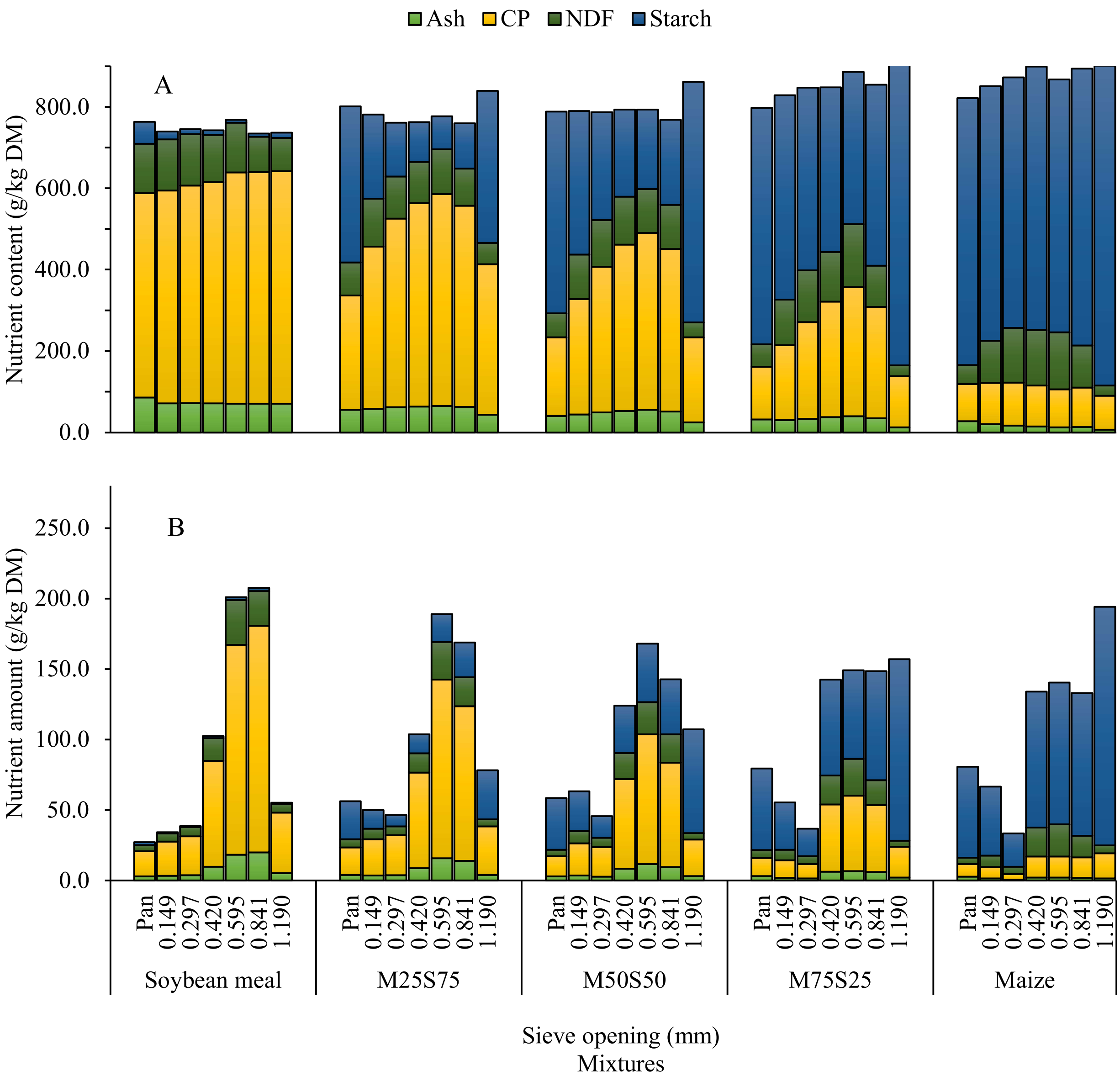


Fig. 7.6 The nutrient content (A) and the nutrient amount (B) per unit dry matter (DM) of particles retained on different sized sieves of hammer milled maize (M), soybean meal (S) and three mixtures (%M:S; 25:75, 50:50, 75:25) of the two.

7.3 Effect of particle size on *in vitro* digestibility

Static methods to measure *in vitro* digestion have been, and are still, used for food, feed and drug evaluation purposes (Boisen and Fernández, 1997; Kaukonen et al., 2004; Maldonado-Valderrama et al., 2010) because of their simplicity, cost/time savings and acceptable reproducibility. *In vitro* digestion models have been found to be useful in predicting the outcomes of *in vivo* digestion trials (Furuya et al., 1979; Huang et al., 2003; Noblet and Jaguelin-Peyraud, 2007; Bohn et al., 2018; Sanchón et al., 2018). The effect of particle size on the digestibility of nutrients is evident in both *in vitro* (Lyu et al., 2021a,b) and *in vivo* trials (Kim et al., 2005; Lahaye et al., 2008; Ball et al., 2015; Rojas and Stein, 2015). Reducing particle size increases the surface-to-volume ratio which improves enzyme access, interaction with the substrate and the release of nutrients from different food/feed matrices. Different to *in vivo* trials, commonly used *in vitro* simulation methods lack the chewing process of animals or humans. *In vitro* trial protocols almost all require that samples should be ground or minced to smaller particles prior to start the *in vitro* analysis (e.g. Boisen and Fernández, 1995; 1997; Minekus et al., 2014).

In Chapter 3, the *in vitro* analysis protocol of Boisen and Fernández (1995; 1997) were used to determine the *in vitro* digestibility of CP and OM of size fractionated maize and SBM particles. In the protocols, samples are required to be ground over 1mm sieve. In Chapter 3, there were four fractions of maize and one fraction for SBM where the GMD was larger than 1 mm (Table 7.2). To follow the *in vitro* digestibility protocol, these 5 fractions were additionally ground in a laboratory mill to pass 1 mm sieve while the other fractions which were already smaller than 1 mm, were not additionally ground. Unexpectedly, I found that reducing the particle size of the fractions larger than 1 mm before *in vitro* digestion increased the digestibility coefficient of OM by up to 0.746 in maize and 0.047 in SBM. To corroborate or contradict these preliminary observations, all fractionated particles were ground over a 1 mm sieve before *in vitro* analysis in Chapter 4 (Table 7.2). Again, significant effects were observed of the additional grinding for both OM and CP digestibility. However, grinding affected the *in vitro* digestibility differently among the various fractions. The effect of particle size on *in vitro* digestibility was especially found for the material with particles > 595 μm. The effect of grinding on OM digestibility increased as the particles became larger. The *in vitro* digestibility coefficients of OM increased up to 0.68/0.06 for maize/SBM and the digestibility coefficients of CP increased up to 0.07 for SBM after grinding particles larger than 595 μm. This confirmation of the findings from Chapter 3 and Chapter 4 led to a further investigation, the results of which are reported in Chapter 6. Here the aim was to determine the influence of particle size on the values for OM and CP digestibility generated by the Boisen and Fernández (1995) assay. For this, the *in vitro* OM and CP digestibility of maize and SBM were analysed with samples milled sequentially over a 1.50, 1.00, 0.75 and 0.50-mm screen. Unexpectedly, the *in vitro* digestibility of nutrients was not affected although the particle size was shown to decrease with decreasing screen size.

Table 7.2 *In vitro* digestibility coefficient of organic matter and crude protein of hammer milled maize and soybean meal (SBM) fractions with (+) or without (-) additional grinding over a 1.0 mm sieve.

Ingredient	Chapter	Sieve opening	Particle size mass fraction (%)	*In vitro* digestibility: Organic matter, 1 mm milling +	Organic matter, 1 mm milling -	Crude protein, 1 mm milling +	Crude protein, 1 mm milling -
Maize	3	Whole sample	100	0.870			
		0.0 (pan)	5.1	0.935			
		0.210	4.1	0.798			
		0.420	7.8	0.710			
		0.841	13.7	0.798*	0.476		
		1.680	13.4	0.888*	0.302		
		2.380	41.6	0.907*	0.212		
		3.360	14.3	0.907*	0.161		
	4	Whole sample	100	0.826	0.346		
		0.0 (pan)	7.4	0.897	0.890		
		0.42	5.9	0.776	0.749		
		0.841	11.8	0.767*	0.546		
		1.68	14.8	0.831*	0.315		
		2.38	43.2	0.872*	0.216		
		3.36	16.9	0.856*	0.172		
Soybean meal	3	Whole sample	100	0.809			
		0.0 (pan)	1.9	0.858			
		0.074	2.6	0.811			
		0.149	5.8	0.778			
		0.297	20.8	0.774			
		0.595	30.1	0.775			
		0.841	31.3	0.761			
		1.19	7.5	0.830	0.783		
	4	Whole sample	100	0.794	0.764	0.937*	0.900
		0.0 (pan)	5.1	0.799	0.807	0.941	0.951
		0.21	8.4	0.771	0.778	0.932	0.934
		0.42	14.1	0.773*	0.791	0.925	0.934
		0.595	27.7	0.797*	0.778	0.940*	0.914
		0.841	32.9	0.800*	0.755	0.945*	0.881
		1.19	11.8	0.818*	0.762	0.949*	0.875

* Values significantly differ (P < 0.05) to corresponding without additional ground samples.

The fact that fine grinding over 1 mm sized screen before *in vitro* digestion analysis affected the OM/CP *in vitro* digestibility in the Chapters 3 and 4, whereas there was no significant effect observed in the Chapter 6 can be explained by four aspects. Firstly, different materials were analysed for *in vitro* digestibility in these chapters. In Chapter 6, whole ground maize and SBM were analysed, which is common practice in the feed industry. While in Chapters 3 and 4, fractionated maize and SBM were analysed. Using the digestibility data from Chapter 4 and the PSD of hammer milled material as weighted parameter, the quantity of OM and CP digested from each sieve can be calculated, which provide an estimate of the *in vitro* digestibility of the whole ground/hammer milled maize and SBM. Table 7.3 shows the comparison between the analysed OM and CP *in vitro* digestibility of whole samples and the calculated values based on data from individual fractionated materials. For the ingredients that were hammer milled first and then additionally laboratory milled, the differences between the analysed and calculated OM and CP *in vitro* digestibility values for both maize and SBM are minor (max 2.9% units for maize and 1.3% units for SBM). However, for the hammer milled material, especially in the maize, large differences were observed between the measured OM *in vitro* digestibility and the calculated values (max 51.3% units for maize with 30 g/kg moisture addition).

Table 7.3 Comparison of *in vitro* digestibility coefficients of organic matter and crude protein (g/g dry base) between ground (1 mm) whole maize and soybean meal and the calculated mean of the sum of the weighted digestibility coefficients of seven individual fractions of these ingredients [1]

Digestible nutrients	Ingredient	Moisture addition[2] (g/kg)	1 mm milling[3]				
			+			-	
			Analyzed	Calculated[4]	Difference (% units)	Calculated	Difference (% units)
Organic matter	Maize	0	0.83	0.86	2.9	0.37	45.9
		30	0.84	0.86	1.5	0.33	51.3
		60	0.81	0.82	1.6	0.33	47.9
	Soybean meal	0	0.79	0.79	0.7	0.78	1.1
		30	0.80	0.80	0.2	0.77	3.1
		60	0.80	0.79	0.1	0.77	2.3
Crude protein	Soybean meal	0	0.93	0.95	1.3	0.91	2.2
		30	0.94	0.94	0.3	0.90	4.3
		60	0.94	0.94	0.2	0.91	3.2

[1] Data was from Lyu et al. (2021a).
[2] Moisture was added (30, 60 g/kg) to maize and soybean meal for determination of effects on hammer-milling.
[3] The material was hammer milled, with (+) or without (-) additionally milling over a 1.0 mm sieve.
[4] Calculated by $\sum p_i m_i$, where p_i is the mass fraction on the i^{th} sieve/pan and m_i is the *in vitro* digestibility coefficients of organic matter or crude protein of the material retained on the i^{th} sieve with i = 1, 2, 3, ...7).

Secondly, after grinding over 1.0, 0.75 and 0.50 mm, the particle sizes of maize and SBM were quite small with the largest particle sizes being observed in maize ground with 1 mm sized screen of 260.3 μm (Table 6.1). Further reducing the particle size can only improve the *in vitro* digestibility to a limited extent. As shown in Chapter 4, digestibility values were observed to be not significantly affected when particle size was generally smaller than 595 μm (Fig. 4.7). *In vivo* trials also found that when brown rice in the diet of weaning and growing pigs was replaced by maize, the apparent total tract digestibility of gross energy, DM and CP increased when GMD decreased from 800 to 600 μm. However, no further improvements were observed in the 400 μm diet (Li et al., 2019).

Thirdly, evaluating the PSD of the finely milled maize and SBM in Chapter 6, it can be noticed that the particles larger than 595 μm make up less than 5% of the whole material. This means that, although reducing the size of particles that are > 595 μm can increase the *in vitro* digestibility, the small percentage of this material cannot make a large difference in the final digestibility. In Chapter 3 and 4, where a large effect of particle size on *in vitro* digestibility was found, the particles larger than 595 μm made up around 88% for maize and 70% for SBM particles.

Furthermore, the assays of Boisen and Fernández (1995; 1997) also specify that digested material is filtered using a glass filter crucible (pore size 40-90 μm) containing 0.4-0.5 g Celite as filter aid. As such, particles that are already smaller than ~90 μm before and those that are reduced in size during digestion are measured as having been digested and, therefore, digestibility values can be overestimated. Overall, before the *in vitro* nutrient digestibility, the PSD should be considered especially the mass fraction of particles that is > 595 μm and < 90 μm. Table 7.4 presents the total mass fraction of particles <105 and >595 μm of compound feeds and other feed ingredients after grinding over a 1 mm screen as per specification of the assay of Boisen and Fernández (1995; 1997). Due to the limitation in existing sieve sizes, the mass fraction of particles smaller than 105 μm were determined instead of 90 μm (Table 7.4). It can be noticed that a large percentage of material of a variety of feed ingredients and feeds consists of particles < 105 μm and > 595 μm when ground over a 1 mm sieve. Especially in the wheat bran that contains 75.7% of particles < 105 μm and >595 μm, followed by oats of 60.8% and rye of 49.8%. Although for the compound feeds and the other feed ingredients, the effect of mass fraction of particles > 595 μm on the *in vitro* digestibility of nutrients, and the effect of filtration on retaining particles < 90 μm, to the knowledge of authors, have not been investigated. Based on the existing research results of maize and SBM, we can speculate that the more particles < 105 and > 595 μm, the greater the impact on the *in vitro* digestibility values. Grinding characteristics of materials, not only between but also within materials (e.g. dent and flint maize) may yield variable PSD with more or less larger/smaller particles, which should be taken into account when measuring the *in vitro* digestibility of nutrients.

Table 7.4 Mass fraction of particles larger than 595 μm and smaller than105 μm in compound feeds and other feed ingredients after comminuting with 1 mm screen.

Material	Mass fraction (%)
Weaned piglets compound feed	28.9
Lacto sow compound feed	25.1
Poultry compound feed	24.6
Maize	8.2
Soybean meal	28.3
Barley	43.8
Rye	49.8
Peas	44.1
Oats	60.8
Wheat	27.5
Wheat bran	75.7
Rapeseed meal	16.1
Sunflower seed meal	26.5

7.4 Conclusions and recommendations

Feed particle characteristics play an important role in feed processing and in the nutrient absorption by animals. New determination methods such as wet sieving and image analyses may be applicable in future evaluation of physical feed characteristics where it should be noted that each method has its own advantages and limitations in application.

Physical characteristics and nutrient content of fractionated maize and SBM particles differ after hammer-milling. Moisture addition increased the plasticity of maize and SBM, resulting in increased energy consumption of hammer-milling and increased GMD of ground material. However, moisture addition showed limited effects on the physical and nutritional characteristics of fractionated maize and SBM, whereas, compared to separately hammer-milling maize and SBM, milling the mixture of the two with different mixing ratio had a significant effect on the breaking behaviour.

The *in vitro* digestibility of OM and CP were affected by particle size in fractionated maize and SBM, and the effects were mainly observed in particles > 595 μm. Additional grinding fractionated particles > 595 μm significantly increased the *in vitro* digestibility of OM and CP. Grinding samples over a 1 mm screen before determination of *in vitro* digestion is commonly expected to not influence the digestibility measurements. However, the PSD of comminuted samples should be considered, especially the quantity of particles that > 595 μm and < 90 μm. For finely ground material, particle agglomeration on sieves should be taken into account during the PSD determination procedure especially when the material contains high amounts of starch granules.

Based on the results of the present study, it is suggested that mixing ingredients before grinding and grinding material with lower MC helps to reduce the energy consumption. This is applicable in hammer mill operations, where other milling operations such as the use of a roller mill or a multicracker system warrants further research. From the perspective of energy saving and improving *in vitro* digestibility, further reducing particle size < 595 μm is not recommended in maize and SBM.

The conclusions obtained from the present thesis are based on the *in vitro* experiments; it is necessary to use animal models to verify the effect of breaking behaviour of feed ingredients e.g. maize, SBM, etc on the animal growth performance in subsequent studies.

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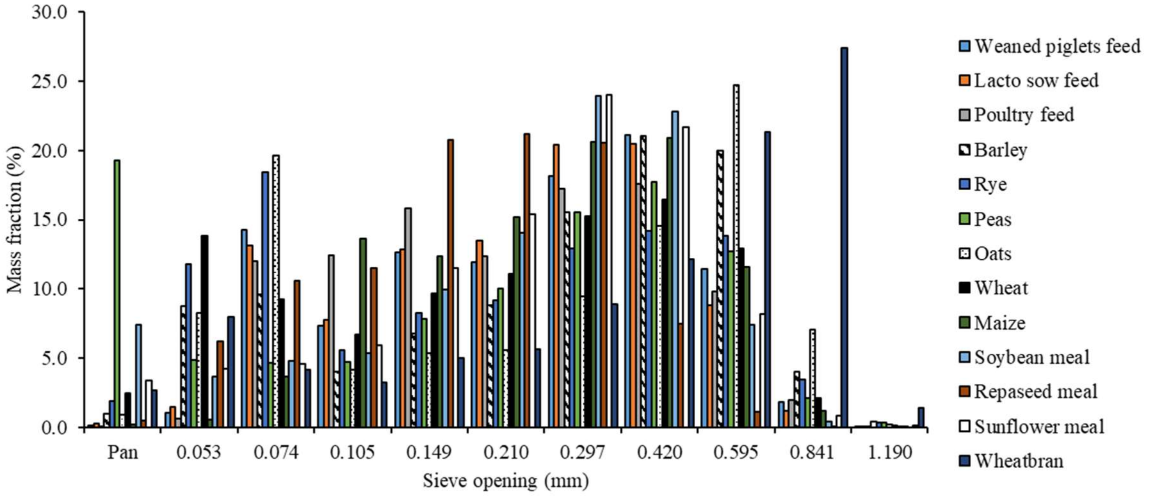
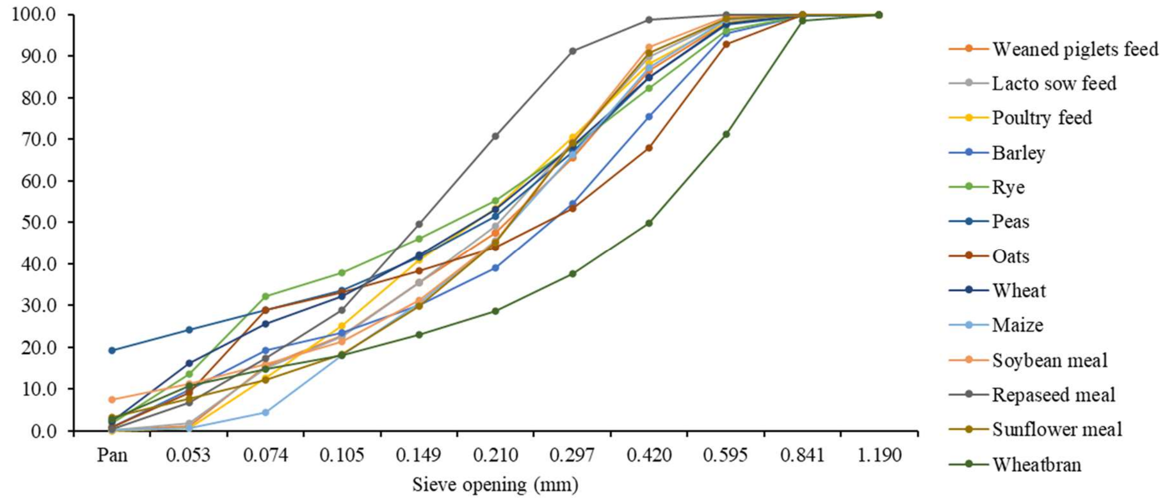
Supplementary information for:

General discussion





Mass (bar) and cumulative mass (line) distribution of compound feeds and other feed ingredients after comminuting with 1-mm sized screen in a laboratory mill.

Summary

Feed processing is an indispensable part of the sustainable development of society and the circular agricultural system. Feed processing uses various technologies to transform co-/by-products or waste from human food production into valuable feeds and feed ingredients enabling the production of animal-sourced foods (e.g. meat, eggs, milk). Continued rapid population growth and increased demand for animal-sourced food are presenting challenges for the sustainable development of the global population and the environment. It is important to use feed ingredients as efficiently as possible to meet current and future food demands. As a standard feed technology, hammer-milling is a critical step affecting feed efficiency. Currently our understanding of breaking behaviour of feed ingredients during hammer-milling is limited. After grinding, not only the particle size (e.g. geometric mean diameter, GMD) of ingredients is reduced, but also the physical characteristics of particles (equivalent particle size (EPS), morphology) and nutrient distribution are changed, which can affect the digestibility and kinetics of nutrient digestion. Currently, inconsistent effects of grinding ingredients on animal growth are found in the literature.

The objective of studies reported in this thesis was to gain insight into the breaking behaviour of maize and soybean meal (SBM) after hammer-milling, via the physical and chemical characteristics of size-fractionated particles, relating to the *in vitro* digestibility of nutrients.

A meta-analysis showed that the calculated EPS (e.g. arithmetic mean diameter, volume mean diameter) was more correlated to pig performance data (feed conversion ratio) than GMD (Chapter 2). Despite the routinely used dry sieving method, other methods (e.g. wet sieving, image analysis, laser diffraction) showed the possibility to determine and express the physical characteristics of particles: not only mean particle size but also other particle characteristics such as projected area, circularity, aspect ratio, etc. In the literature, when the effect of grinding method on particle size and subsequent pig growth performance was studied, there is an assumption that the nutrient of feed ingredients/diets are distributed evenly and the digestibility is the same, which is doubtable. The nutrient content and physical characteristics of size fractionated particles in maize and SBM after hammer-milling were analysed, and their relation to *in vitro* digestibility were explored. In addition, particles of individual size fractions were characterised by dry sieving, wet sieving and image analysis, and expressed as EPS (Chapter 3). The physical properties, nutrient content and the *in vitro* digestibility of fractionated particles differed. Relationships between the *in vitro* digestibility of organic matter (OM) and the physical characteristics were observed via the regression models. Circularity was more related to the *in vitro* digestibility of OM than GMD.

Considering that in practice, there are many factors that may affect the breaking behaviour of ingredients during hammer-milling, the effects of moisture content (MC) and mixing ratio on the physical, chemical characteristics and *in vitro* digestibility of nutrients of fractionated maize and SBM were investigated (Chapters 3 and 4). In Chapter 3, after adding 30 and 60 g/kg water, to maize and SBM, the MC of maize increased from 122.7 to 144.2 and 161.8 g/kg, respectively, with the MC of SBM increasing from 117.1 to 142.7 and 164.4 g/kg. The water addition increased the energy consumption of hammer-milling and resulted in a larger GMD. However, the nutrient content, physical characteristics of fractionated particles were less affected by the MC of the maize and SBM. In Chapter 4, maize, SBM and their mixtures with different ratios (% maize:SBM; 25:75, 50:50, 75:25) were hammer milled and then size classified. Mixing ratio showed significant effects on the breaking behaviour with interactions between maize and SBM being observed during hammer-milling. The energy consumption of grinding mixtures was lower than grinding the same amount of material separately.

In Chapter 3, the commonly used *in vitro* analysis protocol of Boisen and Fernández (1995; 1997) were used to determine *in vitro* digestibility of crude protein (CP) and OM of size fractionated maize and SBM particles. Serendipitously it was found that digestibility values were affected by particles size of the maize and SBM as in both protocols, samples are required to be ground over 1-mm sized sieve. Again, in Chapter 4, the same observation was made. Additional grinding over a 1.0 mm screen of maize and SBM particles collected on sieves >595 μm significantly affected the *in vitro* digestibility values of OM and CP. Further research into this effect on *in vitro* analysis of the digestibility of nutrients was conducted in Chapter 6. Maize and SBM samples were successively ground over 1.50, 1.00, 0.75 and 0.50 mm sized screens in the laboratory mill. The *in vitro* digestibility of OM and CP were not affected although the particle size was shown to decrease with screen size decreasing from 1.00 to 0.50 mm. It was further shown that the grinding of feeds and feed ingredients over a 1 mm sieve as specified in the *in vitro* digestibility assay of Boisen and Fernández (1995; 1997) showed a significant mass fraction exceeding 595 μm that may affect digestibility values.

The results presented in this thesis indicate that physical and nutritional characteristics as well as the *in vitro* digestibility of nutrients of fractionated maize and SBM particles differed after hammer-milling. The effects of mixing ratio of ingredients on such characteristics could be substantial, although, the effect of moisture addition was limited. Mixing ingredients before grinding and grinding material with lower MC helps to reduce the energy consumption during hammer-milling. New determination methods such as wet sieving and image analysis, laser diffraction may be applicable in future evaluation of physical feed characteristics although it should be noted that each method has its own limitations in application. For example, wet sieving consumes much time, part of the measurable particle size range of the image analysis and laser diffraction method is not suitable for feed particles. Another major finding of this thesis is that before *in vitro* analysis, the particle size distribution of the sample should be determined as the fraction exceeding 595 µm may affect the digestibility values, at least for maize and SBM. The conclusions obtained from the present thesis are based on the *in vitro* experiments. Future studies can use animal models to verify the effect of breaking behaviour of feed ingredients e.g. maize, SBM, etc on the animal growth performance.

Acknowledgement

While writing this acknowledgement, I realize that my Ph.D. studies are almost over. Four years may not be long considering my whole life, but these 4 years of my study at Wageningen University are undoubtedly the most memorable and significant four years in my life so far. Studying towards a Ph.D. is not always easy. However, I have met so many people who helped, supported, and loved me, which makes it possible for me to achieve my dream of obtaining a doctor degree. Here, I have the opportunity to express my gratitude to them.

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About the author

Curriculum Vitae

Fang Lyu was born on April 3rd, 1993 in Liao Ning, China. After graduating from high school in 2011 she started the bachelor study Agricultural Engineering at the China Agricultural University. In 2015 she continued her studies at university with the master Processing and Storage of Agricultural Products. In 2015, she finalized her MSc thesis entitled "A model to predict pellet feed quality using BP neural network based on MIV variable selection and genetic algorithm optimization" and obtained her MSc degree. During her master study period, two scientific papers were published, and she was awarded with a China National Scholarship for Graduate Students. The same year in November, she was awarded with a scholarship from the China Scholarship Council and started to work as a PhD candidate at the Animal Nutrition Group under the supervision of Prof. dr. ir. Wouter H. Hendriks. The results of her PhD research are presented in this thesis.

Contact: caulyufang@gmail.com

List of publications

Lyu, F., Thomas, M., Hendriks, W.H., van der Poel, A.F.B., 2020. Size reduction in feed technology and methods for determining, expressing and predicting particle size: A review. Anim. Feed Sci. Technol. 261, 114347.

Lyu, F., van der Poel, A. F. B. Hendriks, W. H., Thomas, M., 2021. Particle size distribution of hammer milled maize and soybean meal, its nutrient composition and in vitro digestion characteristics. Anim. Feed Sci. Technol. 281, 115095.

Lyu, F., Hendriks, W.H., van der Poel, A.F.B., Thomas, M., 2021. Particle size distribution, energy consumption, nutrient composition and *in vitro* digestion characteristics of hammer milling maize and soybean meal as affected by moisture content. Anim. Feed Sci. Technol. (under review)

Lyu, F., Hendriks, W.H., van der Poel, A.F.B., Thomas, M. Breaking behaviour and interactions in maize and soybean meal while grinding in a hammer mill. Adv. Powder Technol. (under review)

Lyu, F., Thomas, M., van der Poel, A.F.B., Hendriks, W.H. The importance of particle size on organic and crude protein *in vitro* digestibility of maize and soybean meal. Anim. Feed Sci. Technol. (accepted)

Lyu, F., Yang, J., Kong, D.D., Chen, X., Yue, Y., Fang, P., Wang, H.Y., 2016. Analysis of processing characteristics of different barley cultivars used in feed (In Chinese). Feed Ind. 37, 6-14.

Lyu, F., Kong, D.D., Chen, X., Yue, Y., Fang, P., Wang, H.Y., 2017. Experimental research on friction characteristics of mash feed (In Chinese). Feed Ind. 38, 7-10.

Training and supervision plan

Training and supervision		Graduate school
Name PhD candidate	Fang Lyu	
Group	Animal Nutrition	
Co-promotor	Dr. ir. A.F.B. van der Poel	
	Dr. ir. M. Thomas	
Promotor	Prof. dr. ir. Wouter H. Hendriks	

Education and training	Year
The Basic Package (3 ECTS[1])	2018
WIAS Introduction Day	2018
Course on philosophy of science and/or ethics	2018
Course on essential skills	
Disciplinary Competences (13 ECTS)	
Research proposal	2018
Design of experiments	2019
ANU-31306 Feed Technology	2018
MAT-50303 R for Statistics	2018
Professional Competences (7 ECTS)	
Project and time management	2018
Research Data management	2018
Supervising BSc and MSc thesis students	2019
Searching and organising literatures for PhD	2020
Scientific writing	2019
Career perspective	
Societal Relevance (2 ECTS)	
Societal impact of your research	2018
Presentation Skills (3 ECTS)	
Animal nutrition Research Forum/Netherlands/oral	2018
Animal nutrition Research Forum/Belgium/oral	2019
WIAS science day /poster	2020
Teaching competences (6 ECTS)	
Supervising one MSc student (major)	2020
Supervising one MSc student (minor)	2020
Supervisor for practical of principle of animal nutrition course	2019, 2020
Supervisor for practical of Introduction to animal science course	2019, 2020
Total = 33 ECTS	

[1]One ECTS credit equals a study load of approximately 28 hours.

Colophon

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