

Quality of South African soybean meal as a factor affecting the efficacy of exogenous protease in broiler diets

By

REUBEN KAARE HOLMES

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Faculty of Natural and Agricultural Sciences

Department of Animal Science

UNIVERSITY OF PRETORIA

Pretoria

SUPERVISOR:

Prof. C. JANSEN VAN RENSBURG

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I, Reuben Kaare Holmes, declare that this dissertation, which I hereby submit for the degree of MSc Agric Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Abstract

The poultry feed industry relies heavily upon soybean meal (SBM) as a relatively affordable, high quality source of protein. Soybean meal composition and quality may vary between batches, mainly contributed to variation in production practice, cultivar and processing technique. Dietary supplementation with a mono-component, exogenous protease, has the potential to reduce feed cost, improve the health and welfare of poultry and reduce the already substantial carbon footprint of poultry production. The effects and interaction of exogenous protease in soybean-maize based diets, however, is unpredictable and cost-effective inclusion is not always realised. Matrices have to be adjusted continually to accommodate the differences in nutritional composition. Studies on the effects of exogenous protease on ileal amino acid digestibilities have yielded contradicting results. Given that the South Africa crushing industry is relatively young, having an understanding of the relationship of protease on apparent ileal amino acid digestibility (AIAAD) and SBM quality is important. However, insufficient data is available in order to more fully understand this relationship. To improve our understanding of the nutritional composition and quality of local SBM, two surveys were conducted in which SBM samples were collected from various soybean processors in South Africa. Survey 1 comprised of SBM samples which were collected from seven of the largest processors in South Africa from June to October 2020. Survey 2 comprised of SBM samples from five of the largest processors from September 2019 to March 2020. Both sets of samples were analysed for nutritional composition, including amino acid (AA) concentration and quality parameters. From samples collected for Survey 2, one low quality (LQ), one high quality (HQ) SBM sample and one over-processed sample (OP) were selected. An under processed (UP) sample was also obtained which served as a negative control. These four samples were incorporated into maize-soybean based diets, either with or without an exogenous protease and fed to broilers in a digestibility study aimed at investigating the interaction between SBM quality and protease efficacy.

In the two surveys conducted, significant differences were observed among at least one of the seven sources for Survey 1 and one of the five sources for Survey 2 in all nutritional components and quality parameters except for zinc, iron or copper. The findings highlighted substantial variability in South African SBM indicating challenges in achieving consistency in SBM composition due to various factors including cultivar, planting and harvesting techniques and origin. The significant variability observed between processors indicates that ensuring consistency in SBM composition and quality is challenging. The efficacy of protease varied across treatments containing different SBM qualities. Protease supplementation significantly increased the apparent ileal digestibility of crude protein (CP). The impact

of protease on AIAAD was most pronounced in the treatment. with UP, followed by HQ, LQ, and then OP. This suggests that protease supplementation may be effective in increasing the AIAAD of some AAs in SBMs that have not undergone sufficient thermal exposure. This may also suggest that it may not always be justified to use a protease to increase the AIAAD of nutritionally important AAs, such as essential AAs, in diets containing SBM that contains insufficiently processed SBM.

With protease supplementation it may not be accurate to assume improvement in CP digestibility based on fixed matrix values recommended by the manufacturer. Nutritionists have to regularly adjust matrices according to batch and supplier. Protease supplementation led to higher ileal CP digestibility across all SBM samples, but this did not consistently translate to increased digestibility of all AAs. The varying effects of protease supplementation on AIAAD indicates an interaction between processing level and protease efficacy on AA digestibility. Protease supplementation had a greater effect on SBM with lower CP content.

Therefore, it is, incorrect to assume a linear relationship between CP digestibility and AA digestibility. The use of protease in soybean-maize diets could potentially enhance the utilization of dietary protein. However, its effectiveness varies. Further research into how protease functions on SBM would be beneficial, given that its impact on SBM of different qualities is unpredictable and challenging to quantify.

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List of Abbreviations

AA	Amino acids
ADF	Acid detergent fibre
AIAAD	Apparent ileal amino acid digestibility
AID	Apparent ileal digestibility
ANFs	Anti-nutritive factors
CF	Crude fibre
CP	Crude protein
DM	Dry matter
EE	Ether extract
GM	Genetically modified
HQ	High quality
KOH	Potassium hydroxide
KOHPS	Protein solubility in potassium hydroxide
LQ	Low quality
ME	Metabolisable energy
NDF	Neutral detergent fibre
NIRS	Near infrared reflectance spectroscopy
NSP	Non-starch polysaccharides
OP	Over-processed
P	Phosphorous
PDI	Protein dispersibility index
PP	Phytic phosphorous
SA	South Africa
UA	Urease activity
UP	Under-processed
USA	United States of America

CHAPTER 1

Introduction

The quest for food security due to projected world population growth (Gerland *et al.*, 2014), environmental awareness and changing consumer preferences have all been major drivers of the changes in agricultural practice (Michel-Guillou & Moser, 2006). Consequently, the genetic development of production animals with high growth rates, increasing body weights and quality products has ensued. The production of these “modern” animals requires specialised strategies that not only meet the needs of the animal but the needs of the environment and the consumer. Feed that is affordable and of a high nutritional quality and which is farmed sustainably, will form an integral part of modern agricultural production. The industry has turned to various feed ingredients and additives to achieve this. One of the ingredients that has proven to be an indispensable source of protein in non-ruminant feeds is soybean meal (SBM). As a result, the growth and development of soybean farming and processing has expanded globally (Dei, 2011).

During recent years, local South African soybean production and processing experienced unprecedented growth. South Africa (SA) was producing between 450 000 and 500 000 tons per annum in 2010, yielding 2.5 to 3 tons per hectare under dry-land conditions (NAMC, 2011). Currently SA is producing over 2 700 000 tons per year (USDA, 2023). This growth was in part due to the adaptation of imported machinery during the 2010 to 2014 period to make it more suitable for processing of the typical South African soybean cultivars, the beans of which are larger and of a different shape (RussellStone Group, 2017). Low levels of locally produced SBM and the use of machinery that could not adequately process and exploit the true nutritional potential of local cultivars hindered the industry from growth. The relatively small soybean industry in SA also means that research that is focused on locally produced SBM was limited. With the adapted machinery, four commercial-scale plants were completed from 2010 to 2014 and their successful operation resulted in an increase in the amount of soybean planted and subsequently paved the way for the construction of five more processing plants. South Africa has nine processing plants operating in the Gauteng, Free State, Mpumalanga, Limpopo and Kwa-Zulu Natal provinces with a combined crushing capacity of 1.93 million tons (Bonsu, 2019). With four of the plants located in the Gauteng region and two in Mpumalanga within proximity to Gauteng, these regions are considered ‘self-sufficient’ in terms of producing and processing soybeans. In the past, coastal regions relied more heavily on imports because the prices were typically cheaper for imported SBM compared to SBM of local, inland origin (RussellStone Group, 2017). The soybeans that were processed were not

always of local origin and often beans were imported to supplement local production, depending on harvest yields. It would have been feasible for coastal regions to source locally processed SBM but that was only when local farmers produced enough soybeans to meet the capacities of the plants. According to Sundani (2013), in 2013 significant potential for growth in local soybean production was identified and was expected that at some point, following trends and projections, local production would be sufficient for the South African market. At the end of 2013, SA became a net exporter of oilseeds as local processor demand was exceeded. After 10 years of persistent growth in the sector, SA has been set to achieve a record soybean export figure (Reidy, 2023).

South African soybean processing plants make use of either of the three most common processing techniques; solvent extraction, mechanical extraction or a combination of both. Solvent extraction is the most common technique as it is the most efficient with only 1.5 % oil residue after extraction (Johnson & Smith., 2018). All extraction processes are preceded by specific treatments unique to the processing plant and governed by the final product that has been requested by the customer. These treatments include heat treatment, crushing and flaking and dehulling (Dunford, 2012). Heat treatment of soybeans is particularly important as the raw bean contains antinutritional factors (ANFs) that decrease the digestibility and bioavailability of the protein fraction. Trypsin inhibitors, phytates, lectins, oligosaccharides, and non-starch polysaccharides are all present in soybeans and are inactivated or destroyed during exposure to heat (Gilani *et al.*, 2012). Trypsin inhibitors are the most ubiquitous and if not destroyed by heat treatment can cause metabolic issues in broilers (Samtiya *et al.*, 2020). A threshold exists for optimum exposure to heat. If the soybeans are insufficiently cooked, the content of trypsin inhibitors may not be reduced enough, which decreases the amount of digestible protein, reducing the quality of the SBM (Aletor & Ojo, 1989). If the soybeans are exposed to too much heat, the protein fraction may also be reduced due to Maillard reactions where sugars and amino groups of the proteins combine in the presence of heat. A large proportion of the variation in SBM arises from differences in temperature and length of exposure to heat during processing (Oliveira *et al.*, 2021).

Despite having the ability to properly process local soybeans, processors still found a lot of variation in quality and nutritional composition between batches of SBM, as is experienced by processors worldwide (Sihlobo, 2019). Other factors including cultivar, region of cultivation, harvesting techniques and storage and method of processing are some of the many factors that lead to variation in the final product (de Coca-Sinova *et al.*, 2008; Wang *et al.*, 2011). This presents a challenge for nutritionists and producers seeking least cost formulation. The use of exogenous protease is an important nutritional strategy employed to exploit the nutritional potential of SBM and compensate for batches of SBM with varying nutritional

composition or with lower available protein (Cowieson & Adeola, 2005). Exogenous protease supplementation in soybean-maize based diets fed to poultry has shown to increase the availability of protein and amino acids (AAs) in the diet (Cowieson & Haahr, 2019), reduce feed costs and reduce the carbon footprint by decreasing the rate of wasteful excretion of undigested nutrients (Milosevic *et al.*, 2013) which help meet the demands for modern food production (Schader *et al.*, 2015). The increases in ileal digestibility of individual AAs are, however, uncertain and increases in ileal DM and CP digestibility do not always reflect increases in ileal AA digestibility or any change in a manner that may be predicted (Caine *et al.*, 1998; Zanella, 1999; Ghazi *et al.*, 2002; Angel *et al.*, 2011; Doskovic *et al.*, 2013; Milosevic *et al.*, 2013). This is further compounded by the inconsistency of the SBM products to be supplemented with protease.

Further insight into the extent of variation of the SBM produced in SA and the use of exogenous protease in diets using locally produced SBM will be beneficial. Due to the relatively new industry of SBM production in SA, there is scope for more nutritional data on SBM produced across the major processors in SA. Larger datasets, when combined and analysed, would provide more accurate results on which nutritionists and researchers can base decisions on the inclusion of SBM in broiler feed.

Aims, objectives and hypotheses

This study had two aims; firstly to provide two data sets on locally produced SBM from the largest processors in SA. Secondly, to assess the effect that SBM of differing quality and nutritional composition has on the efficacy of protease in a broiler diet. The objectives of the study were as follows:

1. Collect SBM samples from soy processors across SA and analyse them for nutritional composition and quality.
2. Select SBM samples of different qualities and incorporate them into diets fed to 21-day old broilers in a digestibility study. Diets would be fed with and without a monocomponent protease supplementation.
3. Assess for significant differences between treatments containing SBM of different nutritional compositions and qualities, both with and without protease supplementation, focusing on ileal digestibility of nutrients.

The null hypothesis posited that there is minimal variation in the nutritional composition and quality of SBM from different sources in SA, and that the efficacy of protease supplementation on the ileal digestibility of nutrients in soybean-maize based diets remains unaffected by SBM quality.

Conversely, the alternative hypothesis suggests significant variability in the nutritional composition and quality of SBM sourced from different producers in SA, and that the effectiveness of protease supplementation on nutrient digestibility in these diets will be influenced by SBM quality.

This study sought to clarify these hypotheses through comprehensive data collection and analysis, aiming to provide valuable insights for optimizing broiler diets and enhancing the utilization of locally produced SBM.

CHAPTER 2

Literature Review

This literature review covers the current state of poultry production worldwide and in SA including current and predicted production figures, current and future challenges, the role of SBM in poultry feed and a brief history of soybean cultivation globally and in SA. Soybean processing is discussed in slightly more detail as well as the ANFs present in raw soybeans. The parameters with which SBM quality is assessed and reported has also been discussed. Finally, the role and efficacy of protease in maize-soybean diets and its effect on DM, CP and AA digestibility was reviewed.

2.1 Poultry production

Poultry production plays a crucial role in the lives of millions of people across the world. Globally, the poultry industry has experienced unrivalled growth in the agricultural sector over the past few decades. An estimated 34.4 billion chickens make up the global sector (FAO, 2023). Poultry production provides the largest portion of protein worldwide and as of recent has overtaken pork as the most consumed meat globally and is expected to maintain its position in future (Shahbandeh, 2022). Being the most consumed protein source globally has led to increased demand for feed that is affordable, accessible, of a high quality and with a low carbon footprint. Soybean meal has become a very important feed ingredient in helping achieve this. However, there are still challenges with the use of SBM, including variation in nutritional composition, high nitrogenous output and the requirement of land for soybean cultivation.

Poultry production is important to people through all income classes in both urban and rural areas. This contribution is not limited to nutrition but is also economically and socially important (Hurst *et al.*, 2005). Poultry systems can convert a large variety of agro-products into high quality edible products for humans, mainly in the form of meat and eggs, providing essential nutrients such as energy, protein and fat. Manure from poultry production is an important non-nutritive product which is used extensively in crop fertilisation in commercial and subsistence applications (Scanen, 2007). In developed countries with higher average incomes, specialised broiler production systems are responsible for about 92% of the global poultry sector with layer birds contributing only 6% of production (FAO, 2023) and very few contributions from informal and smallholder systems. In developing countries informal systems

contribute a lot more. In low- and middle-income countries across Asia and Africa, about 60-90% of total poultry stocks come from rural flocks (Guèye, 2000; Akinola & Essien, 2011). Sonaiya (2007) estimated 30-80% contributions through backyard systems accounting for 98% of the poultry meat consumed in villages. These rural flocks contribute to livelihoods through food security and poverty alleviation by providing meat, eggs, and fertiliser as well as an opportunity for income through the sale of these products (Mottet & Tempio, 2017). Rural flocks also contribute on a cultural level due to its symbolic nature in many societies (Guèye, 1998). The contribution to gender equality is also noteworthy as more than 70% of rural chicken owners in Sub-Saharan Africa are woman (Guèye, 2000). For many African woman the household and agricultural responsibilities rest upon their shoulders and poultry production provides a less oppressive and demanding form of food and/or income generation (Ogunlela & Mukhtar, 2009).

Poultry production plays an equally important role in SA. The industry is the largest single contributor to the agricultural sector with 2019 recording 20% of the total agricultural gross value coming from poultry production. Of the gross value of animal products, poultry accounted for some 40% (SAPA, 2019). The consumption of poultry products in SA by far surpasses that of any other meat being produced or imported. This demand is met by local production as well as a large amount of importation of bone-in portions from Brazil, the USA and the EU (Nkukwana, 2019). Local poultry meat production reached 1.51 million metric tons in 2020 and almost 400 000 metric tons of bone-in meat was imported in 2020 to meet market demands (USDA, 2020). It therefore has a significant impact on food protein provision within the country with 64% of consumption stemming from local production (SAPA, 2019).

2.2 World challenges

Among the major challenges facing the world, insecurity and climate change are likely the most pressing. These challenges are directly and/or indirectly related to each other and are important driving forces directing research for many scientific disciplines including agricultural sciences and poultry production (Kleyn & Ciacciariello, 2021). The agricultural sector is currently unable to reduce its already large carbon footprint, whilst increasing future outputs to meet rising food demands.

Food security and climate change

The world population is estimated to reach around 9.6 billion by the year 2050 (Alexandratos and Bruinsma, 2012; Raut, 2019). Similar predictions were made by the United Nations projecting a population of 9.15 billion by the same year (Raut, 2019). These estimations are a concern as pressures on natural resources increase which limit the growth of agriculture needed to support the growing population

(FAO, 2017). Rojas-Downing *et al.* (2017) estimates that the agricultural sector contributes as much as 14.5% of the cause of global climate change, with poultry production alone responsible for 11%.

High levels of nitrogen in manure, large-scale antibiotic use and the release of greenhouse gasses through poultry farming and poultry feed production are the major factors in the poultry industry that contribute to climate change (Thornton, 2010; Gerber *et al.*, 2013). Water pollution is also a common occurrence in areas surrounding poultry processing plants due to the discharge of polluted wastewater containing chlorine, high levels of pathogens which include *Campylobacter* and *Salmonella*, nitrogen and phosphorous. Lastly, the poultry industry contributes indirectly to climate change through activities such as crop production and processing necessary for production of feeds and the associated loss of land and forests to agricultural land (Gerber *et al.*, 2013; Vetter *et al.*, 2017).

Challenges within the poultry industry

The poultry industry faces difficulties that are linked to natural resource pressure, climate change, consumer preferences and welfare requirements. Although the carbon footprint of poultry is among the lowest of the agricultural sub-sectors (Kleyn & Ciacciariello, 2021), it is still significant.

An enormous demand has been created for poultry products due to the efficiency of production in relation to other livestock. This demand has caused market competitiveness and product prices to decrease (Davis, 2015). The inability of some countries to produce poultry products locally at such low prices results in the dumping of products into these poorer countries (Nkukwana, 2019). Moreover, this demand has been compounded by factors such as dwindling resources, current industry developments, high feed prices, demographics, trade policies, the push for decreased environmental impact, escalating preferences for safer, healthier food and improved animal welfare conditions (Mottet & Tempio, 2017; Kleyn & Ciacciariello, 2021) and any challenge endemic to a certain area. Much research and development are therefore conducted at commercial, small-scale and subsistence levels of poultry production to address global climate and population challenges and counteract the damage caused (FAO, 2006).

The poultry industry has great potential in playing a key role in the mitigation of climate change, world poverty and food insecurity, despite the issues discussed (Rojas-Downing *et al.*, 2017). Overcoming these challenges will require the involvement and collaboration of government and private industry in all areas of the production chain. One of the links in the production chain that has great potential in making a difference is nutrition (Ravindran, 2012).

The influence of nutrition on the environment, the animal, the farmer, and the consumer, is far-reaching and begins as early as the production of the feed crop and it extends until within days of the consumption of feed products. The impact on the environment may be direct such as through residues of harmful substances used in feed (Rosenblatt and Schmitz, 2016), or indirect through deforestation for the expansion of soybean plantations for feed production (Lawrence & Vandecar, 2015). The production and use of large amounts of animal feeds has had a negative impact on the environment which has inspired research that has contributed to overcome some of the issues. In fact, there is great potential for the future of nutrition science to positively contribute to and alleviate current challenges (FAO, 2017).

One of the main contributors to climate change is the release of greenhouse gasses and/or its precursors; nitrous oxide from manure, and ammonia from poultry (Thornton, 2010). Different feed materials and additives can contribute, to a lesser or greater extent, to the amount of nitrogen (N) excreted and ammonia released by the birds (Ritz *et al.*, 2004). Generally, up to 5% of consumed N and 55% of consumed phosphorous (P) sources may be excreted (Chalova *et al.*, 2016). This also presents a great economic loss as protein is an expensive component of feedstuffs and has relatively high inclusion rates in poultry feeds (Ravindran, 2012). An estimated 65-70% of poultry production costs stem from feed alone. With high feed costs already a challenge in the industry, any loss of nutrients in the feed further compounds the economic battle that many poultry producers face. High feed costs and lack of availability of suitable raw materials are major production constraints that smallholder systems face (Schader *et al.*, 2015).

Current developments in the nutrition sector are aimed primarily at production efficiency. Efficiency in feeding, broadly speaking, is achieved by means of feeding less amounts of feed or specific nutrients whilst sustaining production levels. New strategies being investigated include replacing harmful feed additives, finding alternate or novel feed sources, improving gut health, improving feed quality and availability, understanding more about growth and body composition and improving animal genetics through genetic selection (Elwinger *et al.*, 2017).

2.3 Cultivation of soybeans

Commonly referred to as a miracle crop, the soybean plant has proved to be a valuable resource in agriculture. Of all legumes soybeans are the most widely cultivated and the most important, economically speaking, with the three largest producers and processors globally being the USA, Brazil and Argentina (Hart, 2017). As an oilseed with a concentrated protein component of high quality it is harvested predominantly to be processed into oil and a source of protein. Soybeans and their by-products are an

important source of plant protein for human and animal consumption (Nahashon & Kilonzo-Nthenge 2011). Soya oil is second only to palm oil as the most widely produced and utilized plant-based oil (Sugiyama *et al.*, 2015).

Due to the high nutritional quality, relatively low cost of production, diverse growing conditions and high yields, soybeans have and will continue to help alleviate the current and future challenges of world hunger, poverty, climate change and the challenges facing the agricultural industry (FAO, 2004; Dei 2011). The protein component of soybeans is particularly important because of its high quality and desirable AA profile (Baker & Han, 1994). In the raw form, however, soybeans contain ANFs that render nutrients less available for digestion and may cause health challenges when consumed. The ANFs can be inactivated through heat treatment and processing in specialised plants that produce various proteinaceous raw material types and soya oil (Gilani *et al.*, 2012).

Soybeans are grown in temperate, tropical and subtropical regions (Islam *et al.*, 2019). Yields differ greatly between these regions with growth and yield being favoured in temperate, warm moist conditions with rainfall between 550-850 mm over the entire growing season or in dry areas under suitable irrigation. Soybeans are most sensitive to drought and very high temperatures specifically in the flowering and pod formation stages where desirable temperatures are between 13 and 30°C (Pannar Seed (Pty) Ltd, 2010). Photosensitivity or photoperiod sensitivity, which is the ability of day length to control or dictate plant growth, is an important factor in the growth of soybeans and considered one of the most important factors in limiting yield (de Beer & Prinsloo, 2013). Soybeans are highly photosensitive and even slight variations beyond the adaptations of the plant affects plant growth and yield. Sensitivity to photoperiod is a limiting factor in the adaptation of individual cultivars (de Beer & Prinsloo, 2013). Cheng *et al.* (2018) pointed out that genetic engineering must be employed to exploit yield potential and maintaining yield stability of soybean production in water-limited environments to guarantee the supply of food for the growing human population and for livestock. Additionally, new soybean varieties that are resistant to diseases and pests are being developed. Generally, soybeans may grow in most soil types, however optimal growth occurs in warm, well-drained sandy loam but require good levels of soil organic matter with lower pH levels (Pannar Seed (Pty) Ltd, 2010).

2.3.1 History of soybean cultivation

The domestication and subsequent dissemination of the soybean plant and numerous cultivars is believed to have occurred in China in the 18th century. Its introduction into the USA, Europe and Brazil soon

followed as its value as a crop became apparent (Hymowitz & Shurtleff, 2005). England was the first country outside of East Asia to import large amounts of soybeans. A great need for vegetable oil drove the increase of soybean processing in Europe. The first plant processing soybean on a large scale was reported to be in Germany in 1911 and a few years later in North Carolina when a cottonseed oil mill began experimenting with processing soybean. Following the success of the experiment the first commercial processing of soybeans began in Decatur, Illinois, USA, in 1920. The turmoil of the events of World War II, the ever-growing demand for vegetable oil and newfound research on the importance of protein in the nutrition of humans and animals drew great attention to soybean processing. This would slowly result in the dominance of soybean processing amongst all other oilseeds, a dominance that continued growing over the years to come. However, it was not until the late 19th century that large-scale production in the USA and Europe began. For the continents of South America and Africa, widespread commercial production began in the mid to late 20th century (Chang *et al.*, 2014). Soybean production worldwide became the worlds most cultivated legumes with an average annual increase in production of 4.6% from 1961 to 2007. A unique feature of soybeans compared to other crops is that it is sensitive to day length or photoperiod. It is also sensitive to temperature, therefore choosing the cultivar adapted to suit the growth climate is essential to maximise yield and quality. The necessity for the development of the right cultivar was one of the major factors in delaying the spread and large-scale production of soybean in countries with climates that differ greatly from China and the USA (Hymowitz, 2008). Just as the spread of soybean cultivation and processing worldwide required research, time, industrialisation and development of infrastructure, so too were there requirements that needed to be met before SA could increase its scale of production (RussellStone Group, 2017).

2.3.2 . Soybean production in South Africa

The first documentation of soybeans in SA, according to du Toit (1942), was at their introduction in 1903. The crop fell into the hands of farmers that knew little to nothing as to the correct cultivation practices of soybeans. Progress in soybean production was poor and a lot of difficulties were experienced. At the time, the Department of Agriculture and Forestry set up initiatives aimed at trying to develop an understanding of the crop and thus formulate production methods (du Toit, 1942). For the very purpose of research and improvement in the ways of animal feed, the Feed Committee (established in 1942), the Department of Agriculture and Forestry, the Oil Expressers Association and, in 1945, the Animal Feed Manufacturers Association were organised. The Animal Feed Manufacturers Association was established under the Oil Expressers Association as an initiative to “undertake and facilitate the importation and distribution of protein-rich feeds” (Viljoen, 1944). In the early 1940’s an approximate 75% of protein crops used were

imported and shipping capacity was a concern in SA at the time (NAMC, 2011; Sihlobo, 2013). Growth between the 1950's to late 1980's was slow despite the continuous growth in interest in soybeans. Its value as a crop was understood worldwide but its use as a feed in SA was under appreciated. In the 1970's, fish meal was largely used to supplement animal feed requirements, but many became critical about the levels of fishmeal being supplemented. Fishmeal producers soon proposed that SBM could aptly substitute a large portion of the fishmeal used in animal feeds (Scholtemeijer, 2021). Regardless of the growing interest, little progress in increasing production levels was made by 1990, despite several attempts at research and development (Scholtemeijer, 2021). Total yield for the country remained below 50 000 tons in the mid-1980's. During the 1990's a large shift towards soybean production began and, from 1995 to 2000, production levels more than doubled (Crop Estimates Committee, 2000). By the 1990's a large majority of the worldwide soybeans produced were various genetically modified (GM) soybean cultivars. Many countries in the Southern African region, such as Zimbabwe, opposed the use of GM crops and the use of biotechnology, but in Act 15 of 1997 for Genetically Modified Organisms, the South African government sanctioned the use of transgenic crops. The exponential growth trend continued from 2000 to 2019, with production increasing from around 150 000 tons to over 1.2 million tons (Crop Estimates Committee, 2000; Crop Estimates Committee, 2020). The most recent data published shows the 2022-2023 marketing year yielded roughly 2.75 million metric tons of soybeans, following the trend of continued growth of soybeans in the oilseed sector maintaining SA as the largest producer on the African continent (Cilliers, 2023). In 2022 to 2023, Free State was the highest soybean producing province in SA, with 573 500 hectares planted, followed closely by the Mpumalanga with 305 000 hectares planted. In North West, Gauteng and Kwa-Zulu Natal respectively 155 000, 44 000 and 42 000 hectares were planted. (CEC, 2023).

For the soybean cultivars used in SA, the progress in genetic modification and cultivar development has mostly been achieved through the work of the National Soybean Cultivar Trials. The trials, which spanned a 34-year period beginning in 1978 and ending in 2011, resulted in the development of 35 conventional and 33 Roundup-Ready cultivars and one root-knot nematode tolerant cultivar (de Beer & Prinsloo, 2013). Roundup-Ready means that the plant has been developed with a specific gene that renders the plant tolerant to glyphosate herbicide, commonly used in soybean productions (Carpenter & Gianessi, 1999). This breakthrough is considered to have had the largest positive impact on the industry (de Beer and Prinsloo, 2013). According to de Beer and Prinsloo (2013), 90% of the cultivars being used across SA currently are Roundup Ready cultivars. Worldwide, GM soybean cultivars are predominantly planted because of their superior growth performance but also because of their superior health benefits over non-GM cultivars. Genetically modified cultivars are selected to contain lower saturated and trans-fatty acids and greater AA

concentrations which have beneficial health implications both for humans and animals (Baker *et al.*, 2011).

2.3.3 Soybean processing in South Africa

The establishment of the soybean processing or ‘crushing’ industry in the early 2010’s played a major role in the growth of soybean production realised in the past decade (RussellStone Group, 2017). Before 2012, locally produced soybeans were mostly destined for export and thus most soybean by-products were imported. This situation was unfavourable, and producers were eager to find a way to process home-grown beans locally, creating an opportunity for the soybean industry to flourish. For many years it was believed that the beans produced from South African cultivars lacked the quality fit for processing. Furthermore, the beans from South African cultivars were unique, with a typically larger bean size, and could not be appropriately processed by conventional crushing machinery available at the time (RussellStone Group, 2017).

In the late 2010’s there was an urgency to grow the soybean production and processing industries. In August 2007, the National Industrial Policy Framework was established to address the decline in SA’s industrial and manufacturing sectors and to help achieve the goal set out by the government to halve unemployment by 2014 (DTI, 2008). This framework was formed as part of an Industrial Policy Action Plan which was first set up in August 2007. Each Industrial Policy Action Plan runs for a 3-year period and different areas of focus are set out with each plan. A focal point of the 2013/2014 – 2015/2016 Industrial Policy Action Plan was on local agro-processing and one of the key action programmes was the development of the Soybean Action Plan with the purpose of linking primary producers and processors and slowly decreasing the need for imports as, according to Sihlobo (2019), the main constraint facing South African producers is import competition (DTI, 2008). The implementation of the Soybean Action Plan was a timely undertaking as four of the countries’ largest processors had construction completion dates in 2011 and 2013 with a combined production capacity of 3810 tons per day (Dlamini *et al.*, 2014). In 2012, key commercial enterprises foresaw the potential and started importing premium quality processing machinery that had been altered and developed to process the beans from South African cultivars and produce high-quality soya by-products. Four commercial scale plants were then established. The success of the first four commercial scale plants that were established, paved the way for the construction of five more plants. The adapted processing equipment proved to be successful and very shortly after completion these plants achieved relatively high levels of output of consistently high quality (RussellStone Group, 2017). A total of nine commercial processing plants are operating in the Gauteng,

Free State, Mpumalanga, Limpopo and Kwa-Zulu Natal provinces with a combined crushing capacity of 1.93 million tons (Sihlobo & Kapuya, 2015). With four of the plants located in the Gauteng region and two in Mpumalanga close to Gauteng, these regions are, for SBM, considered ‘self-sufficient’ in terms of the use of locally processed soybeans. Because the prices are typically cheaper for imported SBM compared to SBM of local, inland origin, the coastal regions still rely on imports (Russell Stone Group, 2017). However, the soybeans that are processed in the Gauteng region may not always be of local origin and often beans are imported to supplement local production, depending on harvest yields. It would be feasible for coastal regions to source locally processed soya meals but only when local farmers can produce enough soybeans to meet the capacities of the processing plants. According to Sundani (2013), in 2013 significant potential for growth in local soybean production was identified and it was expected that at some point, following trends and projections, local production would be enough. The continued development of drought tolerant and high yielding cultivars and the subsequent use of these cultivars will play a crucial role in helping to achieve this.

2.3.4 Antinutritional factors in soybeans

Due to chemical compounds present in soybeans that limit the use of raw soybeans for monogastric nutrition, soybeans are processed before consumption is possible. This processing produces by-products that are suitable for nutritional applications (Liener, 1995). The primary focus of a soybean processing plant is the production of soya meal, the majority of which is used in animal feeds as a protein source. The greatest portion of soybeans produced in SA are processed primarily for use in commercial animal feed industries (Sihlobo, 2019).

An ANF is any substance which limits nutrient bioavailability or interferes with normal nutrient intake, digestion, assimilation and utilisation. This interference may have a minor or serious adverse effect on health depending on the scenario but will almost always affect production efficiency and lead to morbidity and/or death (Samtiya *et al.*, 2020). Four groups of ANFs exist and are classified based on the effect that it has on the animal. The groups include metal ion scavengers, antivitamins, ANFs that interfere with protein utilisation and a group for any other type of compounds (Adeyemo & Onilude, 2013). Soybeans contain ANFs from all four categories including phytic acid, protease and trypsin inhibitors, lectins, oligosaccharides and non-starch polysaccharides. Of particular focus in the context of this study are protease inhibitors and oligosaccharides. Table 2.1 provides typical concentrations of ANFs in raw soybeans and SBM.

Trypsin inhibitors

Inhibitors of protein metabolism include protease inhibitors, Kunitz trypsin inhibitor and the Bowman-Birk inhibitor (Liener, 1995). These inhibitors prevent normal activity of intestinal proteases including trypsin and chymotrypsin thus thwarting protein metabolism processes (Carvalho *et al.*, 2013). This is performed by competitive inhibition where the inhibitors bind irreversibly to intestinal proteases preventing the binding of protein substrates leading to the accumulation of large protein molecules which cannot be absorbed in their complex forms. This results in malnutrition and poor animal performance due to metabolic and digestive disease (Avilés-Gaxiola *et al.*, 2018). A common symptom is hypertrophy of the pancreas which impairs its secretory function, decreasing the utilisation of nitrogenous substances thus increasing endogenous nitrogenous losses (Pacheco *et al.*, 2014).

Phytic phosphorous

Phytic acids contain highly charged phosphate groups which make phytic acid extremely reactive. When present in foods, these charged phosphate groups bind with divalent cations such as iron, zinc, calcium, copper and magnesium and causing chelates rendering them nutritionally unavailable (Bohn *et al.*, 2008). Furthermore, at later stages of digestion, phytic acids can bind to peptides and AAs and form insoluble complexes and thus proteolytic enzyme action is inhibited (Deak & Johnson, 2007; Wanasundara, 2011). Heat treatment does not breakdown or inactivate phytic acids, however, the supplementation of phytase enzymes has proven effective in the breakdown of phytic acids (Wanasundara, 2011).

Oligosaccharides – Raffinose, Stachyose

Categorised broadly, oligosaccharides are a group of complex sugars containing a few monosaccharide monomers forming a more complex sugar structure often occurring as part of glycoproteins and glycolipids (Pan *et al.*, 2018). In the case of soybeans, raffinose oligosaccharides, particularly raffinose and stachyose, are found as a storage or transport carbohydrates. In the monogastric animal the enzymes capable of digesting raffinose oligosaccharides are absent. This leads to the passage of raffinose oligosaccharides into the large intestine where they undergo anaerobic fermentation which produces large amounts of gas and can cause intestinal discomfort, negatively disrupt microorganism make-up and cause overall loss in digestive efficiency (Karr-Lilienthal *et al.*, 2004; Rubio *et al.*, 2010). Raffinose oligosaccharides are broken down to varying levels during typical processing procedures. The extent of breakdown depends on processing procedures used (Wang *et al.*, 2007).

Lectins

Lectins are glycoproteins that can bind to sugar components (Maenz *et al.*, 1999). Lectins can also bind to the epithelial cells that line the intestine, ultimately altering gut permeability, compromising absorption. Absorption of calcium, iron, P and zinc are particularly affected (Liener, 1995). Lectins are however almost entirely destroyed by heat treatment (Liener, 2009).

Table 2.1: Concentration of antinutritional factors in raw soybeans and soybean meal (Peisker 2001; Carrão-Panizzi *et al.*, 2008.)

Parameter	Raw Soybean	Soybean meal
Trypsin inhibitor (mg/g)	45 - 50	1 - 8
Lectins (mg/kg)	3500	10 - 200
Oligosaccharides (g/kg)	140	150
Saponins (mg/kg)	5	6
Isoflavones (mg/kg)	2700	3700

2.3.5 Soybean processing methods

The principal purpose of soybean processing is the extraction and harvest of the seed oil and protein contents. Extraction of the oils is possible using solvents, enzymes, pressure, heat and extraction through mechanical expelling. The most widely used commercial extraction methods are mechanical and solvent extraction (Cheng *et al.*, 2018). Mechanical extraction or ‘pressing’, was the founding method for oil extraction. Mechanical extraction involves the application of heat and pressure which disrupts the structure of the oil bodies and releases the oily matrix of the oil cells. The products of mechanical extraction are typically used in food and industrial applications (De Lima *et al.*, 2008). Mechanical extraction is a clean and hygienic process, producing soy protein products suitable for human consumption, whilst soya protein products from solvent extraction methods are not suitable for human consumption as the process is not sufficiently hygienic and chemical residues of the solvent remain in the product in high concentrations (Woerfel, 1995). Solvent extraction methods are suitable for use in the feed industry and are significantly more efficient in extracting the oil from the beans (Bargale *et al.*, 1999). Because solvent extracted soya protein products can be fed to animals, the greater extraction efficiency, and the high demand of soya protein products from the feed industry has resulted in solvent extraction being the most utilised method.

Solvent Extraction

Most modern soybean processing plants make use of solvent extraction processes whereby the soybeans are cracked, heated, and flaked before the oil is extracted with hexane. Once the oil has been removed,

the flakes are toasted and ground into a meal. During this production process, temperatures of 135 to 140°C are critical to deactivate the anti-nutritional factors naturally present in raw soybeans. The challenge in SBM processing is to apply enough heat to destroy as much ANFs as possible while avoiding damage to nutrients, to produce the most nutritious product possible (González-Vega *et al.*, 2011). Suboptimal processing results in inadequate deactivation of ANFs whilst excess heating results in heat sensitive lysine and other AAs being damaged due to Maillard reactions (Oliveira *et al.*, 2021).

Before processing, the soybeans are transferred from bulk storage silos to a temporary storage bin. From there the beans are transferred to a conditioner. Conditioning is the first process whereby the beans are uniformly heated causing the moisture to relocate to the surface of the bean. This helps to soften the bean hull. Moisture levels are an important factor in the dehulling process. When moisture levels are well above 10%, hull removal becomes difficult. When the moisture levels drop below 10%, the beans become brittle and prone to cracking into pieces which are too fine to process (Erickson, 1995).

After conditioning, the beans undergo dehulling by being rapidly heated over a very short period causing the hull surface temperature to increase rapidly and the hull is loosened and removes itself from the bean. The heating serves the dual purpose of dehulling as well as preparing the bean for oil extraction. Soy proteins coagulate upon heating, isolating the oil which in turn makes oil extraction easier. The hulls are then screened on a separator, which separates most of the hulls from the dehulled beans (Ali, 2010).

The beans then undergo cracking into smaller pieces using rollers. This step also helps to separate any remaining hulls. To separate the final mix of hulls and cracked bean fractions, the mix is moved to an aspirator where the dehulled bean pieces and all leftover hull pieces are finally separated. The soybean hulls are then either worked back into the meal or they are processed, sold as an animal feed, (Stein *et al.*, 2008) or sold to be processed and used in various other industries. If the customer demand is for the SBM to contain a higher crude fibre (CF) level, the hulls may be mixed into the meal. If the hulls are not included, they are heat treated and milled and can be used as an ingredient in mostly ruminant and some swine diets (Liu & Li, 2017). From the aspirator the bean pieces are ‘flaked’ by being rolled by flaking rolls. The thickness of the flake is important and is a preference of the customer. At this point in the processing procedure, expanders may be utilised. Expanding is the process whereby the flaked beans undergo one more treatment prior to the direct extraction of the oil. The flakes are expanded through an additional step of compression and exposure to live steam. This causes collets, or types of pellets, to be formed. The internal seed oil boils to the surface and percolates out (Lusas, 2016). The use of expanders is not standard practice among the processors within SA, however there are a few plants that have and make use of expanders within their process (RussellStone Group, 2017).

At this point the oil extraction begins. The collets are submerged in a mixture of solvents, with hexane as the most concentrated solvent present in the mix. Whilst submerged, the solvent mixture permeates into the collets and forces the oils and solvent into solution inside the collet forming miscella. The miscella then settles from the collet into the solvent (Ali, 2010). The following processes serve to separate the solvent, oil and meal fractions: A desolventiser-toaster containing steam heated trays cause the solvent to vaporise. The meal is dried by a dryer cooler and the temperature of the meal is reduced. The vaporised solvent is then condensed within an evaporator in which water is used as a coolant. This process is typically named ‘first stage evaporation’ (Witte, 1995). Second stage evaporation then occurs whereby a rising and falling film evaporator uses steam to heat the solvent. The solvent is then totally separated from the oil in a falling film disk and stripping column which uses a highly heated steam to perform the stripping. The solvent and water condense; the solvent is not soluble in water and therefore provides the opportunity for the separation of the solvent and water through decanting the lighter solvent from the top of the mixture. The remaining water may contain solvent in trace amounts and is therefore heated to evaporate the solvent entirely. The solvent and water are then ready for consecutive rounds of extraction. The meal which has been dried and cooled may now undergo grinding and milling to produce the requested particle size (Hensarling & Jacks, 1983).

The focal product of any soybean processing plant is SBM, however all by-products may hold good value and, in the correct market environment, may be marketed to maximise returns. Marketable by-products include crude oil, soya hulls, gums, and a large variety of soap stock (RussellStone Group, 2017).

2.4 Soybean meal

Due to the high concentration of high-quality fat and protein the primary use of soybeans is for processing to produce SBM and soya oil. Soybean meal is produced when the oil in the bean is mechanically or solvent extracted, and the leftover oil ‘cakes’ are further processed into a meal. Both the soya oil and meal are valuable, marketable commodities, although the production of the meal is the focus of most soybean processors. Soybean meal is a rich source of AAs suitable for use in the feed industry. The oil and the meal are used in the food and feed industries respectively (Pettersson & Pontoppidan, 2013).

2.4.1 Nutritional composition

Soybean meal is the largest source of plant protein in animal feeds and has a highly favourable nutrient composition. It has the largest market share of all protein meals included in animal feeds (Nahashon & Kilonzo-Nthenge, 2011). Most of the meal is used in the production of livestock feeds with a very small percentage being used for human food products such as protein alternatives and soymilk. It is estimated that 46% of SBM is to be used in poultry diets (Voora *et al.*, 2020).

The high-quality protein, with its desirable AA profile and high digestibility following proper processing, constitute the main reason as to the prominence of soybean and soybean by-products in animal feeds. The AA profile of SBM complements that of most cereals to provide a very close match to the AA requirements of poultry.

Crude protein is the most frequently analysed nutrient for soya products (Van Eys, 2014). In Table 2.2 is shown different chemical compositions of SBM with different CP concentrations. Typically, soybean seeds contain 40% CP and 20% EE whilst SBM contains around 40-49% CP and 2% fat. The relatively high lysine content, around 6.2 g/16 g N, is limited by cysteine and methionine content (2.9 g/16 gN). Results from AA analyses of raw soybean seeds and SBM from different sources performed by Van Eys, (2014) and Banaszkiwicz (2000) are shown in Table 2.3. Most of the CF is contained within the hull which is removed during processing, therefore the CF content is lower in SBM than in soybean seeds. In some cases the hulls are reworked back into the meal in which case the CF concentration will be much higher than in SBM with the added hulls. Crude fibre concentration in soybean seeds range from 18-22 % of dry matter (DM) whilst CF in SBM drops to between 3-8 % of DM in SBM with the hulls totally removed (Van Eys, 2014). The acid detergent fibre (ADF) content may range from 6-10% of DM in raw soybeans and 3-7% of DM in SBM whilst the neutral detergent fibre (NDF) content in raw soybeans can range from 13-17% of DM and drop to 7-11% of DM in SBM. Crude fat content, from ether extract (EE), ranges from 18-20 % of DM in raw soybeans whilst it drops down to ranges as low as 1-3% of DM in SBM. The concentration of ash may range from 3-6% of DM in soybean seeds and 4-7% of DM in SBM (Banaszkiwicz, 2000). The concentrations of starch between raw soybeans and SBM do not differ greatly and can range between 4-7% of DM. Nutrient concentrations of SBM from different origins are shown in Table 2.3 and AA concentrations of soybeans seeds and SBM of two different origins is represented in Table 2.4.

2.1.1 Quality testing

The nutritional value or quality of a feed ingredient is affected by its nutritional function - the ability to

Table 2.2: Nutritional composition of raw soybean seeds and soybean meal of different compositions (Banaszkiewicz, 2000; Van Eys, 2014)

Chemical composition (% dry matter basis)	Soybean seeds	Soybean meal (44% crude protein, %)	Soybean meal (49% crude protein)	Soybean meal
Crude protein	37.1	43.8 - 49.9	52.8 - 56.3	44.0
Crude ash	4.90	5.60 - 7.20	5.20 - 9.10	6.65
Ether extract	5.12	0.55 - 3.00	1.00 - 3.30	2.18
Crude fibre	18.4	4.30 - 7.20	3.10 - 4.10	6.75
Neutral detergent fibre	13.0	12.3 - 8.19	7.40 - 12.2	15.5
Acid detergent fibre	7.22	8.90 - 11.9	5.20 - 6.70	9.50
Nitrogen free extract	24.0	34.3	33.2	31.8
Starch	4.66	5.51	5.46	6.30

meet the nutritional demands of the animal. Nutritionists will thus select combinations of feedstuffs that provide suitable amounts of nutrients to meet production criteria. Ingredient quality also depends on the bioavailability of the nutrients in question (Zentek & Goodarzi Boroojeni, 2020). Nutrient level and composition do not wholly indicate the quality of a feedstuff and its suitability as feed ingredient. The quality of the feedstuff is also dependent on other important factors including, but not limited to digestibility, palatability, particle size and flow ability (Dale, 1996). Of particular interest in the case of SBM is nutrient digestibility owing to the ANFs present in untreated soybeans. Various analyses have been developed and adopted as acceptable measures of quality with most directly indicating the digestibility of protein whilst other may indirectly provide more information on quality. The most common analyses include urease activity (UA), trypsin inhibitor activity (TIA), protein dispersibility index (PDI), protein solubility in potassium hydroxide (KOHPS), phytic phosphorus (PP) and its proportion to non-phytic phosphorus, reactive lysine, and its proportion to non-reactive lysine (Araba and Dale, 1990; Parsons *et al.*, 1991; Willis, 2003; Van Eys, 2014). The choice of quality analysis differ greatly among producers and feed compounders and not all tests are applied on a regular basis (Dozier,

Tale 2.3: Concentration of nutrients in samples of soybean meal of different origins on a dry matter basis (Tangendjaja, 2020)

Nutrient	Origin		
	Argentina	Brazil	United States of America
Dry matter (%)	88.9	88.5	88.7
Crude protein (%)	46.5	48.6	46.6
Crude fibre (%)	3.81	4.04	3.58
Ether extract (%)	1.56	1.58	2.01
Ash (%)	6.84	6.46	6.29
Neutral detergent fibre (%)	9.07	10.1	8.56

Table 2.4: Amino acid concentration of soybean seeds and soybean meal of different sources (Banaszkiewicz, 2000; Van Eys *et al.*, 2014)

Amino acids (% dry matter basis)	Soybean seeds	Soybean meal 44% crude protein	Soybean meal, g/16g Nitrogen
Arginine	2.45 - 3.10	3.49 - 3.78	6.79
Cysteine	0.45 - 0.67	0.66 - 0.75	1.57
Histidine	1.00 - 1.22	1.21 - 1.32	2.58
Isoleucine	1.76 - 1.98	2.15 - 2.78	4.24
Leucine	2.20 - 4.00	3.66 - 3.92	8.21
Lysine	2.50 - 2.66	2.99 - 3.22	6.49
Methionine	0.50 - 0.67	0.60 - 0.69	1.50
Phenylalanine	1.60 - 2.08	2.35 - 2.03	4.93
Threonine	1.40 - 1.89	1.89 - 2.03	3.99
Tryptophan	0.51 - 2.44	0.66 - 0.75	1.05
Valine	1.50 - 2.44	2.24 - 2.67	5.22

2011). For results of quality tests to have real value and to be comparable between producers it is important to have standardised methods and equipment. This standardisation is becoming increasingly

important as international trade in soybean products grows and competition amongst suppliers increases (Van Eys, 2014).

Nutritional composition and quality of soybean products are published by various entities providing a general standard which enterprises can utilise when trading (Van Eys, 2014). In addition to the general nutritional composition tables, many organisations have since included additional standards that give more information on the quality of the soybean products, which may include common parameters such as UA, protein dispersibility and trypsin inhibitor measures. The standards do not represent a minimal level of quality that producers are obligated by law to follow but merely provide a yardstick and have very little impact on processing operations in international trade (Shurtleff & Aoyagi, 2009).

Despite the processing equipment and processes utilised being similar for all processing plants, the management and application of the processes vary greatly between and within plants, depending on preferences, the need to limit costs and cultivar and quality of the soybean (Erickson, 1995). Soybean quality is affected by cultivar, the region of cultivation as well as the cultivation, harvesting, storage and handling practices (García-Rebollar *et al.*, 2016). Table 2.5 shows the most common quality analyses performed on SBM and the acceptable ranges.

Urease activity index

Of all the quality analyses, UA index is the most widely utilised. The index infers protein quality as a factor of the concentration of urease present in the sample being tested (Chen *et al.*, 2020). The UA index is reported as the increase in pH (in pH units) resulting from the release of ammonia consequence to the breakdown of urea by the remaining urease that has escaped breakdown through the process of thermal processing. There are two methods that interpret the pH increase; one that is used in the European Union, and the other is the method used by the American Oil Chemist Society. The former measures the amount of acid that is needed to maintain a constant pH. The latter simply measures the increase in pH of the total sample media. Results between the two methods and the interpretations thereof will differ slightly (Van Eys, 2014). It is generally accepted that a pH rise of 0.05 to 0.2 indicates optimally processed SBM (Dudley-Cash, 1998; Stein *et al.*, 2008). Under-heating is represented by values above a 0.2 pH rise whilst values below 0.05 indicate over-heating (Stein *et al.*, 2008). Urease activity index is however not useful in determining if SBM has been excessively heated but rather if the SBM has been sufficiently heated to remove ANFs to a level that is safe for consumption (Witte, 1995). Urease activity is thus considered an indirect measure and is therefore correlated to and used in conjunction with other measures of protein quality. Urease destruction through thermal processing is highly correlated to the destruction of trypsin

Table 2.5: Processing parameters of soybeans and appropriateness of quality testing and acceptable quality parameter values (Greenwood & Kim, 2021)

Processing parameter	Trypsin inhibitor activity	Urease activity index	Protein solubility in potassium hydroxide	Protein dispersibility index	Reactive lysine ratio
Under Processing	Very Appropriate	Very Appropriate	Low Use	Appropriate	Not Appropriate
Over Processing	Not Appropriate	Not Appropriate	Appropriate	Low Use	Very Appropriate
Target Values	<2.5 or 5mg/g	<0.05to<0.30 pH rise	78 – 84%	40 – 45%	>90%

inhibitors and other ANFs. High urease values may be correlated to high TIA. Since trypsin inhibitors are the most abundant ANF in soybeans, it is fitting that often the values are reported together rather than independently (Chen *et al.*, 2020).

Trypsin inhibitor activity

The analysis of TIA, using method Ba 12a-2017 (Chen, 2020), is relatively rapid analyses and widely utilised. The method involves preparation of mixtures of sample substrate, trypsin, and inhibitors of varying levels (Pacheco *et al.*, 2014). These mixtures are then combined in either a substrate last (S-last) or enzyme last (E-last) fashion using either the full volume assay (10 mL total sample, reagent enzyme mixture) or half volume assay (5 mL total sample reagent, substrate mixture). The latter method potentially increases assay sensitivity and reduces the amount of reagent needed. The full volume assay is regarded as the standard method (Cheng *et al.*, 2020). The procedure is colourimetric and the difference in absorbance between the presence or absence of an inhibitor forms the basis of the assay (Kunitz, 1947). Reported in mg/g, it is generally accepted that TIA values below 2.5 mg/g are sufficient to avoid detrimental effects to animal health (Van Eys, 2014). Trypsin inhibitor activity is generally regarded as an indicator for all other ANFs present in SBM. Trypsin inhibitor values indicating that sufficient amounts of trypsin inhibitor have been inactivated may also indicate sufficient inactivation of all other ANFs of concern in SBM (Vagadia, 2017).

Studies performed by Liener (1995) and Rackis *et al.*, (1985) showed how increasing heat treatment increased animal health and nutritional value.

Despite both UA and TIA being useful, reliable and highly correlated measures for determining whether overheating has taken place or not, they fail to indicate or infer the amount of overheating when other tests cannot be performed. The colour of the meal may provide insight to the occurrence of browning from Maillard reactions due to overheating. Testing protein solubility in potassium hydroxide is useful in understanding levels of overheating (Batal *et al.*, 2000).

Protein solubility in potassium hydroxide

Protein solubility in KOH, is a useful analysis to differentiate between SBM that is over-heated and SBM that has been correctly processed. Potassium hydroxide solubility is, however, not useful in quantifying the level or amount of overheating. It is also not useful when gauging under-processing of SBMs (Van Eys, 2014). The method requires solubilisation of the sample in a mix of KOH solution (Araba & Dale, 1990). The nitrogen content of the sample is first determined, then nitrogen content which has dissolved in the liquid is determined. The difference is expressed as a percentage of nitrogen in the sample. As the level of heat treatment increases, the KOH solubility decreases. The range of acceptable solubility levels is 70-85% (Heuzé, 2016). Values below the accepted range adversely affect animal performance whilst values above the acceptable range indicate optimum processing levels and optimum animal response. The solubility values are therefore correlated to animal performance (Mahesh *et al.*, 2017). Potassium hydroxide solubility is not informative when investigating cases where insufficient processing has taken place. In such cases using the PDI will be suitable.

Protein dispersibility index

The PDI gives an indication of the solubility of soybean proteins in water and well adapted to all soya product types (Batal *et al.*, 2000). It is suitable in evaluating both under and over-heating. The measure is given as the amount of soya protein that is dispersed in water after a mixture of water and the soya protein source has been blended. The analysis is performed by determining total nitrogen content of the soya product. The amount of soluble nitrogen is determined by placing a 20 g sample of the soya product in a blender with 300 mL of de-ionised water at a temperature of 30°C. The mixture is blended for 10 minutes at 8500 revolutions per minute where after it is filtered and centrifuged for 10 minutes at 1000xg. The nitrogen content of the supernatant is then analysed and expressed as a percentage of the total nitrogen content of the soya product sample as per method Ba 11-65 (AOCS, 2022). It is the simplest of all quality tests and reported to be the most consistent method. Van Eys (2014) indicates that acceptable

PDI values range from 15–30%, while Batal *et al.* (2000) initially reported 45% protein dispersibility a suitable value. Experimental data suggest that the PDI value is more sensitive than UA or KOHPS, and most soybean processors and soybean product users believe PDI values as best for assessing quality of protein (Van Eys, 2014).

Total phosphorous and phytic phosphorous

Total P, or available P is the P that is absorbed by the animal from the diet. Phosphorous levels follow digestible AA and metabolizable energy (ME) in importance when determining feed quality (Van Eys, 2014). When evaluating and comparing soybean products, accurate determination of total P indirectly through P and PP evaluations is critical (Selle & Ravindran, 2007). Additionally, the high cost of supplemental P in poultry feeds, the fact that up to 60% of P in soy products is unavailable to monogastric (Nelson *et al.*, 1968) animals and that SBM is a notable source of P, makes analysis of P important when determining SBM quality (Camden *et al.*, 2001).

Phytic P levels refer to the portion of P that is bound to phytin molecules forming phytic acid (*myo*-inositol hexaphosphate), the major storage form of P, and is poorly utilised by poultry. Many commercial producers supplement feeds with phytase to increase P availability (Selle *et al.*, 2009). In addition to being an indication of bioavailable levels of P, PP will provide insight into digestible protein levels. Phytic P analysis has been shown to affect protein metabolism in later stages of digestion (Bohn *et al.*, 2008). Phytic acid contains phosphate groups that are capable of binding to peptides and AA constituents, thus proteolytic enzyme action is inhibited (Deak & Johnson, 2007).

Reactive lysine

Lysine is considered an essential AA as poultry cannot synthesise lysine in the body. It is also the second most limiting AA and the most limiting AA in terms of growth in poultry and is therefore used as a reference for adjusting the content of other AA inclusions in maize-soybean based diet formulations for poultry (Khwatenge *et al.*, 2020). Muscular development, growth and performance depend heavily on supply of lysine and an understanding of levels of bioavailable or *reactive* lysine is helpful (Cemin *et al.*, 2017).

During heat treatment of soybeans, heat sensitive lysine is damaged through Maillard reactions and lysine-sugar complexes are created which are insoluble and indigestible. Analysis of SBM, through near infrared reflectance spectroscopy (NIRS) and wet chemistry, is performed to determine how much of the lysine is still reactive. This gives an indication of the severity of the over processing that took place. Kim *et al.*

(2012) demonstrated that varying levels of reactive lysine were observed when samples of soybeans were exposed to varying degrees of heat treatment. There is thus a correlation between degree of heat treatment during processing and reactive lysine content i.e. the higher the degree of heat treatment the less the concentration of reactive lysine. Optimum values of reactive lysine: total lysine ratio are above 90%, however the majority of soybean samples globally were analysed to have reactive lysine percentages around 72% (Rutherford, 2015).

The most commonly used quality parameters and the typical values of each from three of the world's largest producers of are shown in Table 2.6.

Digestible amino acids

The levels of digestible AAs and ME are important when determining the quality and value of any ingredient. Digestible AA content is second only to ME as being the largest cost item when formulating feeds (Lopez *et al.*, 2020). Amino acid digestibility values are particularly important in the context of SBM. Soybean meal is one of the most consistent and highest quality protein sources for animal nutrition. However, some variation does occur in both the nutrient concentration and quality (digestibility or bioavailability) among different samples and sources of SBM (Nahashon & Kilonzo-Nthenge, 2011). Differences in processing methods have a major effect on quality and this may lead to important differences among crushing plants. Undercooking of soybeans leads to higher concentrations of ANFs, reduces palatability and poorer AA and energy digestibility. Overcooking results in the loss of methionine and lysine and, in severe cases, a reduction in digestibility. In addition to processing method, one of the most studied sources of variation in quality is the geographic origin of soybeans and soybean products (de Coca-Sinova *et al.*, 2008; Wang *et al.*, 2011).

2.5 Exogenous enzymes to improve quality of soybean meal

Properly processed SBM provides a versatile, high-quality and cost effective protein source that can be used as either the sole or supplemental protein source in animal feeds. Prices for SBM are, however, increasing with a trend towards continued price increases in the future (Chen and Yan, 2022). For SBM to maintain its role as a cost-effective protein source and to hold its place as popular feed ingredient in the face of non-conventional, alternative protein products now surfacing, there needs to be an increase in the efficiency of utilisation of the nutrients within the

Table 2.6: Mean quality parameter values of soybean meal samples from different origins (Aguirre *et al.*, 2022)

Quality Parameter	Origin		
	Argentina	Brazil	United States of America
Urease Activity, (mg N/g)	0.01	0.01	0.01
Protein dispersibility index (%)	11	12.9	14.6
Potassium hydroxide protein solubility (%)	74.6	77.6	80.4
Trypsin inhibitor activity (mg/g DM)	1.89	2.36	2.61

meal (Olukosi *et al.*, 2019). Currently the use of microbial exogenous enzymes is playing a major role in helping to increase the nutritional value of SBM. It has been established that the use of protease enzymes is effective in increasing the protein availability in addition to heat treatment (Selle & Ravindran, 2007, Pettersson & Pontopidan, 2013; Erdaw *et al.*, 2016).

Almost all chemical reactions occurring within an organism require the presence of enzymes. Enzymes are special proteins that act as biological catalysts. All living organisms synthesise enzymes and these enzymes play critical roles in catalysing chemical reactions in almost all metabolic pathways within an organism. Enzymes are also synthetically made and used in many industries including nutrition (Erdaw *et al.*, 2016).

Although exogenous enzymes have been included in studies conducted as early as the 1920s (Hervey, 1925), first commercial use dates back to 1984 (Bedford & Partridge, 2010) where barley-based feeds were supplemented with enzymes used in brewing. The use of phytase for improving phosphorous and to some degree calcium, in livestock diets paved the way for research into other enzymes. Initial use of enzymes in poultry diets was by way of admixtures of two or more enzyme preparations, most commonly phytase and carbohydrase. Now the use of a single enzyme as a mono-component additive is becoming common (Cowieson & Adeola, 2005). Protease, specifically as a mono-component additive for poultry and pig nutrition, has become available over the last decade whereas it has regularly formed part of enzyme admixtures for the past 25 years (Fru-Nji *et al.*, 2011). The purpose of enzyme use was primarily to achieve greater efficiency of nutrient utilisation of the feed thereby reducing feed costs by reducing the levels of nutrient inclusion in feed. Further research revealed added benefits not only for the farmer and animal but for the environment as well (Thornton, 2010).

2.5.1 Mechanisms of action of enzymes

Enzymatic reactions are smaller processes that form part of larger reactions. In most metabolic reactions in an organism, there comes a point where a given molecule needs to be converted into a form that is easily or quickly broken down for the reaction to continue or occur fast enough for proper physiological functioning ultimately sustaining life (Roskoski, 2015). Enzymes achieve this by helping to decrease the amount of energy that is required for that part of the reaction to take place. A deviation from the required amounts of products or from the time at which or over which the products are produced compromises metabolic pathways and almost always leads to sickness and/or death (Engelking, 2015).

Almost all enzymes are proteins and stereotypically globular in structure (Grahame *et al.*, 2015). They act alone or in multi-enzyme complexes. The structure of an enzyme is an important aspect to its nature and mode of action. The substrate to which an enzyme binds is highly specific, however there are some exceptions (Segel, 2013). The specificity of an enzyme to a substrate upon which it acts is brought about through the enzymes' unique structure which is shaped in such a way that it represents the exact complement of the shape of the substrate, this theory is commonly known as the "Lock and Key" model. This allows the substrate to bind or "fit" the enzyme in an appropriate way at a region on the enzyme which is called the "active site". The active site contains residues of AAs which form the bonds between enzyme and substrate and is comprised of the binding site, where the physical binding occurs, and the catalytic site, where the reaction is catalysed. Another proposed model is that of the binding occurring under an induced fit, where incomplete binding to AAs on the active site induced changes in the shape of the enzyme until it forms a direct fit at which point the substrate is completely bound. This model suggests that the binding site will not always be perfect since the shape of the enzyme is dynamic and always changing under the influences of continuous reactions (Roskoski, 2015).

2.5.2 Factors affecting enzyme activity

Enzyme activity and effectiveness is subject to several factors. These factors include temperature, hydrogen ion concentration (pH), enzyme concentration, substrate concentration, and the presence of inhibitors or activators (Engelking, 2015). There is a small environmental threshold in which enzymes operate optimally. The temperature and the pH of the environment in which the enzyme is found is critical. As the temperature increases, the rate of the reaction increases until a temperature optimum is reached where enzyme activity is at its peak. From this point onwards, any increase in temperature slowly decreases the activity of the enzyme and in cases of severe heat exposure enzymes denature or

permanently lose their structure or function (Segel, 2013). Enzyme activity can be manipulated to achieve a variety of functions. Enzyme activity can be decreased using inhibitors. Inhibitors will bind to the active site of the enzyme and as a general function alter the substrate to enzyme binding capabilities (Di Cera, 2009). In the context of trypsin inhibitor in raw soybeans and SBM, competitive inhibition takes place. Competitive inhibition occurs when a molecule, closely resembling the substrate, binds to the enzymes active site creating a physical barrier between enzyme and substrate binding. Trypsin inhibitor binds to trypsin where proteins from digesta would have been bound for the digestive process. This indirectly inhibits chymotrypsin conversion/ inhibition, resulting in an antinutritional effect (Mazzei *et al.*, 2016).

2.5.3 Exogenous protease

Exogenous enzymes collectively refer to enzymes of a synthetic nature that are produced and supplemented in animal feeds and are typically of bacterial or fungal origin. Endogenous enzymes, on the other hand, are secreted by the animal. Exogenous enzymes are supplemented to target specific nutrients (Alabi *et al.*, 2019). The class of proteases is large with a large variety of enzymes classified into different protease families with differing modes of action. It is important to choose suitable and appropriate proteases according to the dynamics and functioning of the enzyme itself, the targeted substrate and the needs and outcomes of the producer (Cowieson & Haahr, 2019). Despite its evidence as a tool to improve the digestibility of DM, CP and AAs as well as mitigate the effects of ANFs, further research is required in order to provide a comprehensive understanding of the mode of action of mono-component protease use in soybean-maize based diets fed to broilers (Angel *et al.*, 2011).

Protease classification

All enzymes are characterised according to their mode of action. Broadly categorised, proteases are hydrolases which hydrolyse peptide bonds. Proteases are further broken down into families and sub-families. Proteases are differentiated by the AA sequence present at the active site. The main families include cysteine, serine, aspartic, glutamic, threonine, and metallo proteases which are further broken down into endo and exo-peptidases (McDonald & Barrett, 1987). Endopeptidases cleave proteins from within the protein chains, breaking up and solubilising protein fragments whilst exo-peptidases cleave on the outends of the peptide chains releasing AAs, dipeptides, and tripeptides. The site at which cleavage takes place is also specific to the protease. Trypsin is an example of a highly site specific protease which cleaves at lysine and arginine (Rawlings & Barrett, 1991). Commercially available proteases used in feeds include neutral and alkaline proteases such as serine proteases, which act optimally in neutral pH

environments. Differentiation of proteases may also be by the species from which they are isolated. Commercially produced proteases are isolated from either fungi or bacteria (Neitzel, 2010).

Effects of exogenous proteases on nutrition

The commercial use of enzymes, such as phytase, began in the early 1980s (Bedford and Partridge, 2010). In the late 1990s proteases began to form part of commercial diets, initially as admixtures. It is only in the last decade that widespread use of protease as a mono-component only became relevant (Cowieson and Adeola, 2005; Fru-Nji *et al.*, 2011). Achieving greater efficiency of feed nutrient utilisation and thus reducing feed costs and environmental impact through decreased nutrient requirement as a result of improved nutrient digestibility is the primary intention of protease use (Ghazi *et al.*, 2002). Proteases may have direct and indirect effects on animal nutrition. Direct effects are characterised by the intended purpose of enzyme use i.e. breakdown of complex proteinaceous molecules into smaller constituents improving rate and extent of hydrolysis of peptides thus increasing protein digestibility as well as the breakdown of proteinaceous ANF's such as trypsin inhibitor and lectins, additionally increasing the digestibility of protein. Indirect effects include significant effects on non-proteinaceous nutrients such as the improvement of digestibility of energy indirectly through increases in fat and starch digestibility as well as the benefits of improved gut health and a reduced environmental impact (Peek *et al.*, 2009; Wan *et al.*, 2018).

In both early and more recent studies, the potential in commercial protease application became apparent. In a study by Marsman *et al.*, (1997), soybean-maize based diets with and without protease inclusion were fed to broilers in order to assess changes in apparent ileal amino acid digestibility (AIAAD). Apparent ileal AA digestibility increased from 83.7% to 85.2% in the diets containing protease. In a similar study within the same year, Bernard and McNab (1997), showed that soybean-maize based diets fed to three week old broilers containing protease had increased AIAAD. Numerous other findings from studies using maize-soybean based diets reported similar increases in apparent ileal digestibility (AID) of CP and AIAAD when protease was used. Freitas *et al.* (2011) observed a 1.8% increase, also with a lower inclusion of protease. Cowieson and Roos (2014) observed a mean increase in AID of 3.74% across all AAs. More recently, Erdaw (2016) reported a mean increase of AIAAD of 5.7% across all AAs. Lastly, Park *et al.* (2020) reported a mean increase of 3.00% across three test diets at a lower protease inclusion.

The benefits of protease use in commercial diets are clearly illustrated in literature, however the use of protease in commercial diets is unpredictable and not always justified. Both past and more recent studies have reported variable effects in ileal DM, CP and AA digestibilities in soybean-maize test diets for

broilers where proteases were included (Doskovic *et al.*, 2013). These studies have been performed to test the many factors which contribute to the efficacy of protease in soybean-maize based diets. Some of the factors included but are not limited to the digestive capabilities of individual birds, protease concentration or dosage, species from which the enzymes are isolated, *in vitro* compared to *in vivo* studies, the effect of differences in origin and quality of the protein component and different levels of heat treatment during processing (Angel *et al.*, 2011; Milosevic *et al.*, 2013). Furthermore, more research has been performed on monocomponent protease inclusion in maize-soybean based broiler diets than on other enzymes such as phytase or protease in admixtures (Bedford and Morgan, 1995). Overall, no adverse effects of monocomponent protease inclusion have been reported in studies, however, enzyme inclusion does not always improve DM or nutrient digestibility, decrease the negative effects of ANFs or compensate for low CP content in feed. Observations from studies previously discussed have shown increases in AIAAD for all AAs, however, some studies have shown the contrary. Yu *et al.* (2002) observed no significant increase in AIAAD when proteases were fed to three week old broilers. The increase or decrease in AIAAD as a result of protease use is not uniform or proportional across all AAs. Studies on AIAAD have shown that protease use may result in the increase in digestibility of some AA and a decrease or no significant increase in others and not only an increase in the AID of all AAs. Zanella (1999) reported significant increases in AID of tryptophan and leucine whilst a reduction in AID was observed for methionine. Bertechini *et al.*, (2020) only observed a significant increase in the AID of alanine, arginine, cysteine, glycine, histidine, methionine, proline, threonine, tyrosine and valine.

Cowieson *et al.*, (2016) investigated the effect of different sources of feed protein on nutrient digestibility and performance in diets with and without a protease. A number of test diets were used, containing either canola meal or SBM and fed to broilers with and without protease. It was found that AID of N was increased for both protein sources when a protease was included, however, ileal digestible energy was increased and intestinal integrity improved only for the diets containing SBM as the main protein source. This suggests that levels of protease activity may be specific to the substrates to which they are presented. In a similar study, Yu *et al.*, (2002) evaluated the interaction of protein source and enzyme supplementation and the effect it has on protein digestibility. Despite all protein sources having had equal N levels, protein digestibility varied for all protein sources. For the diets that included protease it was shown that the dietary protein source also had an effect on the activity of protease. Within the same dietary protein source, i.e. SBM, variation in efficacy of protease in these diets has been demonstrated. Fru-Nji *et al.*, (2011) conducted a study, in which diets with SBM of low and high CP content were fed with and without protease in order to test the effect of CP content on protease efficacy. It was found that only the diet with low CP content had a significant increase in AID of CP (4.9%). De Coca-Sinova *et al.*, (2008)

assessed the AID of N and AAs in feeds with SBM of varying CP content. It was found that AID of AAs and N varied more in the diets with higher CP content. Greater protease effects in low CP diets were also observed by Dessimoni *et al.*, (2019) when multiple treatment diets of varying CP levels were compared. Angel *et al.*, (2011) reduced the dietary CP levels by 10% in relation to the control diet and also concluded that protease activity was increased. Conversely, Cowieson *et al.*, (2020) reported that no interaction was found between protease and SBM of two different origins which was tested to be significantly different nutritionally. Salazar-Villanea *et al.*, (2022) showed that there was also an interaction between level of thermal processing of soybeans and nutrient digestibility as well as an interaction between level of soybean processing and protease supplementation and the effect it had on AIAAD and AID of CP. An increase in processing time and using a protease may not be suitable to compensate for improperly processed SBMs. Also related to processing, Marsman *et al.*, (1997) observed that different methods of thermal processing resulted in higher or lower AID of CP. Extrusion significantly improved AID of CP compared to toasting methods, however no significant differences were found in AID of CP between the diets with both extruded or toasted SBM that were fed with or without protease.

In a study by Ghazi *et al.*, (2002) the effect of species of enzyme isolation was assessed. It was shown that AID of N increased when a protease isolated from *Aspergillus* species was used and that the AID of N did not increase when protease isolated from a *Bacillus* species was used. Testing the effect of dosage concentration of a commercially available enzyme mixture, designated 'A' and one test mixture, designated 'B', both with protease activity, found that the efficacy of the enzymes was dependent on dosage (Kocher *et al.*, 2002). For enzyme mixture A, using the recommended concentration resulted in no significant difference in AID of CP, whilst a dosage of five times the recommended amount resulted in a significant increase in CP AID. For enzyme B, no significant difference was found using enzyme B at the recommended dose whilst at five times the recommended dose, AID of CP significantly decreased.

Conflicting results have been obtained when comparing *in vitro* and *in vivo* DM and CP digestibilities. Using a commercially available enzyme, Yu *et al.*, (2007) evaluated the effects on performance of a mono-component enzyme inclusion in low protein diets tested *in vitro* and *in vivo*. A significant difference in CP and DM digestibility was found in *in vitro* test diets with enzymes supplementation however no differences in *in vivo* digestibilities were noted. A recent study conducted by Zheng *et al.*, (2023) assessed the efficacy of four different protease types; acidic, alkaline, keratinase and neutral fed separately in maize-soybean diets tested *in vitro* and *in vivo*. It was found that supplementation with protease of all types significantly reduced AIAAD while alkaline, keratinase and neutral proteases increased *in vitro* CP digestibility whilst decreased CP AID. This means that information from *in vitro*

investigations into protease use may be limited in terms of its relevance to *in vivo* applications (Caine *et al.*, 1998). To the contrary, de Coca-Sinova *et al.*, (2008) compared an *in vitro* assay to that of results of an *in vivo* investigation and concluded that for SBM, and *in vitro* assay may be satisfactorily used to predict *in vivo* digestibility of DM and CP.

Fru-Nji *et al.*, (2011) conducted a study to compare the effect of broiler sex on AID when test diets were fed with or without protease. It was found that in one study, performance in both males and females was improved in the diets containing protease and in the second study, AID of CP was increased (on average for both sexes) from 76.9% to 82.8%. The digestive capacity of individual broilers was shown to be a significant factor contributing to digestibility of protein in SBM and should not be disregarded (Cowieson *et al.*, 2020).

2.6 Conclusion

Variation in the nutritional composition and quality of SBM is influenced by many factors. The composition of SBM sampled from different crops may vary greatly, mainly due to the fact that these processing plants source raw soybeans from different regions where producers use different cultivars and implement different farming practices. Furthermore, another major factor causing this variation is the processing technique used by the processors, which is governed largely by the nature of the final product requested by the customer. This variation may affect the way in which a protease acts on substrates in a given diet consumed by birds that have individual digestive capabilities. One of the parameters used to identify differences between SBMs is CP content. However CP content may not consistently indicate AA content and differing levels of CP in SBM may have an effect on the efficacy of a protease on AID of AAs and CP.

CHAPTER 3

A Survey of Soybean Meal from South Africa's Largest Processors

3.1 Introduction

The production and use of soybeans have become an integral part of the agricultural sector worldwide with a projected 85% of harvested soybeans destined to livestock feed (Voorra *et al.*, 2020). The soybean industry in SA is a lot younger than that of many other countries with production of soybeans only starting to see its first growth during the 1990's with less than 100 000 tons being produced per year from non-genetically modified cultivars (Bahta & Willemse, 2016). Exponential growth in production was experienced from 2000 to 2005 with production per year growing from 150 000 tons to 400 000 tons (CEC, 2018) stimulated by continued growth in other countries as well as the sanctioning of the use of transgenic crops, stipulated in Act 15 of 1997, in SA. The industry grew steadily until the early 2010s when a spike in soybean production occurred and exponential growth followed in subsequent years. This came a result of the first large-scale establishment of the soybean processing industry in SA (RussellStone Group, 2017). Agro processing formed part of the focal points of government initiatives from 2007 (DTI, 2008) to 2014 drawn up to address the industrial and manufacturing sectors to improve and localise industries across various sectors. Soybean processing eventually became one of the key areas of focus according to the 2013/2014 – 2015/2016 Industrial Policy Action Plan to maximise local processing of soybeans which were previously mostly destined for export (DTI, 2014).

Serious development of the soybean processing industry only began in early 2010. Previously it was believed that locally produced soybeans lacked the quality required for value adding, compounded by the fact that beans from South African cultivars could not be appropriately processed with conventional crushing machinery available at the time (RussellStone Group, 2017). This meant that there was a poor understanding of the nutritional make-up and quality parameters of SBM and the information that was available did not reflect the true potential of South African cultivars when processed. Enough accurate information on the nutritional makeup of SBM from local soybeans was insufficient and inaccurate for precise formulation. When the potential was realised farmers and the government began to focus on localising and developing the industry. Key commercial enterprises took advantage of this opportunity and imported soybean processing machinery that had been adapted and developed to process the unique profile of beans cultivated in SA which enabled them to produce high quality soybean by-products destined for the South African market (De Beer and Prinsloo, 2013). As processors began exploiting the full potential

of SBM from local beans it became important to develop a database of accurate information from the new processors in order to understand the SBM products produced as well as the variation that would arise due to different processing protocols and the further development of new cultivars.

3.2 Objectives and Hypotheses

The aim of the study was to compile a comprehensive database, comprised of data from two surveys that outlay the nutritional information and quality parameters of SBM produced by seven of the largest soybean processors in SA. The objectives were:

1. Collection of SBM samples from various processors across SA.
2. Analyse the SBM samples for nutritional composition and test for significant differences between samples.

The null hypothesis was that there is little to no variation in nutritional composition and quality of SBM from different sources in SA. The alternative hypothesis was that there are large amounts of variation in nutritional composition and quality of SBM from different sources in SA.

Survey One

3.3 Materials and methods

3.3.1 Collection of soybean meal samples

Samples of SBM were collected from seven processing plants (here on referred to as Sources 1-7) over winter and spring - specifically the months of June to October 2020. These samples were taken from local crushers only based in Gauteng, Mpumalanga, Free-State and North-West Province. It was confirmed that the crushers had only sourced locally produced soybeans from the batches from which samples were being taken. Between seven and twelve samples were taken from different batches in each of the plants. Ten samples were collected from Source 1, seven samples from Source 2, nine samples from Source 3, seven samples from Source 4, twelve samples from Source 5, eleven samples from Source 6 and twelve samples from Source 7. Not all producers allowed the required amount of 12 samples to be taken. All samples were collected at random on separate occasions to achieve the best representation of the SBM that is distributed commercially. Each sample collected weighed 20 kg. The samples were taken directly from

bulk bags located in the stores. Four sub samples of 2.5 kg each were taken from the 20 kg bag using a small feed sampler.

3.3.2 Analyses of samples

Each of the four sub samples were sent to three different laboratories: the Animal Production and Health Department of the Polytechnic University of Madrid, Evonik Africa (Pty) Ltd and DSM Nutritional Products Iberia, S.A. Using wet chemistry the Animal Production and Health Department of the Polytechnic University of Madrid analysed for moisture, using method 925.04 (AOAC, 1996); DM, according to method 930.15-1930 (AOAC, 1999); CP using method 990.3 (AOAC, 2006); CF according to method Ba6a-05 (AOCS, 2017); NDF according to method 2002.04 (AOAC, 2006); EE according to method Am 5-04 (AOCS, 2017); ash according to method 923.03 (AOAC, 2017); sucrose, stachyose and raffinose were analysed according to method 926.13 (AOAC, 1999); Ca, P, K, Mg, Na, Zn, Mn, Fe and Cu were all analysed using method 985.29/45.407 (AOAC, 1996). Wet chemistry was also used to analyse: urease activity (UA), using method 941.04 (AOAC, 2017); protein dispersibility index (PDI) according to Ba 11-65 (AOCS, 2022); trypsin inhibitor activity (TIA) was analysed using method Ba 12a-2022 (AOCS, 2022); protein solubility in potassium hydroxide (KOH) according to method 994.12 (AOCS, 2022) and phytic phosphorous levels (PP) according to method 934.01 (AOAC, 2006). Using NIRS, Evonik Africa (Pty) Ltd analysed the concentration of AA's and Processing Condition Indicator (PCI), which is parameter developed and used solely by Evonik which gives an overall indication of level of processing. Also using NIRS, DSM Nutritional Products Iberia, S.A.; analysed KOHPS and protein digestibility index.

3.3.3 Statistical analysis

The data obtained from the wet chemistry and NIRS analysis were analysed statistically with the Proc mixed model system (Statistical Analysis System, 2021) for the mean effects. Means and standard error were calculated and significance of difference ($P < 0.05$) between means was determined by Fischers test (Samuels, 1989). Repeated Measures Analysis of Variance with the Mixed model was used for repeated week or period measures.

The linear mixed model used is described by the following equation:

One way

$$Y_{ijk} = \mu + S_i + L_j + B_k + SL_{ij} + e_{ijk}$$

Where Y_{ijk} = variable studied during the period

μ = overall mean of the population

S_i = effect of the i th source

L_j = effect of the j th level

B_k = effect of the k th block

SL_{ij} = effect of the ij th interaction between source and level

e_{ijk} = error associated with each

3.4 Results

3.4.1 Nutritional composition

The nutritional composition of samples from different processors is shown in Table 3.1. For DM, Sources 4 and 6 were significantly ($P < 0.05$) higher than Source 7. Sources 1, 2, 3, 5 and 7 were all significantly lower than Source 4. Both Sources 5 and 7 were shown to be significantly lower than Source 6.

The CP concentrations of Sources 5 and 7 were significantly ($P < 0.05$) higher than Source 1. Sources 2, 3, 4 and 6 were found to be significantly lower than Source 5.

Sources 1, 2, 3, 4 and 6 were significantly ($P < 0.05$) greater in CF concentration than Source 5. The CF concentration of Source 7 was significantly lower than Sources 1, 2, 3, 4 and 6.

For the concentration of NDF, Sources 1, 2, 3, 4 and 6 were significantly ($P < 0.05$) higher NDF concentrations than Source 5. Sources 2, 3 and 4 were significantly higher NDF concentrations than Source 7.

For the concentration of EE between samples, Sources 3, 4 and 5 differed significantly ($P < 0.05$) from Source 1 and from Source 2. Sources 5 and 6 were significantly higher than Sources 3 and 4. Furthermore, differences were found between Sources 4, 5 and 6 and Source 7 with Source 7 being significantly lower than Source 5 and Source 4 being significantly lower than Source 7.

Sources 2, 3, 4, 5 and 7 were shown to be significantly ($P < 0.05$) higher than Source 1 in terms of the concentration of ash. Source 6 was significantly lower than Sources 2, 4 and 5.

Concentrations of stachyose, sucrose and raffinose between samples of the different sources are

represented in Table 3.2. The stachyose concentrations for Sources 2, 3, 5 and 7 were significantly ($P < 0.05$) higher than Source 1. Sources 2, 3, 4, 5 and 7 were significantly higher levels of stachyose than Source 6. The sucrose concentration levels of Sources 1, 6 and 7 were significantly higher than the levels in Source 3. For raffinose concentrations, Sources 3 and 4 were

Table 3.1: Nutrient composition (%; dry matter basis) of soybean meal samples from different sources (\pm standard error of the mean)

Source	Dry matter	Crude protein	Crude fibre	Neutral detergent fibre	Ether extract	Ash
1	89.8 ^c (± 0.266)	46.0 ^c (± 0.243)	4.05 ^{ab} (± 0.126)	8.87 ^{ab} (± 0.415)	2.94 ^{bc} (± 0.152)	5.79 ^b (± 0.077)
2	90.0 ^{bc} (± 0.318)	46.6 ^{bc} (± 0.291)	4.18 ^{ab} (± 0.151)	9.57 ^a (± 0.496)	2.90 ^{bc} (± 0.183)	6.36 ^a (± 0.092)
3	89.9 ^{bc} (± 0.281)	46.6 ^{bc} (± 0.256)	4.17 ^{ab} (± 0.133)	9.62 ^a (± 0.438)	2.29 ^d (± 0.16)	6.16 ^{ab} (± 0.081)
4	90.9 ^a (± 0.318)	46.8 ^{bc} (± 0.291)	4.41 ^a (± 0.151)	9.71 ^a (± 0.496)	2.06 ^e (± 0.182)	6.21 ^a (± 0.092)
5	89.8 ^c (± 0.243)	47.8 ^a (± 0.222)	3.35 ^c (± 0.115)	7.34 ^c (± 0.379)	3.46 ^a (± 0.139)	6.21 ^a (± 0.071)
6	90.5 ^{ab} (± 0.254)	46.4 ^c (± 0.232)	3.95 ^b (± 0.12)	8.54 ^{ab} (± 0.396)	3.19 ^{ab} (± 0.145)	5.95 ^b (± 0.074)
7	89.4 ^c (± 0.243)	47.2 ^{ab} (± 0.222)	3.55 ^c (± 0.115)	8.26 ^b (± 0.379)	2.57 ^{cd} (± 0.139)	6.13 ^a (± 0.071)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values were obtained using wet chemistry, Animal Production and Health, Polytechnic University of Madrid.

Source 1, n=10; Source 2, 4, n=7; Source 3, n=9; Source 5, 7, n=12; Source 6, n=11.

significantly higher than Source 1 in terms of raffinose concentration. Source 1 was significantly lower than Source 4. Also, for raffinose concentrations, Source 4 was significantly higher than Sources 2, 3, 5 and 6.

Concentrations of calcium, phosphorus, potassium, magnesium, sodium, zinc, manganese, iron and copper for the various SBM samples are shown in Table 3.3. For calcium concentration, Sources 2, 3 and 5 were significantly ($P < 0.05$) higher than Source 1 and Source 6. Calcium concentrations in Sources 2 and 3 were found to be significantly higher than Source 4 and Source 7. Sources 2, 3 and 5 were shown to have significantly higher total P concentrations than Sources 6 and 4. Also for total P concentrations, Source 7 was significantly ($P < 0.05$) higher than Sources 1, 4 and 6. Potassium concentrations of Sources 3, 4 and 5 were significantly lower than the concentrations of Source 1. The potassium concentrations of Source 6 were significantly higher than the concentrations for Sources 3 and 4. Sources 1, 2, 3, 5, 6, and

7 had significantly higher magnesium concentrations than Source 4. Sources 1 and 7 had lower magnesium concentrations than Source 6. Values for Sources 2, 3, 5, 6 and 7, in terms of sodium concentration, were significantly lower than the sodium concentration of Source 4. Also for sodium concentration, Source 1, 6 and 7 had significantly higher values than Source 2. Sources 3 and 5 had significantly lower sodium concentrations than Source 1. No significant ($P>0.05$) differences in zinc concentration were observed between any of the Sources. The manganese concentration of Source 2 was significantly higher than Source 6. No significant differences in iron concentration were observed between any of the Sources. No significant differences in copper concentration were observed between any of the Sources.

Table 3.2: Concentrations (%; dry matter basis) of sugars in soybean meal samples from different sources (\pm standard error of the mean)

Source	Stachyose	Sucrose	Raffinose
1	4.5 ^{bc} (± 0.15)	9.14 ^a (± 0.233)	0.92 ^c (± 0.067)
2	5.19 ^a (± 0.179)	8.81 ^a (± 0.279)	1.08 ^{bc} (± 0.08)
3	4.95 ^a (± 0.158)	8.35 ^b (± 0.246)	1.17 ^b (± 0.070)
4	4.91 ^{ab} (± 0.179)	8.66 ^a (± 0.279)	1.40 ^a (± 0.08)
5	5.02 ^a (± 0.137)	8.84 ^a (± 0.213)	1.10 ^{bc} (± 0.061)
6	4.32 ^c (± 0.143)	9.19 ^a (± 0.222)	0.88 ^c (± 0.063)
7	5.14 ^a (± 0.137)	9.32 ^a (± 0.213)	1.05 ^{bc} (± 0.061)

^{a-c} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values were obtained using wet chemistry, Animal Production and Health, Polytechnic University of Madrid.

Source 1, n=10; Source 2, 4, n=7; Source 3, n=9; Source 5, 7, n=12; Source 6, n=11.

3.4.2 Amino acid concentration

Indispensable amino acids

Table 3.4 represents AA concentrations for the various indispensable AAs in SBM samples from different processing plants.

Sources 1, 2, 3, 4, 6 and 7 were shown to have significantly lower ($P<0.05$) arginine concentrations than Source 5. Both Sources 4 and 7 had significantly higher arginine concentrations than Sources 1, 2, 3 and 6.

The concentration of histidine of Source 5 was reported to be significantly higher than Sources 1, 2, 3,

Table 3.3: Mineral concentrations (dry matter basis) in soybean meal samples from different sources (\pm standard error of the mean)

Source	Calcium (g/kg)	Total phosphorous (g/kg)	Potassium (g/kg)	Magnesium (g/kg)	Sodium (g/kg)	Zinc (mg/kg)	Manganese (mg/kg)	Iron (mg/kg)	Copper (mg/kg)
1	0.22 ^d (\pm 0.008)	0.68 ^{bc} (\pm 0.012)	2.31 ^a (\pm 0.03)	0.28 ^b (\pm 0.006)	0.05 ^{ab} (\pm 0.006)	54.5 (\pm 1.445)	31.7 ^{ab} (\pm 0.647)	140 (\pm 16.875)	12.2 (\pm 0.4985)
2	0.26 ^{ab} (\pm 0.01)	0.71 ^{ab} (\pm 0.014)	2.22 ^{abc} (\pm 0.04)	0.28 ^{ab} (\pm 0.007)	0.02 ^d (\pm 0.007)	54.0 (\pm 1.728)	33.7 ^a (\pm 0.774)	111 (\pm 20.17)	13.4 (\pm 0.5958)
3	0.27 ^a (\pm 0.009)	0.70 ^{ab} (\pm 0.013)	2.17 ^c (\pm 0.031)	0.28 ^{ab} (\pm 0.006)	0.03 ^{cd} (\pm 0.006)	52.8 (\pm 1.524)	33.3 ^{ab} (\pm 0.682)	99.4 (\pm 17.788)	12.7 (\pm 0.5254)
4	0.22 ^d (\pm 0.01)	0.64 ^c (\pm 0.014)	2.18 ^c (\pm 0.036)	0.24 ^c (\pm 0.007)	0.07 ^a (\pm 0.007)	54.0 (\pm 1.728)	32.5 ^{ab} (\pm 0.774)	104 (\pm 20.17)	13.1 (\pm 0.5958)
5	0.24 ^{bc} (\pm 0.008)	0.69 ^{ab} (\pm 0.011)	2.20 ^{bc} (\pm 0.027)	0.28 ^{ab} (\pm 0.005)	0.04 ^c (\pm 0.005)	52.0 (\pm 1.32)	32.9 ^{ab} (\pm 0.591)	117 (\pm 15.405)	12.9 (\pm 0.455)
6	0.21 ^d (\pm 0.008)	0.65 ^c (\pm 0.012)	2.27 ^{ab} (\pm 0.028)	0.29 ^a (\pm 0.005)	0.04 ^c (\pm 0.005)	52.3 (\pm 1.378)	31.6 ^b (\pm 0.617)	107 (\pm 16.09)	13.0 (\pm 0.4753)
7	0.22 ^{cd} (\pm 0.008)	0.71 ^a (\pm 0.011)	2.23 ^{abc} (\pm 0.027)	0.27 ^b (\pm 0.005)	0.04 ^c (\pm 0.005)	54.0 (\pm 1.32)	32.9 ^{ab} (\pm 0.591)	99.6 (\pm 15.405)	13.0 (\pm 0.455)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values were obtained using wet chemistry, Animal Production and Health, Polytechnic University of Madrid.

Source 1, n=10; Source 2, 4, n=7; Source 3, n=9; Source 5, 7, n=12; Source 6, n=11.

4, 6 and 7. The concentration of histidine in Sources 4 and 7 was reported to be significantly higher than Sources 6 and 1.

For the concentration of isoleucine, Source 1 was significantly ($P < 0.05$) lower than Source 2. Sources 1, 2, 3 and 6 were found to be significantly lower in isoleucine concentration than Source 4 and Source 5. The isoleucine concentration of Source 7 was significantly higher than Sources 1, 3 and 6.

Sources 1, 2, 3, 4, 6 and 7 were shown to have significantly ($P < 0.05$) lower leucine concentrations than Source 5. Also for the concentration of leucine, Sources 4 and 7 had a significantly higher concentration than Sources 1, 2, 3 and 6. Lastly Source 1 had significantly lower leucine concentrations than Source 2.

The concentration of tryptophan of Sources 4, 5 and 7 were shown to be significantly ($P < 0.05$) higher compared to Sources 1, 2, 3 and 6.

The lysine concentrations of Sources 1, 2, 3, 4, 6 and 7 were found to be significantly ($P < 0.05$) lower than Source 5. The lysine concentration of Source 7 was significantly higher than the lysine concentrations of Sources 1, 2, 3, 4 and 6.

The concentration of methionine in Sources 1, 2, 3 and 6 were significantly ($P < 0.05$) lower than both Source 4, 5 and Source 7.

For the concentration of phenylalanine, Sources 4, 5 and 7 were reported to have significantly ($P < 0.05$) higher concentrations than Sources 1, 2, 3 and 6. Source 7 was significantly lower in phenylalanine concentration than Source 5.

Sources 5 and 7 had significantly ($P < 0.05$) higher threonine concentrations compared to Sources 1, 2, 3 and 6. Also, for threonine concentration, Source 4 had significantly higher levels than Sources 1, 3 and 6. Lastly Sources 3 and 1 were significantly lower than Source 2.

Sources 1, 2, and 3 all had significantly ($P < 0.05$) higher valine concentrations than Sources 4, 5, 6 and 7. Valine concentration of Source 1 was shown to be significantly less than Source 2. Source 4 was significantly higher than Sources 5, 6 and 7.

Dispensable amino acids

Table 3.5 represents AA concentrations for the various dispensable AAs in SBM samples from different processing plants.

The alanine concentrations of Sources 1, 2, 3 and 6 were reported to be significantly ($P < 0.05$) lower than Sources 4, 5 and 7. Source 1 was lower in alanine concentration than Source 2.

Sources 1, 2, 3 and 6 were found to have significantly ($P < 0.05$) lower concentrations of aspartic acid than Sources 4, 5 and 7. Source 1 and 3 had significantly lower aspartic acid concentration than Source 2.

For the concentration of cysteine, Sources 1, 2 and 3 were significantly ($P < 0.05$) higher than Sources 4, 5, 6 and 7. Lastly, Source 7 was found to have a significantly lower cysteine concentration than Sources 4, 5 and 6.

Sources 1, 2, 3 and 6 were found to have significantly ($P < 0.05$) lower methiocysteine concentrations than Sources 5 and 7. Also for methiocysteine concentration, Source 4 was found to have significantly higher concentrations than Sources 1, 3 and 6.

Sources 1, 2, 3 and 6 were found to have significantly ($P < 0.05$) lower concentrations of glutamic acid than Sources 4, 5 and 7

Sources 1, 2, 3 and 6 had significantly ($P < 0.05$) lower glycine concentrations than Source 5. Sources 1, 3 and 6 were shown to have significantly lower glycine concentrations than both Sources 4 and 7.

Sources 1, 2, 3 and 6 were found to be significantly ($P < 0.05$) lower in proline concentration than Sources 4, 5 and 7. Also, Source 1 had significantly lower proline concentration than Source 2.

Sources 1, 2, 3, 4, 6 and 7 were found to have significantly ($P < 0.05$) lower serine concentration than Source 5. Sources 1, 3 and 6 were found to have significantly lower serine concentrations than source 4. Source 7 was found to have significantly higher serine concentrations than Sources 1, 2, 3 and 6

3.4.3 Amino acid concentration as percentage of crude protein

Indispensable amino acids

Table 3.6 represents AA concentrations for the various indispensable AAs in SBM samples from the different processing plants. All sources had significantly ($P < 0.05$) lower arginine percentages than Source 5. Source 4 was significantly lower compared to Sources 1, 2, 6 and 7. Sources 1, 6 and 7 were shown to have higher arginine percentages than Sources 3 and 2.

The concentrations of histidine in Sources 1, 2, 3, 5, 6 and 7 were found to be significantly ($P < 0.05$) higher than source 4. Sources 2 and 5 had significantly higher concentrations of histidine than Source 3.

Sources 1, 3, 4 and 6 had significantly ($P < 0.05$) lower isoleucine concentrations compared to Sources 5 and 2. Source 7 had significantly lower isoleucine concentration than Source 2.

In terms of leucine concentration, Sources 1, 2, 3, 4, 6 and 7 was significantly ($P < 0.05$) higher compared to Source 5. Sources 1, 2, 3, 6 and 7 were significantly higher, in terms on leucine concentration, compared to Source 4.

Table 3.4: Concentration (%; dry matter basis) of indispensable amino acids in soybean meal samples from different sources (\pm standard error of the mean)

Source	Arginine	Histidine	Isoleucine	Leucine	Tryptophan	Lysine	Methionine	Phenylalanine	Threonine	Valine
1	3.385 ^c (± 0.014)	1.197 ^c (± 0.005)	2.057 ^d (± 0.011)	3.476 ^d (± 0.016)	0.636 ^b (± 0.003)	2.831 ^c (± 0.013)	0.630 ^b (± 0.003)	2.303 ^d (± 0.011)	1.801 ^c (± 0.007)	2.269 ^a (± 0.01)
2	3.383 ^c (± 0.016)	1.215 ^b (± 0.006)	2.109 ^b (± 0.013)	3.526 ^c (± 0.019)	0.642 ^b (± 0.003)	2.856 ^c (± 0.015)	0.638 ^b (± 0.003)	2.341 ^c (± 0.013)	1.825 ^b (± 0.009)	2.259 ^a (± 0.011)
3	3.354 ^c (± 0.014)	1.120 ^b (± 0.005)	2.076 ^c (± 0.012)	3.484 ^c (± 0.017)	0.636 ^b (± 0.003)	2.829 ^c (± 0.013)	0.635 ^b (± 0.003)	2.324 ^{cd} (± 0.011)	1.800 ^c (± 0.008)	2.246 ^a (± 0.01)
4	3.465 ^b (± 0.016)	1.230 ^b (± 0.006)	2.150 ^a (± 0.013)	3.587 ^b (± 0.019)	0.654 ^a (± 0.003)	2.842 ^c (± 0.015)	0.648 ^a (± 0.003)	2.396 ^a (± 0.013)	1.846 ^{ab} (± 0.009)	2.213 ^b (± 0.012)
5	3.544 ^a (± 0.012)	1.246 ^a (± 0.005)	2.157 ^a (± 0.01)	3.639 ^a (± 0.014)	0.662 ^a (± 0.002)	2.956 ^a (± 0.012)	0.644 ^a (± 0.002)	2.416 ^a (± 0.01)	1.862 ^a (± 0.007)	2.196 ^c (± 0.009)
6	3.405 ^c (± 0.013)	1.205 ^c (± 0.005)	2.077 ^c (± 0.011)	3.504 ^c (± 0.015)	0.642 ^b (± 0.003)	2.854 ^c (± 0.012)	0.637 ^b (± 0.003)	2.321 ^{cd} (± 0.011)	1.816 ^c (± 0.007)	2.190 ^c (± 0.009)
7	3.496 ^b (± 0.012)	1.231 ^b (± 0.005)	2.135 ^b (± 0.01)	3.584 ^b (± 0.014)	0.654 ^a (± 0.002)	2.922 ^b (± 0.012)	0.651 ^a (± 0.002)	2.382 ^b (± 0.01)	1.851 ^a (± 0.007)	2.176 ^c (± 0.009)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd

Source 1, n=10; Source 2, 4, n=7; Source 3, n=9; Source 5, 7, n=12; Source 6, n=11.

All sources had significantly ($P < 0.05$) higher tryptophan concentrations than Source 4. Sources 1, 5 and 6 had significantly higher tryptophan concentrations than Source 3 and Source 2 had significantly lower tryptophan concentrations than Source 5.

Source 1 had a significantly ($P < 0.05$) higher lysine concentration than Sources 5, 6 and 7. Sources 5 and 6 were found to have significantly lower lysine concentrations than Sources 1, 2, 3 and 4. Source 7 had a significantly lower lysine concentration than all the other sources.

Sources 1, 2, 3, 6 and 7 were all found to be significantly ($P < 0.05$) higher in methionine concentration than Sources 4.

Sources 1, 2, 3, 4, 6 and 7 were reported to be significantly ($P < 0.05$) lower in terms of phenylalanine concentration compared to Source 5. Source 4 was reported to have a significantly lower phenylalanine concentration than Source 7.

For the concentration of threonine, Sources 1, 2, 3, 5, 6 and 7 were found to have significantly ($P < 0.05$) higher values than Source 4. The proportion of threonine in Sources 1, 2 and 6 was found to be significantly higher than the concentration of threonine in Source 3.

Also for valine concentration, Sources 1, 2, 3, 5, 6 and 7 were shown to be significantly ($P < 0.05$) higher than Source 4. For the concentration of valine, Sources 1 and 3 were significantly lower than Source 5.

Dispensable amino acids

Table 3.7 represents AA concentrations for the various dispensable AAs in SBM samples from the different processing plants.

The concentration of alanine in Sources 1, 2, 3, 5, 6 and 7 were found to be significantly ($P < 0.05$) higher than the alanine proportion in Source 4. Sources 1, 6 and 7 were found to have significantly lower alanine concentrations than Source 5.

The concentrations of aspartic acid in Sources 1, 3, 4 and 6 were shown to be significantly ($P < 0.05$) lower than Source 5. Also for the proportion of aspartic acid, Source 4 was found to be significantly lower than Sources 2, 6 and 7. Lastly, Sources 1, 2 and 7 had significantly higher values than Source 3.

Table 3.5: Concentration (%; dry matter basis) of dispensable amino acids in soybean meal samples from different sources (\pm standard error of the mean)

Source	Alanine	Aspartic acid	Cysteine	Methiocysteine	Glutamic acid	Glycine	Proline	Serine
1	1.975 ^c	5.208 ^c	0.704 ^a	1.310 ^c	8.221 ^b	1.933 ^c	2.308 ^c	2.304 ^d
	(± 0.009)	(± 0.025)	(± 0.003)	(± 0.005)	(± 0.038)	(± 0.008)	(± 0.01)	(± 0.01)
2	2.005 ^b	5.286 ^b	0.703 ^a	1.322 ^b	8.332 ^b	1.965 ^b	2.340 ^b	2.334 ^{cd}
	(± 0.01)	(± 0.03)	(± 0.004)	(± 0.006)	(± 0.046)	(± 0.01)	(± 0.012)	(± 0.012)
3	1.994 ^b	5.215 ^{bc}	0.697 ^a	1.314 ^c	8.255 ^b	1.953 ^c	2.313 ^{bc}	2.306 ^d
	(± 0.009)	(± 0.03)	(± 0.004)	(± 0.006)	(± 0.04)	(± 0.009)	(± 0.01)	(± 0.01)
4	2.047 ^a	5.418 ^a	0.688 ^b	1.339 ^{ab}	8.518 ^a	1.992 ^{ab}	2.405 ^a	2.357 ^{bc}
	(± 0.01)	(± 0.03)	(± 0.004)	(± 0.006)	(± 0.046)	(± 0.01)	(± 0.012)	(± 0.012)
5	2.064 ^a	5.433 ^a	0.682 ^b	1.340 ^a	8.604 ^a	2.000 ^a	2.403 ^a	2.400 ^a
	(± 0.008)	(± 0.023)	(± 0.003)	(± 0.005)	(± 0.035)	(± 0.007)	(± 0.009)	(± 0.009)
6	1.993 ^b	5.254 ^b	0.682 ^b	1.321 ^c	8.297 ^b	1.950 ^c	2.325 ^{bc}	2.324 ^d
	(± 0.008)	(± 0.024)	(± 0.003)	(± 0.005)	(± 0.036)	(± 0.008)	(± 0.01)	(± 0.009)
7	2.036 ^a	5.388 ^a	0.675 ^c	1.353 ^a	8.507 ^a	1.983 ^{ab}	2.378 ^a	2.371 ^b
	(± 0.008)	(± 0.023)	(± 0.003)	(± 0.005)	(± 0.034)	(± 0.007)	(± 0.009)	(± 0.009)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).
 Values were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd.
 Source 1, n=10; Source 2, 4, n=7; Source 3, n=9; Source 5, 7, n=12; Source 6, n=11.

For the concentration of cysteine, Sources 1, 2, 3, 6 and 7 were significantly ($P < 0.05$) higher than Source 5. Source 7 had significantly higher cysteine concentrations than Sources 1, 3, 4 and 6.

Sources 1, 2, 3, 6 and 7 were shown to have significantly ($P < 0.05$) higher methiocysteine concentrations than Sources 4 and 5.

Sources 1, 2, 3 and 6 were significantly ($P < 0.05$) lower in glutamic acid concentration than Sources 4, 5 and 7.

The glutamic acid concentrations of Sources 1, 2, 3, 5, 6 and 7 were significantly ($P < 0.05$) higher than source 4. Sources 1, 2 and 6 were found to be significantly lower than Sources 5 and 7.

The glycine concentration of Sources 1, 2, 3 and 6 were significantly ($P < 0.05$) higher compared to Sources 4, 5 and 7. It was also found that the glycine concentration of Sources 5 and 7 were significantly lower than sources 1, 2, 3 and 6.

For proline concentration, Sources 1, 2, 4, 5, 6 and 7 had significantly ($P < 0.05$) higher values than Source 3. Source 5 had a significantly higher concentration of proline than Sources 4, 6 and 7.

Sources 1, 2, 3, 5, 6 and 7 were significantly ($P < 0.05$) higher in serine concentration than Source 4. Source 3 had a significantly lower concentration of serine than Sources 5 and 6.

3.4.4 Quality parameters

Table 3.8 represents the values for UA, PDI, TIA, KOHPS, KOHPS (Evonik analysis), PP, digestible protein, protein digestibility index and processing condition indicator of different SBM samples.

The UA values for Sources 1, 2, 4, 5, 6, and 7 were significantly ($P < 0.05$) lower than the value for Source

Sources 1, 3, 4, 5 and 6 had significantly ($P < 0.05$) lower PDI values than Sources 2 and 7. Significantly ($P < 0.05$) lower TIA values were observed for Sources 1, 2, 3, 4, 6 and 7 when compared to Source 5.

Table 3.6: Concentration as a percentage of crude protein (%; dry matter basis) of indispensable amino acids in soybean meal samples from different sources (\pm standard error of the mean)

Source	Arginine	Histidine	Isoleucine	Leucine	Tryptophan	Lysine	Methionine	Phenylalanine	Threonine	Valine
1	7.357 ^b	2.601 ^{ab}	4.471 ^c	7.554 ^b	1.383 ^a	6.183 ^a	1.370 ^a	5.005 ^{bc}	3.914 ^a	4.720 ^b
	(\pm 0.013)	(\pm 0.005)	(\pm 0.01)	(\pm 0.014)	(\pm 0.003)	(\pm 0.019)	(\pm 0.003)	(\pm 0.009)	(\pm 0.0085)	(\pm 0.007)
2	7.252 ^c	2.604 ^a	4.521 ^a	7.558 ^b	1.375 ^{bc}	6.157 ^{ab}	1.367 ^a	5.017 ^{bc}	3.913 ^a	4.743 ^{ab}
	(\pm 0.015)	(\pm 0.006)	(\pm 0.012)	(\pm 0.017)	(\pm 0.003)	(\pm 0.023)	(\pm 0.003)	(\pm 0.01)	(\pm 0.0102)	(\pm 0.008)
3	7.238 ^c	2.589 ^b	4.480 ^b	7.518 ^b	1.372 ^c	6.152 ^{ab}	1.370 ^a	5.015 ^{bc}	3.886 ^b	4.727 ^b
	(\pm 0.013)	(\pm 0.005)	(\pm 0.01)	(\pm 0.015)	(\pm 0.003)	(\pm 0.02)	(\pm 0.003)	(\pm 0.009)	(\pm 0.0089)	(\pm 0.007)
4	7.215 ^d	2.562 ^b	4.476 ^b	7.468 ^c	1.362 ^d	6.151 ^{ab}	1.350 ^b	4.988 ^c	3.844 ^c	4.703 ^c
	(\pm 0.015)	(\pm 0.006)	(\pm 0.012)	(\pm 0.017)	(\pm 0.003)	(\pm 0.023)	(\pm 0.003)	(\pm 0.01)	(\pm 0.0102)	(\pm 0.008)
5	7.412 ^a	2.607 ^a	4.511 ^a	7.610 ^a	1.384 ^a	6.122 ^c	1.346 ^b	5.052 ^a	3.894 ^{ab}	4.746 ^a
	(\pm 0.012)	(\pm 0.004)	(\pm 0.009)	(\pm 0.013)	(\pm 0.003)	(\pm 0.018)	(\pm 0.002)	(\pm 0.008)	(\pm 0.0078)	(\pm 0.006)
6	7.338 ^b	2.598 ^{ab}	4.476 ^b	7.551 ^b	1.383 ^{ab}	6.110 ^c	1.373 ^a	5.003 ^{bc}	3.913 ^a	4.732 ^{ab}
	(\pm 0.012)	(\pm 0.005)	(\pm 0.009)	(\pm 0.013)	(\pm 0.003)	(\pm 0.018)	(\pm 0.003)	(\pm 0.008)	(\pm 0.0081)	(\pm 0.006)
7	7.367 ^b	2.595 ^{ab}	4.498 ^{ab}	7.552 ^b	1.378 ^{bc}	5.918 ^d	1.372 ^a	5.019 ^b	3.902 ^{ab}	4.732 ^{ab}
	(\pm 0.012)	(\pm 0.004)	(\pm 0.009)	(\pm 0.013)	(\pm 0.003)	(\pm 0.018)	(\pm 0.002)	(\pm 0.008)	(\pm 0.0078)	(\pm 0.006)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd.

Source 1, n=10; Source 2, 4, n=7; Source 3, n=9; Source 5, 7, n=12; Source 6, n=11.

TIA values for Sources 1, 2 and 6 were significantly lower than Source 4. Also, source 2 was significantly lower than Source 3.

Sources 1, 2 and 7 were found to have significantly ($P < 0.05$) higher KOHPS values than Sources 3, 4, 5 and 6.

For the values of KOHPS (Evonik analyses), Source 5 was significantly ($P < 0.05$) higher than all other Sources. Additionally, Sources 2 and 3 were significantly lower than Source 7.

Source 6 and 7 had significantly ($P < 0.05$) high PP levels than sources 1 and 5.

The digestible protein values for Sources 1, 2, 4 and 6 were significantly ($P < 0.05$) lower than Source 3, 5 and 7.

For protein digestibility index values, sources 1, 2, 4 and 6 were found to be significantly ($P < 0.05$) lower than Sources 5 and 7. Additionally sources 4 and 6 were reported to be significantly lower compared to Source 3 for the protein digestibility index.

Sources 2, 5 and 7 were found to have significantly ($P < 0.05$) higher PCI values compared to Source 1. Also, sources 3, 4 and 6 were shown to be significantly lower than Source 2. Sources 5 and 7 were found to have significantly higher PCI values than Source 3.

Table 3.7: Concentration as a percentage of crude protein (%; dry matter basis) of dispensable amino acids in soybean meal samples from different sources (\pm standard error of the mean)

Source	Alanine	Aspartic acid	Cysteine	Methiocysteine	Glutamic acid	Glycine	Proline	Serine
1	4.293 ^b (± 0.006)	11.310 ^{bc} (± 0.013)	1.468 ^b (± 0.003)	2.846 ^a (± 0.007)	17.867 ^{bc} (± 0.027)	4.201 ^a (± 0.0059)	5.016 ^{ab} (± 0.005)	5.008 ^{ab} (± 0.011)
2	4.298 ^{ab} (± 0.008)	11.331 ^{ab} (± 0.015)	1.474 ^a (± 0.004)	2.834 ^a (± 0.008)	17.859 ^{bc} (± 0.032)	4.213 ^a (± 0.007)	5.015 ^{ab} (± 0.006)	5.002 ^{ab} (± 0.013)
3	4.302 ^{ab} (± 0.007)	11.254 ^d (± 0.014)	1.471 ^b (± 0.004)	2.835 ^a (± 0.007)	17.815 ^{cd} (± 0.028)	4.215 ^a (± 0.006)	4.990 ^c (± 0.006)	4.977 ^b (± 0.011)
4	4.262 ^c (± 0.008)	11.281 ^{cd} (± 0.015)	1.466 ^{bc} (± 0.004)	2.788 ^b (± 0.008)	17.736 ^d (± 0.032)	4.147 ^c (± 0.007)	5.008 ^b (± 0.006)	4.908 ^c (± 0.013)
5	4.316 ^a (± 0.006)	11.363 ^a (± 0.012)	1.458 ^c (± 0.003)	2.803 ^b (± 0.006)	17.993 ^a (± 0.024)	4.183 ^b (± 0.005)	5.026 ^a (± 0.005)	5.018 ^a (± 0.01)
6	4.295 ^b (± 0.006)	11.324 ^b (± 0.012)	1.469 ^b (± 0.003)	2.847 ^a (± 0.006)	17.882 ^{bc} (± 0.025)	4.203 ^a (± 0.006)	5.011 ^b (± 0.005)	5.009 ^a (± 0.01)
7	4.289 ^b (± 0.006)	11.354 ^{ab} (± 0.012)	1.481 ^a (± 0.003)	2.851 ^a (± 0.006)	17.925 ^{ab} (± 0.024)	4.179 ^b (± 0.005)	5.012 ^b (± 0.005)	4.995 ^a (± 0.01)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd

Source 1, n=10; Source 2, 4, n=7; Source 3, n=9; Source 5, 7, n=12; Source 6, n=11.

Table 3.8: Quality parameter values of soybean meal samples from seven different sources (\pm standard error of the mean)

Source	Urease activity	Protein dispersibility index	Trypsin inhibitor activity	Protein solubility in potassium hydroxide (%)	Protein solubility in potassium hydroxide (%) **	Phytic phosphorous	Digestible protein (%) **	Protein digestibility index*	Processing condition indicator**
1	0.05 ^b (± 0.063)	13.2 ^b (± 1.096)	2.35 ^c (± 0.264)	79.1 ^b (± 1.387)	84.7 ^a (± 1.738)	0.44 ^b (± 0.015)	83.7 ^b (± 0.6817)	1.78 ^{bc} (± 0.6817)	12.5 ^c (± 0.277)
2	0.07 ^b (± 0.075)	15.7 ^a (± 1.31)	1.91 ^d (± 0.315)	76.9 ^c (± 1.658)	79.9 ^a (± 2.077)	-	83.5 ^b (± 0.8148)	1.82 ^{bc} (± 0.8148)	14.0 ^a (± 0.332)
3	0.08 ^a (± 0.067)	12.5 ^b (± 1.155)	2.81 ^{bc} (± 0.278)	76.5 ^c (± 1.462)	77.0 ^b (± 1.832)	-	85.4 ^a (± 0.7186)	3.76 ^{ab} (± 0.7186)	12.3 ^c (± 0.292)
4	0.05 ^b (± 0.075)	12.3 ^b (± 1.31)	3.21 ^b (± 0.315)	78.5 ^b (± 1.658)	77.7 ^b (± 2.077)	-	83.2 ^b (± 0.8148)	1.21 ^c (± 0.8148)	12.8 ^{bc} (± 0.332)
5	0.04 ^b (± 0.058)	14.8 ^b (± 1.001)	4.02 ^a (± 0.241)	85.3 ^a (± 1.266)	78.3 ^b (± 1.586)	0.41 ^b (± 0.018)	85.5 ^a (± 0.6223)	4.01 ^a (± 0.62230)	13.3 ^{ab} (± 0.253)
6	0.04 ^b (± 0.06)	14.1 ^b (± 1.045)	2.06 ^d (± 0.252)	79.5 ^b (± 1.323)	78.1 ^b (± 1.657)	0.47 ^a (± 0.014)	82.5 ^b (± 0.65)	0.38 ^c (± 0.65)	13.0 ^{bc} (± 0.265)
7	0.06 ^b (± 0.058)	18.8 ^a (± 1.007)	2.53 ^b (± 0.241)	80.8 ^b (± 1.266)	83.3 ^a (± 1.586)	0.49 ^a (± 0.021)	85.8 ^a (± 0.6223)	4.37 ^a (± 0.6223)	13.4 ^{ab} (± 0.265)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values without an ‘*’ or ‘**’ were obtained using wet chemistry, Animal Production and Health, Polytechnic University of Madrid.

* Values were obtained using Near Infrared Reflectance Spectroscopy, DSM Nutritional Products Iberia, S.A

** Values were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd.

Source 1, n=10; Source 2, n=7; Source 3, n=9; Sources 5, 7, n=12; Source 6, n=11.

Survey Two

3.5 Materials and methods

This survey involved the collection of samples of SBM from five of the largest processing plants in SA. These samples were analysed to assess nutritional composition and quality. The primary objective was to investigate and quantify any variations in quality and composition among the samples obtained from different processing plants.

3.5.1 Collection of soybean meal samples

Collection of SBM samples from five South African soybean processing plants (here on referred to as Sources 1-5) located in Gauteng, Mpumalanga, Free-State and North-West Province. Collection occurred over a period of 6 months commencing in September 2019 with the last samples being collected in March 2020. It was confirmed that the crushers had only sourced locally produced soybeans from the batches from which samples were being taken. From four of the processing plants, samples from seven different batches were collected. Five batches from a fifth plant were collected. All samples were collected at random on separate occasions to achieve the best representation of the SBM that is distributed commercially. Each sample collected weighed 20 kg. The samples were taken directly from bulk bags located in the stores.

3.5.2 Analysis of samples

After collection, the samples were immediately taken to and stored in a walk-in cold room set to a temperature of 1°C. As each sample was attained the following five sub samples were taken from each sample: a single retention sample of 100 g; a sample of 200 g sent to DSM Nutritional Products South Africa (Pty) Ltd; a sample of 300 g sent to Evonik Africa (Pty) Ltd; a sample of 600 g sent to the Department of Animal Production and Health, Polytechnic University of Madrid, Madrid, a sample of 600 g sent to DSM Nutritional Products Iberia, S.A. and a sample of 200 g to NutriLab, Department of Animal Science, University of Pretoria. Where analyses were made at more than one laboratory for a given nutrient, the mean value for all results from all laboratories was calculated. This was done to provide the most accurate value for each nutrient with all values from all resources available. It was not part of the aim to evaluate variation between laboratories. As per the initial protocol, it was intended that all 3 samples were to be analysed using wet chemistry. The protocol changed and it was requested by DSM

Nutritional Products South Africa (Pty) Ltd that one of the samples to be analysed in South Africa was to be analysed using NIRS. It was also requested that due to the change in protocol and having NIRS and wet chemistry results that an average be taken of all. This does produce a confounding effect, however this was not taken into account for the analysis.

Using NIRS, DSM Nutritional Products South Africa (Pty) Ltd analysed the following: DM, CP, CF, EE, ash digestible protein and protein digestibility index. Evonik Africa (Pty) Ltd analysed DM, CP, CF, EE, ash, ADF, NDF, starch, sugar, KOHPS, PP, PDI, TIA, P, reactive lysine, PCI and concentration of AAs using NIRS. The Animal Production and Health Department of the Polytechnic University of Madrid, Madrid, used wet chemistry to analyse the following: moisture, using method 925.04 (AOAC, 1996); DM using method 930.15-1930 (AOAC, 1999); CP using method 990.3 (AOAC, 2006); CF according to method Ba6a-05 (AOCS, 2017); NDF according to method 2002.04 (AOAC, 2006); EE according to method Am 5-04 (AOCS, 2017); ash according to method 923.03 (AOAC, 2017); ammonia according to method 941.04 (AOAC, 2017); total N including and excluding ammonia using method 978.02 (AOAC, 1999); sucrose according to method 926.13 (AOAC, 1999); starch according to method 996.11 (AOAC, 2002); KOHPS according to AOAC method 994.12 (2022). All UA values were analysed at Nutrilab, Department of Animal Science, University of Pretoria, using wet chemistry according to method 941.04 (AOAC, 2017). The following quality parameters were analysed using NIRS at DSM Nutritional Products Iberia, S.A: digestible protein and protein digestibility index.

3.5.3 Statistical Analysis

Values that were used for statistical analysis were comprised of all data attained from the various analyses performed on each of the sub-samples at the various laboratories. Mean values for all parameters were calculated using the values of the sub-samples. These mean values were used for statistical analysis.

Differences in nutritional parameters between sources were analysed with the Proc Mixed model (Statistical Analysis System, 2021) for the mean effects. Means and standard error were calculated and significance of difference ($P < 0.05$) between means was determined by Fischer's test (Samuels, 1989). Repeated Measures Analysis of Variance with the Mixed model was used for repeated week or period measures.

The linear mix model used is described by the following equation:

One way

$$Y_{ijk} = \mu + S_i + L_j + B_k + SL_{ij} + e_{ijk}$$

Where Y_{ijk} = variable studied during the period

μ = overall mean of the population

S_i = effect of the i th Source

L_j = effect of the j th level

B_k = effect of the k th block

SL_{ij} = effect of the ij th interaction between Source and level

e_{ijk} = error associated with

3.6 Results

3.6.1 Nutritional Composition

The nutritional composition of samples from different processors are shown in Table 3.9. The DM content of Source 1 was found to be significantly higher ($P < 0.05$) than Source 3 and 5. The DM concentration of Source 2 was significantly higher than Sources 3 and 5. Lastly for DM concentration, Source 5 was significantly lower than Sources 3 and 4.

The CP concentration of Sources 1 and 2 was significantly ($P < 0.05$) lower than Sources 3, 4 and 5. Source 1 was significantly higher than Source 2. Furthermore, Source 2 was significantly lower than Source 1 in CP concentration.

Sources 1, 2 and 5 had significantly ($P < 0.05$) higher CF content than Sources 3 and 4. Source 3 was found to be significantly lower than source 4. Also for CF concentration, Source 2 had a significantly higher concentration than and 5.

Sources 1 and 2 had significantly ($P < 0.05$) higher ADF concentrations than Sources 3 and 4. Source 1 had a significantly higher ADF concentration than Source 5. Source 5 had significantly lower ADF concentration than Source 2. Source 4 was significantly higher than Source 3 and 5.

For concentrations of NDF, it was observed that Sources 1, 2 and 5 were significantly ($P < 0.05$) higher than Sources 3 and 4.

For the concentration EE, Source 2 was significantly ($P < 0.05$) lower than Sources 1, 3, 4 and 5. Source 1 was significantly lower than Sources 4 and 5. Sources 4 and 5 were significantly higher than Sources 3 and Source 4 was significantly higher than Source 5. Source 5 was significantly higher than Sources 1

and 2.

The concentration of ash of Source 3 was significantly ($P < 0.05$) higher than Sources 1, 2 and 4 whilst significantly lower than Source 5.

Table 3.9: Nutrient composition (%; dry matter basis) of soybean meal samples from different sources (\pm standard error of the mean)

Source	Dry matter	Crude protein	Crude fibre	Acid detergent fibre *	Neutral detergent fibre *	Ether extract	Ash	Ammonia	Total nitrogen including Ammonia (%)	Total nitrogen excluding Ammonia (%)	Sucrose *	Starch *
1	90.6 ^a (± 0.277)	46.0 ^c (± 0.138)	4.14 ^{ab} (± 0.131)	5.87 ^a (± 0.114)	10.7 ^a (± 0.443)	2.18 ^c (± 0.109)	6.27 ^c (± 0.037)	0.89 ^c (± 0.003)	43.1 ^c (± 0.152)	42.2 ^b (± 0.4)	10.5 ^b (± 0.097)	0.76 ^b (± 0.03)
2	91.0 ^a (± 0.277)	45.2 ^d (± 0.138)	4.44 ^a (± 0.131)	5.91 ^a (± 0.114)	10.7 ^a (± 0.443)	1.27 ^d (± 0.109)	6.28 ^c (± 0.037)	0.88 ^d (± 0.003)	42.5 ^d (± 0.152)	41.7 ^c (± 0.4)	10.8 ^{ab} (± 0.097)	0.86 ^a (± 0.03)
3	89.7 ^b (± 0.277)	47.8 ^a (± 0.138)	2.56 ^c (± 0.131)	4.54 ^d (± 0.114)	9.07 ^b (± 0.443)	1.92 ^c (± 0.109)	6.41 ^b (± 0.037)	0.94 ^a (± 0.003)	44.9 ^a (± 0.152)	44.8 ^a (± 0.4)	10.9 ^a (± 0.097)	0.73 ^{bc} (± 0.03)
4	90.4 ^{ab} (± 0.328)	47.4 ^{ab} (± 0.163)	2.66 ^b (± 0.155)	4.92 ^c (± 0.135)	10.1 ^b (± 0.524)	3.19 ^a (± 0.129)	6.27 ^c (± 0.044)	0.92 ^b (± 0.004)	44.2 ^b (± 0.179)	43.5 ^b (± 0.474)	10.1 ^b (± 0.115)	0.72 ^{bc} (± 0.035)
5	88.7 ^c (± 0.277)	47.0 ^b (± 0.138)	3.88 ^b (± 0.131)	5.43 ^b (± 0.114)	10.8 ^a (± 0.443)	2.64 ^b (± 0.109)	6.57 ^a (± 0.037)	0.93 ^a (± 0.003)	44.6 ^{ab} (± 0.152)	43.8 ^{ab} (± 0.4)	10.1 ^b (± 0.097)	0.54 ^c (± 0.03)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values shown are mean values of analyses using Near Infrared Reflectance Spectroscopy at DSM Nutritional Products South Africa (Pty) Ltd and Evonik Africa (Pty) Ltd, and wet chemistry at the Animal Production and Health Department of the Polytechnic University of Madrid unless otherwise noted.

* Values obtained only at Evonik Africa (Pty) Ltd and the Animal Production and Health Department of the Polytechnic University of Madrid.

Source 1-4, n=7; Source 5, n=5.

For ammonia concentrations, Sources 3, 4 and 5 were significantly ($P < 0.05$) higher than Source 1 and Source 2. Source 1 was significantly higher than Source 2. Source 4 was significantly lower than Sources 3 and 5.

For the total concentration of nitrogen including ammonia, Sources 3, 4 and 5 were found to have significantly ($P < 0.05$) higher values than Sources 1 and 2. Source 2 was significantly lower than Source 1, and Source 4 was significantly lower than Source 3.

For the total concentration of nitrogen excluding ammonia, Source 1 was significantly ($P < 0.05$) lower than Sources 3 and 5. Source 2 was significantly lower than Sources 3, 4 and 5. Lastly, Source 4 was lower than Source 3. Sources 4 and 5 were significantly lower than Sources 1, 2 and 3. The

concentration of sucrose of Source 1 was significantly ($P < 0.05$) lower than Source 3.

Source 2 was found to have significantly ($P < 0.05$) higher starch concentration than Sources 1, 3, 4 and 5. Source 1 was found to have significantly higher starch concentration than Source 5.

3.6.2 Amino acid concentration

Indispensable amino acids

Table 3.10 represents AA concentrations for the various indispensable AAs in SBM samples from different processing plants.

The following observations were made for the concentration of arginine: Sources 3, 4 and 5 were significantly ($P < 0.05$) higher than Sources 1 and 2. Source 3 was significantly higher than Source 5.

For the concentration of histidine, Sources 1 and 2 were significantly ($P < 0.05$) lower than Sources 3, 4 and 5. Lastly, Sources 3 and 4 were significantly higher than Source 5.

Sources 1 and 4 were significantly ($P < 0.05$) lower than Sources 3 and 5 in terms of isoleucine concentration. Source 1 was significantly lower than source 4 for isoleucine concentration.

For the concentration of leucine, Sources 1 and 2 were found to be significantly ($P < 0.05$) lower than Sources 3, 4 and 5. Source 1 was significantly higher than Source 2. Source 4 was found to be significantly lower than Source 3.

For the concentration of tryptophan, Sources 1, 3, 4 and 5 were shown to be significantly ($P < 0.05$) lower than Source 2. Source 1 was reported to be significantly lower than Sources 3, 4, and 5, but significantly higher than Source 2.

In terms of lysine concentration, Sources 1 and 2 were significantly ($P < 0.05$) lower than Sources 3, 4 and 5. Also, Source 3 was significantly higher than Source 5.

For the concentration of methionine, Source 2 was significantly ($P < 0.05$) lower than Sources 3, 4 and 5. Source 1 was significantly higher than Source 2 whilst significantly lower than Source 4. Source 1 was found to be significantly lower than Sources 3 and 5. Lastly, Source 3 was found to be significantly higher than Sources 4 and 5.

For the concentration of phenylalanine, it was observed that Sources 3, 4 and 5 were significantly ($P < 0.05$) higher than Sources 1 and 2.

Source 2 had significantly ($P < 0.05$) lower threonine concentration than Sources 3, 4 and 5. Sources 3 and 5 had significantly higher concentrations of threonine than Source 4.

Sources 3, 4 and 5 had significantly ($P < 0.05$) higher valine concentrations than Sources 1 and 2. Lastly, Source 5 was found to have significantly higher valine concentrations than Source 4.

Dispensable amino acids

Table 3.11 represents AA concentrations for the various dispensable AAs in SBM samples from different processing plants.

For alanine concentration, Sources 3, 4 and 5 were found to be significantly ($P < 0.05$) higher than Sources 1 and 2. Source 3 was significantly higher in alanine concentration than Source 4.

Table 3.10: Concentration (%; dry matter basis) of indispensable amino acids of soybean meal samples from different sources (\pm standard error of the mean)

Source	Arginine	Histidine	Isoleucine	Leucine	Tryptophan	Lysine	Methionine	Phenylalanine	Threonine	Valine
1	3.29 ^c (± 0.016)	1.20 ^c (± 0.004)	2.06 ^c (± 0.008)	3.43 ^c (± 0.013)	0.63 ^b (± 0.002)	2.76 ^c (± 0.012)	0.63 ^c (± 0.002)	2.28 ^b (± 0.018)	1.78 ^c (± 0.005)	2.16 ^c (± 0.009)
2	3.26 ^c (± 0.016)	1.17 ^d (± 0.004)	2.01 ^d (± 0.008)	3.39 ^d (± 0.013)	0.62 ^c (± 0.002)	2.72 ^c (± 0.012)	0.62 ^d (± 0.002)	2.27 ^b (± 0.018)	1.74 ^c (± 0.005)	2.13 ^c (± 0.009)
3	3.48 ^a (± 0.016)	1.24 ^a (± 0.004)	2.13 ^a (± 0.008)	3.57 ^a (± 0.013)	0.65 ^a (± 0.002)	2.87 ^a (± 0.012)	0.65 ^a (± 0.002)	2.34 ^a (± 0.018)	1.84 ^a (± 0.005)	2.23 ^{ab} (± 0.009)
4	3.45 ^{ab} (± 0.019)	1.24 ^a (± 0.005)	2.10 ^b (± 0.01)	3.52 ^b (± 0.015)	0.65 ^a (± 0.002)	2.83 ^{ab} (± 0.015)	0.64 ^b (± 0.003)	2.34 ^a (± 0.021)	1.81 ^b (± 0.006)	2.21 ^b (± 0.01)
5	3.44 ^b (± 0.016)	1.22 ^b (± 0.004)	2.13 ^a (± 0.008)	3.56 ^{ab} (± 0.013)	0.65 ^a (± 0.002)	2.82 ^b (± 0.013)	0.64 ^b (± 0.002)	2.38 ^a (± 0.018)	1.83 ^a (± 0.005)	2.24 ^a (± 0.009)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).
 Values were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd.
 Source 1-4, n=7; Source 5, n=5.

For the concentration of aspartic acid, Sources 3, 4 and 5 were found to be significantly ($P < 0.05$) higher than Sources 1 and 2. Source 3 was significantly higher in aspartic acid concentration than Source 4.

Source 3 was found to have a significantly ($P < 0.05$) lower cysteine concentration than Sources 1, 2, 4 and 5. Source 2 was found to be significantly lower than Sources 1, 4 and 5.

For methiocysteine concentration, Source 1 was significantly ($P < 0.05$) lower than Source 2, 3, 4 and 5. Sources 3, 4 and 5 were found to be significantly higher than Source 2 and Sources 4 and 5 were significantly lower than Source 3.

For glutamic acid concentrations, Sources 1 and 2 were observed to be significantly ($P < 0.05$) lower than Sources 3, 4 and 5. Source 1 had significantly higher concentrations than Source 2.

Sources 3, 4 and 5 had significantly ($P < 0.05$) higher concentrations of glycine than Sources 1 and 2. Source 4 was significantly lower than Sources 3 and 5.

For proline concentration, Sources 1 and 2 were significantly ($P < 0.05$) lower than Sources 3, 4 and 5. Source 4 was significantly lower than Source 5.

For the concentration of serine, Sources 3, 4 and 5 were significantly ($P < 0.05$) higher than both Sources 1 and Source 2. The serine concentration of Source 1 was higher than Source 2.

3.6.3 Quality parameters

Table 3.12 represents the values for UA, PDI, TIA, KOHPS, reactive lysine, proportion of reactive lysine to total lysine, total P, PP, digestible protein, protein digestibility index and processing condition indicator of different SBM samples.

Sources 3 and 4 had a significantly lower ($P < 0.05$) UA value than Sources 1 and 2, while Source 1 had a significantly higher UA value than source 2.

For the PDI values, Sources 1, 3 and 5 were significantly ($P < 0.05$) higher than Sources 2 and 4.

Sources 1, 3 and 5 had significantly ($P < 0.05$) higher TIA than Source 2 and 4.

Table 3.11: Concentration (%; dry matter basis) of dispensable amino acids in soybean meal samples from different sources (\pm standard error of the mean)

Source	Alanine	Aspartic acid	Cysteine	Methiocysteine	Glutamic acid	Glycine	Proline	Serine
1	1.959 ^c (± 0.006)	5.175 ^c (± 0.02)	0.676 ^a (± 0.054)	1.295 ^c (± 0.006)	8.135 ^b (± 0.033)	1.919 ^c (± 0.006)	2.297 ^c (± 0.008)	2.269 ^b (± 0.008)
2	1.941 ^c (± 0.006)	5.088 ^c (± 0.02)	0.657 ^{ab} (± 0.054)	1.271 ^c (± 0.006)	8.021 ^c (± 0.033)	1.903 ^c (± 0.006)	2.261 ^c (± 0.008)	2.240 ^c (± 0.008)
3	2.033 ^a (± 0.006)	5.398 ^a (± 0.02)	0.521 ^b (± 0.054)	1.345 ^a (± 0.006)	8.511 ^a (± 0.033)	1.975 ^a (± 0.006)	2.383 ^a (± 0.008)	2.357 ^a (± 0.008)
4	2.012 ^b (± 0.007)	5.323 ^b (± 0.024)	0.689 ^a (± 0.064)	1.316 ^b (± 0.007)	8.428 ^a (± 0.038)	1.953 ^b (± 0.007)	2.347 ^b (± 0.01)	2.336 ^a (± 0.01)
5	2.031 ^b (± 0.006)	5.376 ^{ab} (± 0.02)	0.698 ^a (± 0.054)	1.329 ^b (± 0.006)	8.452 ^a (± 0.033)	1.976 ^a (± 0.006)	2.386 ^a (± 0.008)	2.339 ^a (± 0.008)

^{a-c} Within each column, values without a common superscript differ significantly ($P < 0.05$).
 Values were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd.
 Source 1-4, n=7; Source 5, n=5.

For the solubility of protein in potassium hydroxide, Source 4 was found to have significantly ($P < 0.05$) higher values than Sources 1, 2 and 5.

For the concentration of reactive lysine, Source 3, 4 and 5 had significantly ($P < 0.05$) higher values than Sources 1 and 2.

For the proportion of reactive lysine to total lysine, Source 1 had significantly ($P < 0.05$) lower values than Sources 2, 3, 4 and 5. Source 2 was significantly lower than Source 4.

Sources 1 and 2 were found to have significantly ($P < 0.05$) lower total P concentrations than Sources 3, 4 and 5. Source 5 was found to have a significantly higher total P concentration than Source 3.

Sources 1, 3 and 4 were found to have a significantly ($P < 0.05$) higher proportion of PP than Sources 2 and 5.

For the digestible protein value, Sources 1 and 5 were found to have a significantly ($P < 0.05$) lower value than Sources 2, 3 and 4.

Sources 2 and 4 had significantly ($P < 0.05$) higher protein digestibility index values than Sources 1, 3 and 5. Sources 1 and 3 were found to have significantly higher values than Source 5.

Sources 3, 4 and 5 had significantly ($P < 0.05$) higher PCI values than Source 2. The PCI value of Source 1 to be significantly higher than Sources 2 and 4.

Lastly, results for selected nutritional components were selected and compared to results published by the National Research Council (NRC), CVB and National Institute for Agricultural Research (INRA) as shown in Table 3.13. From both surveys, all values obtained for macronutrients, trace minerals and AAs were found to fall within the ranges of values published by the NRC, CVB and the (INRA) with the exception of dry matter and ether extract percentage and glutamic acid concentration which were found to be higher.

Table 3.12: Quality parameter values of soybean meal samples from five different sources (\pm standard error of the mean)

Source	Urease activity	Protein dispersibility index (%)	Trypsin inhibitor activity (mg/g)	Protein solubility in potassium hydroxide (%)	Reactive Lysine (%)	Proportion of reactive lysine to total lysine (%)	Phosphorus (mg/g)	Phytic phosphorous	Digestible protein (%)*	Protein digestibility index	Processing condition indicator
1	0.21 ^a (± 0.04)	12.9 ^b (± 0.57)	0.80 ^{ab} (± 0.16)	74.4 ^d (± 0.96)	2.49 ^c (± 0.02)	87.59 ^c (± 0.29)	6054 ^d (± 42.08)	0.50 ^{bc} (± 0.02)	84.41 ^{bc} (± 0.44)	3.77 ^{bc} (± 0.42)	13.29 ^a (± 0.30)
2	0.14 ^b (± 0.04)	9.36 ^{cd} (± 0.57)	0.10 ^{ab} (± 0.17)	74.8 ^{cd} (± 0.96)	2.47 ^c (± 0.02)	88.28 ^{bc} (± 0.29)	6049 ^{cd} (± 42.08)	0.46 ^c (± 0.02)	86.63 ^a (± 0.44)	5.26 ^a (± 0.39)	11.43 ^c (± 0.30)
3	0.09 ^b (± 0.04)	15.1 ^a (± 0.57)	1.10 ^a (± 0.16)	77.5 ^{bc} (± 0.96)	2.58 ^a (± 0.02)	88.64 ^{ab} (± 0.29)	6372 ^b (± 42.08)	0.54 ^{ab} (± 0.02)	86.14 ^a (± 0.44)	3.34 ^c (± 0.39)	13.14 ^{ab} (± 0.30)
4	0.05 ^{bc} (± 0.04)	8.88 ^d (± 0.68)	0.20 ^b (± 0.34)	81.8 ^a (± 1.14)	2.52 ^{bc} (± 0.02)	89.26 ^a (± 0.34)	6410 ^{ab} (± 49.79)	0.60 ^a (± 0.02)	86.65 ^a (± 0.52)	5.34 ^a (± 0.46)	12.40 ^b (± 0.36)
5	-	15.5 ^a (± 0.57)	0.89 ^{ab} (± 0.15)	74.4 ^d (± 0.96)	2.50 ^{cd} (± 0.02)	88.97 ^{ab} (± 0.29)	6520 ^a (± 42.08)	0.39 ^d (± 0.02)	83.26 ^c (± 0.44)	1.49 ^d (± 0.42)	12.86 ^{ab} (± 0.30)

^{a-d} Within each column, values without a common superscript differ significantly ($P < 0.05$).

Values without ‘*’ or ‘**’ obtained by Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd.

* Values were obtained using wet chemistry.

** Values were obtained using Near Infrared Reflectance Spectroscopy, DSM Nutritional Products Iberia, S.A.

Source 1-4, n=7; Source 5, n=5.

Table 3.13: Mean nutrient composition of soybean meal samples from different sources including NRC, CVB and INRA values for soybean meals (NRC, 1994; CVB, 2016; INRA 2024)

Nutrients (%)	Dry matter	Crude protein	Crude fibre	Acid detergent fibre	Neutral detergent fibre	Ether extract	Ash				
Survey 1 and 2	90.1	46.7	3.78	5.33	9.45	2.55	6.22				
NRC	88.4	47.5	3.90	-	-	1.00					
CVB	88.7	46.8	3.80	5.20	8.60	1.60	6.40				
INRA	88.0	46.2	6.00	7.40	12.50	1.50	6.20				
Minerals	Calcium (g/kg)	Total phosphorous (g/kg)	Potassium (g/kg)	Magnesium (g/kg)	Sodium (g/kg)	Zinc (mg/kg)	Manganese (mg/kg)	Iron (mg/kg)	Copper (mg/kg)		
Survey 1 and 2	2.34	0.68	2.23	0.27	0.04	53.4	32.6	111.4	12.9		
NRC	2.70	0.62	1.98	0.30	0.02	55.0	43.0	170	15		
CVB	3.10	0.68	4.70	3.00	0.20	49.0	37.0	305	15		
INRA	3.40	0.62	2.90	2.80	0.14	55.0	39.0	241	17		
Indispensable amino acids (%)	Arginine	Histidine	Isoleucine	Leucine	Tryptophan	Lysine	Methionine	Phenylalanine	Threonine	Valine	
Survey 1 and 2	3.83	1.21	2.10	3.52	0.64	2.84	0.64	2.34	1.82	2.21	
NRC	3.48	1.28	2.12	3.74	0.74	2.96	0.67	2.34	1.87	2.22	
CVB	3.51	1.26	2.15	3.6	0.61	2.90	0.66	2.43	1.83	2.25	
INRA	3.38	1.24	2.11	3.53	0.64	2.88	0.66	2.34	1.77	2.23	
Dispensable amino acids (%)	Alanine	Aspartic acid	Cysteine	Methiocysteine	Glutamic acid	Glycine	Proline	Serine			
Survey 1 and 2	2.01	5.30	0.67	1.32	8.36	1.96	2.35	2.33			
NRC	-	-	0.72	1.39	-	2.05	-	2.48			
CVB	2.06	5.43	0.70	1.34	8.33	2.01	2.39	2.39			
INRA	2.01	5.22	0.73	1.39	8.25	1.94	2.30	2.17			

- = Values not available.

3.7 Discussion

The results show significant variation in the nutritional composition and quality of SBM from different processors within the same regions and across different regions of South Africa. When compared with literature values from SA and other global regions (Tangendjaja, 2020; Aguirre et al., 2022), the variation observed in Surveys 1 and 2 falls within typical ranges found in SBM from different processors worldwide, indicating expected variability. Significant differences were found among at least one of the seven sources in Survey 1 and one of the five sources in Survey 2 across all nutritional components except for zinc, iron, or copper. The findings from these two surveys, where SBM samples were collected from various processing plants and averaged, demonstrate that South African SBM's nutritional content and quality are comparable to values published by authoritative organizations such as NRC (1994), CVB (2016), and INRA (2024), which serve as comprehensive benchmarks for feedstuff nutrition across diverse regions.

Recent studies by Tangendjaja (2020), comparing SBM nutritional content from Argentina, Brazil, and the USA, and Aguirre *et al.* (2022), comparing quality parameters across these countries plus SA, suggest that South African SBM aligns closely with the nutrient composition and quality of SBM from the USA. Tangendjaja (2020) analyzed 23 samples from Argentina, 20 from Brazil, and 16 from the USA using NIRS for DM, CP, CF, and EE. Comparing these values with averages, highest, and lowest values from Surveys 1 and 2, the DM content was similar across Argentina (88.85%), Brazil (88.46%), and the USA (88.68%), with the mean in this study at 90.10%. Source 2 from Survey 2 had the highest DM value (91.04%), while Source 5 from Survey 2 had the lowest (88.76%).

Brazil exhibited the highest CP values at 48.57%, followed by the study mean of 46.78%, with the USA and Argentina at 46.47% and 46.59%, respectively. Source 3 from Survey 2 recorded the highest CP at 47.85%, while Source 2 from Survey 2 had the lowest at 45.25%. Brazil also showed the highest CF content at 4.04%, followed by the study mean of 3.78%, Argentina at 3.81%, and the USA at 3.58%. Source 2 from Survey 2 had the highest CF content (4.44%), and Source 3 from Survey 2 had the lowest (2.56%). Source 5 from Survey 1 had the highest EE content (3.46%), while Source 2 from Survey 2 had the lowest (1.27%), with the overall mean at 2.55%. In Tangendjaja's (2020) results, the USA had the highest EE content (2.01%), followed by Brazil (1.58%) and Argentina (1.56%).

Aguirre *et al.* (2022) found that the UA of South African SBM, at 0.04 mg N/g, was higher than that of SBM from Argentina, Brazil, and the USA, all of which measured 0.01 mg N/g. In contrast, the current

study reported a mean UA of 0.08 mg N/g. Additionally, this study indicated a lower PDI value of 13.63%, compared to the highest value reported by Aguirre *et al.*(2022): 15.7% for SA, 14.6% for the USA, 12.9% for Brazil, and 11% for Argentina. Regarding KOHPS, SA had the highest value at 81%, followed closely by the USA at 80.4%. The value reported in this study was 78.33%, with Brazil and Argentina showing 77.6% and 74.6%, respectively. Lastly, comparing TIA values, this study recorded the lowest at 1.83 mg/g, while Argentina had 1.89 mg/g, Brazil 2.36 mg/g, and the USA 2.61 mg/g, with SA at 2.62 mg/g.

3.8 Conclusion

The adaptation of existing crushing equipment and subsequent success in the processing of soybean of local and regional origin pioneered the way for the establishment of other soybean processing plants. The increase in demand for soybeans led to an increase in local production. The local soybean cultivars were then being processed under similar processing conditions used by other major processors in other countries. Based on the results of the surveys on the nutritional composition and quality of South African SBM it can be concluded that there is a substantial amount of variation. Further comparison of the results with the results of other studies from SA and other countries as well as with figures published by research associations indicate that South African SBM is comparable to that of SBM from Argentina, Brazil, and the USA. Due to the similarity of processing conditions between the processors from which samples were analysed it may be concluded that the variation in SBM shown may, to a greater extent, be influenced by factors such as cultivar, planting and harvesting techniques, storage time and other inherent factors. The significant variability observed in this study emphasizes the importance of considering origin-specific matrices when formulating diets, both internationally and locally. Therefore, we reject the null hypothesis that there is little to no variation in the nutritional composition and quality of SBM from different sources in SA, and accept the alternative hypothesis that there is substantial variation in nutritional composition and quality among these sources. Each processor should provide the buyer of SBM a comprehensive nutrient profile and quality parameters.

CHAPTER 4

Nutrient digestibility of South African soybean meal of different quality with and without protease

4.1 Introduction

Research on *in vivo* digestibility has been pivotal in advancing nutritional science. Before ileal digestibility studies, AA digestibility was estimated by comparing AA content in excreta with that in the diet (Kuiken & Lyman, 1948; Lemme *et al.*, 2004), but this method did not account for endogenous AA losses. Siriwan (1994) demonstrated significant AA absorption by gut microbiota, prompting the development of more accurate methods like ileal digestibility, pioneered by Lemme *et al.*, (2004). This method compares AA content in digesta from the distal ileum with that in excreta, excluding endogenous losses (Ravindran, 2012). Since the early 2000s, ileal digestibility studies have become standard in quantifying nutrient digestibility, notably in SBM diets extensively used in poultry feed formulation (Ravindran *et al.*, 2017).

Despite numerous studies on SBM's ileal AA digestibility, values vary widely within SBM and across other feed ingredients due to inherent nutrient differences, processing techniques, and laboratory methodologies (Ravindran *et al.*, 2017). Factors influencing SBM digestibility include bean cultivar, origin, growth conditions, harvest, storage, and especially processing conditions (Angel *et al.*, 2011; Milosevic *et al.*, 2013). Digestibility is crucial in SBM due to ANFs present in raw soybeans, such as phytates, tannins, trypsin inhibitors, and oligosaccharides, which hinder nutrient bioavailability (Samtiya *et al.*, 2020). Trypsin inhibitors, for instance, reduce AA availability by inhibiting trypsin's ability to bind with protein substrates, leading to undigested protein molecules in the lower digestive tract (Avilés-Gaxiola *et al.*, 2018). While heat treatment can deactivate 80-95% of trypsin inhibitors, residual activity still impacts physiological functions and performance (Rackis *et al.*, 1985). Processing variations in heat treatment, including temperature, duration, and pressure differences, affect ANF reduction (Aletor and Ojo, 1989).

In addition to heat treatment, exogenous proteases like phytase and, more recently, proteases, have been incorporated into commercial diets to enhance protein digestion efficiency and reduce formulation costs

(Bedford and Partridge, 2010; Cowieson and Adeola, 2005; Fru-Nji et al., 2011). Protease use aims to optimize nutrient utilization by breaking down proteins more effectively (Ghazi *et al.*, 2002).

4.2 Aim, objectives and hypotheses

The aim of the study was to assess the effect of SBM quality on the efficacy of protease in AA digestibility of broilers.

The objectives of the study were:

1. Collection of SBM samples from five different soybean processors across SA.
2. Select SBM samples of different qualities and feed them to 21-day old broilers in a digestibility study. Diets to be fed with and without a monocomponent protease supplementation.
3. Test for significant differences between treatments containing SBM of different nutritional composition and quality, fed with or without a monocomponent protease

The null hypothesis was that the use of protease will increase the ileal digestibility of nutrients in soybean-maize based diets regardless of the quality of the SBM in the diet. The alternative hypothesis was that the use of protease in soybean-maize based diets will be influenced by the quality of the SBM in the diet.

4.3 Materials and methods

The project consisted of two phases. Phase 1 (Survey 2 as detailed in Chapter 3) involved collecting SBM samples from five specified soybean processing plants. Phase 2 comprised an *in vivo* trial with broilers to assess the impact of protease on apparent ileal digestibility when feeding diets containing SBM of different qualities.

4.3.1 Phase 1: Soybean meal sample collection

Samples from five different South African soybean processing plants were collected during the period of June 2019 to March 2020 i.e. Survey 2. From four of the processing plants, samples from seven different batches, were collected, while samples from five batches from a fifth plant were collected. The samples weighed between 25 and 50 kg each. One processing plant (Source 3) was requested to also provide samples of specially prepared under-processed SBM. These samples were not included in the analysis

which took place in Survey 2 but were kept apart to be used in the digestibility trial conducted during Phase 2 (Chapter 3) as a negative control.

Representative sub-samples of 2 kg each were taken from the randomly collected samples, and the under-processed sample. These sub-samples were taken directly from the 20kg samples bags provided by the crushers using a small feed sampler inserted directly into the middle of the feed bag. These sub-samples were further divided into four representative samples and sent to four laboratories; DSM Nutritional Products South Africa (Pty) Ltd; Evonik Africa (Pty) Ltd; the Department of Animal Production and Health of the Polytechnic University of Madrid and Nutrilab, Department of Animal Science, University of Pretoria for analysis of nutrient concentrations and quality parameters.

Using NIRS, DSM Nutritional Products South Africa (Pty) Ltd analysed the following: DM, CP, CF, EE, ash digestible protein and protein digestibility index. Evonik Africa (Pty) Ltd analysed DM, CP, CF, EE, ash, ADF, NDF, starch, sugar, KOHPS, PP, PDI, TIA, P, reactive lysine, PCI and concentration of AAs using NIRS. The Animal Production and Health Department of the Polytechnic University of Madrid, Madrid, used wet chemistry to analyse the following: moisture, using method 925.04 (AOAC, 1996); DM using method 930.15-1930 (AOAC, 1999); CP using method 990.3 (AOAC, 2006); CF according to method Ba6a-05 (AOCS, 2017); NDF according to method 2002.04 (AOAC, 2006); EE according to method Am 5-04 (AOCS, 2017); ash according to method 923.03 (AOAC, 2017); sucrose according to method 926.13 (AOAC, 1999); starch according to method 996.11 (AOAC, 2002), as represented in Tables 4.1 and 4.2; KOHPS according to AOAC method 994.12 (2022). All UA values were analysed at Nutrilab, Department of Animal Science, University of Pretoria, using wet chemistry according to method 941.04 (AOAC, 2017). The following quality parameters were analysed using NIRS at DSM Nutritional Products Iberia, S.A: digestible protein and protein digestibility index.

Based on the results of all quality parameters, represented in Table 4.3, a low quality (LQ) sample, a high quality (HQ) sample and an over-processed sample (OP) were chosen. The LQ was designated as such due to the sample having the lowest KOHPS, the highest TIA value, the lowest total P and the lowest PDI value of all five sources. The HQ sample was designated as such due it having the highest solubility in KOH, the highest total P content, the highest reactive lysine %, the highest proportion of reactive lysine to total lysine and the highest PDI value of all five samples. Although some of the quality values for the sample designated as OP were found to be similar to the HQ sample, the OP was chosen due to it having less total P, less PP, less reactive lysine and a lower proportion of reactive lysine to total lysine than the HQ sample. Also TIA was not detected for the OP sample which suggests sufficient

cooking to remove trypsin inhibitor. These samples were used as the main protein source in broiler diets so that the effect of SBM quality and protease supplementation on ileal digestibility could be assessed.

The requested under-processed sample was designated as UP.

Table 4.1: Nutritional composition of soybean meal samples differing in quality and processing level (%; dry matter basis)

Soybean Meal Quality	Dry matter	Crude protein	Crude fibre	Ether extract	Ash	Acid detergent fibre *	Neutral detergent fibre *	Starch	Sugar
UP	92.6	45.2	6.1	0.7	6.1	8.1	13.6	0.7	9.9
LQ	90.4	45.5	4.2	1.3	6.4	5.8	9.7	0.9	10.8
HQ	89.9	47.5	2.6	3.1	6.2	4.7	9.2	0.6	10.4
OP	91.3	47.9	2.6	2.4	6.4	5.0	11.3	0.8	9.9

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: High quality SBM; OP: Over-processed SBM.

Values shown are mean values of analyses using Near Infrared Reflectance Spectroscopy at DSM Nutritional Products South Africa (Pty) Ltd and Evonik Africa (Pty) Ltd, and wet chemistry at the Animal Production and Health Department of the Polytechnic University of Madrid unless otherwise noted.

* Values obtained only at Evonik Africa (Pty) Ltd and the Animal Production and Health Department of the Polytechnic University of Madrid.

UP, LQ, HQ, OP; n=7.

4.3.2 Phase 2: *In vivo* digestibility trial

4.3.2.1 Broiler rearing

Broiler rearing as well as the digestibility trial were conducted in an environmentally controlled broiler house with concrete flooring situated at Innovation Africa Research Park, University of Pretoria, Hillcrest, Pretoria. Three weeks prior to chick placement, the entire facility and all equipment including steel dividers, bell feeders, tray feeders, fountain drinkers, plastic water drums, bins and feed scoops were thoroughly cleaned using a solution of Virukil (ICA International). The nipple and water lines were thoroughly flushed. The facility and equipment were then left unused and rested for two weeks where after house preparations began.

Six days prior to chick placement, the nipple and water lines were again flushed with water and checked for any leaks or blockages and damaged and/or faulty nipples. Eight 2.5m x 2.5m floor pens were assembled using steel mesh dividers. Each pen had two nipple lines with 12 nipples per line in each pen. In each pen a 7 cm thick layer of pine shavings was evenly placed. Each pen was equipped with four tube feeders. Three days prior to placement the house was preheated to 36°C and fumigated.

On the day of placement (day 0), four floor feeder trays were placed in each floor pen and all floor trays and tube feeders were filled with the pre-starter/starter ration. Fountain drinkers were filled with fresh

Table 4.2: Amino acid composition (%; dry matter basis) of soybean meal samples differing in quality and processing level

	Soybean Meal Quality			
	UP	LQ	HQ	OP
Indispensable amino acids				
Arginine	3.186	3.283	3.462	3.445
Histidine	1.150	1.188	1.240	1.231
Isoleucine	1.966	2.043	2.113	2.090
Leucine	3.287	3.428	3.555	3.511
Tryptophan	0.595	0.623	0.650	0.644
Lysine	2.662	2.756	2.846	2.814
Methionine	0.602	0.622	0.642	0.639
Phenylalanine	2.208	2.306	2.359	2.337
Threonine	1.692	1.763	1.821	1.802
Valine	2.076	2.158	2.232	2.202
Dispensable amino acids				
Alanine	1.885	1.962	2.025	2.010
Aspartic acid	4.959	5.150	5.365	5.324
Cysteine	0.655	0.672	0.696	0.686
Methiocysteine	1.244	1.293	1.335	1.310
Glutamic acid	7.804	8.093	8.494	8.419
Glycine	1.887	1.919	1.969	1.948
Proline	2.205	2.289	2.365	2.341
Serine	2.181	2.264	2.354	2.334

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: High quality SBM; OP: Over-processed SBM. Values were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd.

water (at a temperature of 19°C) and four drinkers were placed in each pen. 1000 Day-old male Ross 308 chicks were collected from National Chicks, Boschkop, Gauteng. Groups of 25 chicks were randomly taken from each of the ten boxes, one chick from a box at each time, and assigned randomly to one of eight floor pens. This was repeated until all forty groups of chicks were assigned to a pen with 125 chicks per pen.

A commercial starter ration in crumble form was fed from day 0 till day 14 and a pelleted commercial grower ration from day 14 until day 21. All birds received the same feed from 0 – 21 days of age. The nipples lines were continuously adjusted to appropriate bird height. For the first seven days, four small fountain drinkers were placed in each floor pen to ensure easy access to water. These drinkers were cleaned and refilled every morning to maintain the freshness of the drinking water. The feed trays were cleaned where necessary and topped up with feed every morning. The tube feeders were also topped up where necessary every morning.

From day 0 till day 7 the birds were exposed to 23 hours of light and 1 hour of dark. After seven days-of-age the birds received 5 hours of darkness each day until the age of 21 days, as per the recommendations for the Ross 308 broiler (Aviagen, 2018).

4.3.2.2 Dietary treatments of the digestibility study

The digestibility trial had a 4x2 arrangement of treatments with SBM of four different qualities and two levels of protease (none or included). The eight treatment diets used in the digestibility study comprised of semi-purified diets containing a basal mix of maize sugar (glucose/dextrose), maize starch, vegetable oil, monocalcium phosphate, limestone, sodium chloride, vitamin-mineral premix (containing a coccidiostat), potassium carbonate, titanium dioxide, and SBM included at 41.0% (DM basis) of the diet, as shown in Table 4.4. The treatment diets contained one of the four chosen qualities of SBM i.e. UP, LQ, HQ and OP. The quality parameter values for each of the SBMs used is represented in Table 4.3 Each diet either contained no protease or the protease enzyme (Ronozyme® ProAct DSM Nutritional Products, Kaiseraugst, Switzerland).

4.3.2.3 Preparation of treatment diets

The feed was mixed and pelleted at Simplegrow Agricultural Services, Centurion, Gauteng. Titanium dioxide was used as an indigestible marker and included in the feed at a concentration of 5 g/kg. This

Table 4.3: Quality parameter values of selected soybean meal samples differing in quality and level of processing

	Soybean Meal Quality			
	UP	LQ	HQ	OP
Urease activity, mg N/g *	0.78	0.04	0.05	0.05
Protein dispersibility index (%)	14.5	8.3	7.6	9.3
Potassium hydroxide protein solubility (%)	71	76	81	81
Trypsin inhibitor activity (mg/g DM)	0.6	1.31	ND	ND
Total phosphorus (mg/kg)	5876	6097	6611	6335
Phytic phosphorous (mg/kg)	3526	3658	3966	3801
Reactive lysine (%)	2.42	2.51	2.52	2.47
Proportion reactive lysine of total lysine (%)	87.20	89.03	89.23	89.01
Digestible protein (%) **	81.43	84.28	81.87	83.31
Protein digestibility index (%) **	-1.01	2.46	6.41	5.65
Processing condition indicator	12	11	12	12

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.
 ND: Not detectable.

Values without an '*' or '**' were obtained using Near Infrared Reflectance Spectroscopy, Evonik Africa (Pty) Ltd.

* Values were obtained using wet chemistry, Nutrilab, Department of Animal Science, University of Pretoria.

** Values were obtained using Near Infrared Reflectance Spectroscopy, DSM Nutritional Products Iberia, S.A.

Table 4.4: Ingredient composition of the experimental diets (g/kg, dry matter basis)

Feed ingredient	Inclusion
Maize starch	526
Soybean meal	410
Soybean oil	30
Monocalcium phosphate	10.6
Limestone (fine)	9.9
Vitamin/mineral premix	4
Titanium dioxide	5
Salt	3.6
Potassium carbonate	0.9
Total	1000

inclusion level ensured even distribution of marker throughout the feed and allowed for high accuracy of titanium recovery from the treatment diets and the ileal contents of the birds post-trial (Short *et al.*, 1996).

The marker was mixed with a maize starch as a carrier to increase the flow ability of the marker and prevent

self-cohesion of the marker that could hinder a homogenous spread throughout the feed. The marker and the carrier were mixed at a proportion of 30:70, respectively. A basal mixture of additives was produced by blending the titanium-carrier mix, coccidiostat, vitamin and mineral premix, salt, limestone and monocalcium phosphate. After dividing the additive mixture into two, Ronozyme® ProAct (DSM Nutritional Products, Kaiseraugst, Switzerland) protease was added to the one part, where the other remained free of protease. Another basal mixture of maize, sugar (dextrose/glucose), maize starch and vegetable oil, was prepared. This mixture was divided into four equal portions to which the four different SBM samples were added. After creating the initial four mixtures, each was divided in half to produce the final eight basal diets. To these diets, additive mixtures containing either protease or none were added. All mixtures were thoroughly blended in an appropriately sized paddle mixer for five minutes. Subsequently, the eight treatment diets underwent pelleting at high temperatures, specifically between 80-90°C, with humidity maintained at 14%. Throughout the pelleting process, samples were collected from the outlet of the pelleter at the beginning, middle, and end stages. These feed samples were subjected to analysis for DM concentration, determined using the CAN-MET-008C method for moisture content, CP concentration measured by the Dumatherm method, and titanium dioxide concentration analyzed using the ICP-OES method by ChemNutri (Olifantsfontein, South Africa). Additionally, AA concentrations were determined using method ASM 021 by the Agricultural Research Council (Irene, South Africa), as detailed in Tables 4.5 and 4.6. Titanium dioxide levels recovered in the treatment diets were lower than the inclusion level (5 g/kg dry matter basis). This may have been caused by improper addition of ingredients due to human error, improper mixing of the ingredients and human error during the titanium recovery. On day 20, a 500g sample was taken from all treatment diets using a small feed sampler driven directly into the middle of the feed bag. These samples were analysed as explained in section 4.3.2.4 Digestibility Trial.

Table 4.5: Nutrient composition and titanium dioxide concentration of the treatment diets (dry matter basis)

Treatment	Quality	Protease	Dry matter (%)	Crude protein (%)	Titanium dioxide (g/kg)
1	UP	0	88.5	24.9	2.37
2	UP	1	90.3	24.0	2.17
3	HQ	0	88.8	24.2	2.30
4	HQ	1	89.5	25.4	2.28
5	LQ	0	89.2	25.6	2.26
6	LQ	1	89.2	25.7	2.48
7	OP	0	89.6	25.9	2.20
8	OP	1	89.4	25.1	2.54

Values were obtained using wet chemistry, ChemNutri, Olifantsfontein, South Africa

Table 4.6: Concentration of amino acids of the treatment diets (% , dry matter basis)

Amino acid	Treatment 1 UP - 0	Treatment 2 UP - 1	Treatment 3 HQ - 0	Treatment 4 HQ - 1	Treatment 5 LQ - 0	Treatment 6 LQ - 1	Treatment 7 OP - 0	Treatment 8 OP - 1
Arginine	1.85	1.98	2.06	1.90	1.99	2.08	1.90	1.85
Serine	1.15	1.18	1.23	1.19	1.17	1.17	1.32	1.10
Aspartic acid	2.19	2.32	2.40	2.23	2.32	2.41	1.91	2.14
Glutamic acid	4.00	4.10	4.21	3.95	4.13	4.27	4.08	3.85
Glycine	0.97	1.04	1.06	0.99	1.02	1.05	1.10	0.95
Threonine	0.88	0.90	0.90	0.87	0.87	0.90	0.93	0.81
Alanine	0.99	1.02	1.04	1.03	1.01	1.02	1.04	0.95
Tyrosine	0.85	0.91	0.88	0.69	0.87	0.84	0.77	0.74
Proline	1.33	1.34	1.35	1.40	1.47	1.48	1.54	1.38
HO-Proline	0.02	0.04	0.20	0.03	0.03	0.02	0.02	0.02
Methionine	0.35	0.37	0.01	0.25	0.26	0.16	0.46	0.27
Valine	1.20	1.24	1.32	1.31	1.42	1.47	1.47	1.31
Phenylalanine	1.14	1.18	1.22	1.21	1.30	1.31	1.33	1.17
Isoleucine	1.11	1.15	1.19	1.16	1.28	1.31	1.33	1.15
Leucine	1.97	1.96	2.01	2.04	2.15	2.16	2.03	1.96
Histidine	1.39	1.35	1.47	1.50	1.30	1.41	1.62	1.33
Lysine	1.49	1.57	1.56	1.48	1.64	1.67	1.59	1.44

Values were obtained using wet chemistry, Agricultural Research Council, Irene, South Africa.

4.3.2.4 Digestibility Trial

Three weeks prior to chick placement, ninety-six poultry metabolic cages were cleaned and disinfected thoroughly using a high-pressure cleaner and a cleaning solution containing Virukil (ICA International). Thereafter the cages were rested unused for a period of two weeks. Two days prior to chick placement, the cages were placed in the same broiler facility where the birds were reared. The cages were arranged in a three-tier configuration. Each cage measured 125 cm in length, 65 cm in width and 80 cm in height, with a faecal collection tray at the bottom and a feed trough in the front. Each cage had its own gravity fed drinking system with a 5 L water reservoir connected to a water line containing five nipples. Cages were clearly marked with a tag showing the cage number and colour coded to match the colour assigned to each of the treatment bag labels i.e. UP, LQ, HQ or OP SBM with protease or non-coloured without protease.

On day 18, all birds were individually weighed and birds with body weights closest to the mean body weight of the population were selected. 768 birds were randomly allocated to 96 cages. Eight birds were placed in a single cage to ensure that enough ileal content can be collected per treatment. Birds allocated to each cage were weighed to ensure that the total body weight of birds in all pens did not differ significantly from each other. The birds remained in cages for four days from day 21 to 24 days-of-age, while being fed their respective treatment diets *ad libitum*. This allowed for enough time for passage of rearing diet and adjustment to the new environment and treatment diet. The lighting program was altered to allow for one more extra hour of daylight (20 hours daylight) to stimulate feed intakes leading up to ileal sampling at 24 days-of-age.

On the morning of the 24th day, feed intake was physically stimulated by movement and shifting of the feed in all the feeders every 15 minutes for a period of two and a half hours. Beginning at cage number 1, all birds from each cage were removed and placed into a gassing box. All eight birds were exposed to a gaseous mixture of 25% CO₂, 10% N₂ and 65% O₂ for a period of no less than 4 minutes to ensure complete anaesthesia and unconsciousness. Thereafter, the anaesthetised birds were euthanised by being exposed to gaseous CO₂ at 100% concentration for no less than two and a half minutes to ensure complete asphyxiation and death of the birds. Once all the birds were euthanised, the carcasses were removed from the box and dissected to remove the distal portion of the ileum, 1 cm proximal to the Meckel's diverticulum and 1 cm proximal to the ileocecal junction. The removed ileal samples were immediately placed in a plastic tub labelled with the corresponding cage number. The plastic tub was kept cold by being placed in a mixture of crushed ice and water (Angel, 2011)

Once all ilea were removed from the birds from the same cage, the ilea were transferred from the cups onto cooled granite slabs which were placed on ice to cease all digestive actions. Each ileum was then flushed with 1 mL of cooled distilled water using a syringe fitted with a 16-gauge needle. The bezel of the needle was removed, to prevent puncture of the ileum when flushing allowing an airtight flush. The ileal content from all eight birds in each cage was flushed into the same container which was placed on a mixture of crushed ice and water. After collection of digesta, the marked container was closed and immediately placed in a freezer.

On the following day, all frozen ileal samples were placed in a freeze-drier (Scitek) and kept there for 10 days with the temperature set at -40°C to ensure complete dehydration of the samples. The freeze-dried samples were then ground by using a clean laboratory grinder (IKA). Each sample was ground for a duration of 10-20 seconds, removed, and passed through a $0.5\ \mu\text{m}$ sieve into a foil tray. Any remaining larger particles were removed from the sieve into another foil tray and ground again either using the IKA laboratory grinder or using a pestle and mortar. Between each sample, all the equipment used was cleaned using an air compressor and all surfaces were wiped with tissue paper. The entire grinding and intermittent cleaning processes were performed inside a laboratory extractor.

The 96 pooled ileal digesta samples were, together with the 500g treatment samples, analysed for concentration of DM, CP and titanium (ChemNutri, Olifantsfontein, South Africa) and AA concentrations (Agricultural Research Council, Irene, South Africa). and reported in mg/kg. Dry matter was determined by calculation using moisture determined with the CAN_MET_008C analysis reported in g/100g and CP was determined using the Dumas method reported in g/kg. Recovery of the titanium marker was performed using the ICP-OES analysis and reported in mg/kg.

Ileal AA digestibility values were calculated using the following equation:

$$\text{AID, \%} = [1 - (M_{\text{diet}}/M_{\text{ileal}}) \times (AA_{\text{ileal}}/AA_{\text{diet}})] \times 100$$

Where M_{diet} and M_{ileal} represent the diet and ileal marker concentrations (g/kg, DM basis), respectively and AA_{ileal} and AA_{diet} represent the ileal and diet AA concentrations (g/kg, DM basis), respectively (Adeola *et al.*, 2016).

CP digestibility values were calculated using the following equation:

$$\text{CPD, \%} = [1 - (M_{\text{diet}}/M_{\text{ileal}}) \times (CP_{\text{ileal}}/CP_{\text{diet}})] \times 100$$

Where M_{diet} and M_{ileal} represent the diet and ileal marker concentrations (g/kg, dry matter basis basis), respectively and CP_{ileal} and CP_{diet} represent the ileal and diet CP concentrations (g/kg, DM basis), respectively (Adeola *et al.*, 2016).

DM digestibility values were calculated using the following equation:

$$\text{DMD, \%} = ((\text{Indicator in faeces (g/kg DM)} - \text{indicator in feed (g/kg DM)}) / \text{Indicator in faeces (g/kg DM)}) \times 100 \text{ (McDonald } et al., 2010)$$

4.3.2.5 Statistical analysis

Data were analysed statistically with the Proc Mixed model (Statistical Analysis System, 2021) for the mean effects. Means and standard error were calculated and significance of difference ($P < 0.05$) between means was determined by Fischer's test (Samuels, 1989). Repeated Measures Analysis of Variance with the Mixed model was used for repeated week or period measures.

The linear mix model used is described by the following equation:

One way

$$Y_{ijk} = \mu + S_i + L_j + B_k + SL_{ij} + e_{ijk}$$

Where Y_{ijk} = variable studied during the period

μ = overall mean of the population

S_i = effect of the i th Source

L_j = effect of the j th level

B_k = effect of the k th block

SL_{ij} = effect of the ij th interaction between Source and level

e_{ijk} = error associated with each Y

4.4 Results

Dry matter digestibility was not significantly ($P > 0.05$) increased for any of the sources of SBM when protease was added to the diet, represented in Table 4.7. Also, there were no differences found in ileal digestibility of DM between sources for feeds with or without protease inclusion in the diets ($P > 0.05$).

Table 4.7: Ileal digestibility (%) of dry matter of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	71.0 (± 0.010)	69.8 (± 0.009)	70.4 (± 0.007)
LQ	71.2 (± 0.009)	69.7 (± 0.009)	70.4 (± 0.006)
HQ	71.7 (± 0.009)	70.8 (± 0.009)	71.2 (± 0.006)
OP	71.7 (± 0.009)	71.4 (± 0.009)	71.6 (± 0.006)
Mean	71.4 (± 0.005)	70.4 (± 0.005)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

As shown in Table 4.8, the inclusion of protease resulted in a significant increase ($P < 0.05$) in ileal digestibility of CP for all four quality levels of SBM included in the diets. There were no significant ($P > 0.05$) differences noted for ileal digestibility between the different qualities of SBM, either with or without the supplementation of protease.

Table 4.8: Ileal crude protein digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	76.7 ^a (± 0.915)	73.6 ^b (± 0.871)	74.3 (± 0.632)
LQ	76.7 ^a (± 0.871)	74.4 ^b (± 0.871)	74.4 (± 0.616)
HQ	77.8 ^a (± 0.871)	75.1 ^b (± 0.871)	74.9 (± 0.632)
OP	77.6 ^a (± 0.871)	76.4 ^b (± 0.871)	74.8 (± 0.632)
Mean	77.2 ^a (± 0.441)	74.9 ^b (± 0.436)	

^{a-b} Within each row, digestibility values of feeds with and without protease within the same level of quality of soybean meal (SBM) without a common superscript differ significantly ($P < 0.05$).

UP: under-processed SBM; LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Referring to Table 4.9, for the diets that did not contain protease, ileal alanine digestibility of OP differed significantly ($P < 0.05$) from UP. For the mean values of the diets containing one quality level of SBM, fed with and without protease, a significant difference was found between Sources UP and OP.

Table 4.9: Ileal alanine digestibility of feed (%; dry matter basis) with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean*
UP	74.9 (± 1.830)	73.4 ^B (± 1.830)	74.1 ^B (± 0.766)
LQ	76.2 (± 1.830)	75.7 ^{AB} (± 1.830)	76.0 ^{AB} (± 0.766)
HQ	75.9 (± 1.830)	76.1 ^{AB} (± 1.830)	76.0 ^{AB} (± 0.766)
OP	77.1 (± 1.830)	76.6 ^A (± 1.830)	76.9 ^A (± 0.766)
Mean	76.1 (± 0.541)	75.4 (± 0.541)	

^{A-B} Within each column, values within the same protease inclusion, between soybean meal (SBM) quality levels, and mean values, regardless of protease inclusion, without a common superscript differ significantly ($P < 0.05$).
 UP: under-processed SBM; LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Referring to Table 4.10, it was noted for the diets containing enzymes, that HQ showed significantly ($P < 0.05$) higher ileal histidine digestibility than UP. For the diets that contained UP SBM, digestibility of histidine was greater in the absence of protease than when enzymes were present. For the diets containing HQ SBM, the ileal histidine digestibility was higher for the diets that contained enzymes than the diets that did not. For the mean values of the diets with different quality levels, with or without the supplementation of protease, it was observed that UP and LQ was significantly lower than HQ. It was also observed that LQ differed significantly from OP.

Table 4.10: Ileal histidine digestibility of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	78.7 ^{bb} (± 2.482)	81.14 ^B (± 2.482)	79.9 ^C (± 1.755)
LQ	84.9 ^{AB} (± 2.482)	83.4 ^{AB} (± 2.482)	84.2 ^{BC} (± 1.755)
HQ	89.6 ^{aa} (± 2.482)	89.0 ^{ba} (± 2.482)	89.3 ^A (± 1.755)
OP	85.5 ^{AB} (± 2.482)	85.1 ^{AB} (± 2.482)	85.3 ^{AB} (± 1.755)
Mean	84.7 (± 1.241)	84.6 (± 1.241)	

^{A-C} Within each column, values within the same protease inclusion, between soybean meal (SBM) quality levels, and mean values, regardless of protease inclusion, without a common superscript differ significantly ($P < 0.05$).

^{a-b} Within each row, digestibility values of feeds with and without protease within the same level of quality of soybean meal (SBM) without a common superscript differ significantly ($P < 0.05$).

UP: under-processed SBM; LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

For the ileal digestibility of lysine, shown in Table 4.11, it was noted for the diets containing enzymes,

OP showed significantly ($P < 0.05$) higher digestibility than both UP and LQ, with UP having a higher digestibility than LQ. In the diets that did not contain protease, it was also noted that OP had a higher digestibility value than LQ. Across all sources there were no significant differences in the digestibility of lysine between the diets that contained protease and those that did not. For the mean values of the diets containing different SBM quality levels, fed with or without protease, OP was significantly higher than UP and LQ, with LQ having the lowest digestibility.

Table 4.11: Ileal lysine digestibility of feed (%; dry matter basis) with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	81.2 ^B (± 1.297)	81.4 ^{AB} (± 1.297)	81.3 ^B (± 0.645)
LQ	80.8 ^B (± 1.297)	80.8 ^B (± 1.297)	80.8 ^B (± 0.645)
HQ	82.3 ^{AB} (± 1.297)	81.7 ^{AB} (± 1.297)	82.02 ^B (± 0.645)
OP	84.0 ^A (± 1.297)	83.56 (± 1.297)	83.7 ^A (± 0.645)
Mean	82.0 (± 0.459)	81.8 (± 0.459)	

^{A-B} Within each column, values within the same protease inclusion, between soybean meal (SBM) quality levels, and mean values, regardless of protease inclusion, without a common superscript differ significantly ($P < 0.05$)
 UP: under-processed SBM; LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM

Table 4.12 shows that for diets containing protease, HQ had significantly ($P < 0.05$) higher ileal leucine digestibility than the diet containing OP. For the mean values of the diets containing different levels of SBM quality, fed with or without protease, significant differences were found between HQ and OP.

Table 4.12: Ileal leucine digestibility of feed (%; dry matter basis) with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean*
UP	80.6 ^{AB} (± 0.954)	80.0 (± 0.954)	80.3 ^{AB} (± 0.674)
LQ	81.1 ^{AB} (± 0.954)	80.2 (± 0.954)	80.6 ^{AB} (± 0.674)
HQ	83.2 ^A (± 0.954)	81.1 (± 0.954)	82.1 ^A (± 0.674)
OP	79.5 ^B (± 0.954)	79.7 (± 0.954)	79.6 ^B (± 0.674)
Mean	81.1 (± 0.477)	80.3 (± 0.477)	

^{A-B} Within each column, values within the same protease inclusion, between soybean meal (SBM) quality levels, and mean values, regardless of protease inclusion, without a common superscript differ significantly ($P < 0.05$).
 UP: under-processed SBM; LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

The ileal methionine digestibility of LQ, HQ, OP and the mean value was significantly ($P < 0.05$) increased in the diets containing protease than the diets that did not, as shown in Table 4.13.

Table 4.13: Ileal methionine digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	82.3 (± 3.294)	83.6 (± 2.768)	83.0 (± 2.151)
LQ	87.7 ^a (± 3.294)	83.3 ^b (± 2.768)	85.5 (± 2.151)
HQ	87.7 ^a (± 3.294)	81.9 ^b (± 2.768)	84.8 (± 2.151)
OP	90.6 ^a (± 3.294)	81.2 ^b (± 2.768)	85.9 (± 2.151)
Mean	87.1 ^a (± 1.787)	82.5 ^b (± 1.384)	

^{a-b} Within each row, digestibility values of feeds with and without protease within the same level of quality of soybean meal (SBM) without a common superscript differ significantly ($P < 0.05$).

UP: under-processed SBM; LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Referring to Table 4.14 representing ileal serine digestibility, for the mean values of the diets containing different levels of soybean quality, either with or without protease, OP was significantly ($P < 0.05$) higher than UP.

Table 4.14: Ileal serine digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	72.0 (± 1.414)	70.7 (± 1.414)	71.3 ^B (± 1.00)
LQ	74.1 (± 1.414)	72.3 (± 1.414)	73.24 ^B (± 1.00)
HQ	73.5 (± 1.414)	73.1 (± 1.414)	73.35 ^B (± 1.00)
OP	74.6 (± 1.414)	74.0 (± 1.414)	74.3 ^A (± 1.00)
Mean	73.5 (± 0.707)	72.5 (± 0.707)	

^{A-B} Within each column, values within the same protease inclusion, between soybean meal (SBM) quality levels, and mean values, regardless of protease inclusion, without a common superscript differ significantly ($P < 0.05$).

UP: under-processed SBM; LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.15 shows that HQ had significantly ($P < 0.05$) higher ileal tyrosine digestibility than OP when protease was supplemented in all diets. For diets that did not contain protease, HQ showed significantly higher digestibility than OP. Within the diets containing HQ, the diet supplemented with protease showed

significantly greater tyrosine digestibility than the diet that did not contain any enzyme. Diets containing OP with protease also showed significantly greater digestibility than the diet with OP without protease. For the mean values of the diets with different quality levels, with or without the supplementation of protease, it was observed that HQ had significantly higher ileal tyrosine digestibility than LQ and OP.

Table 4.15: Ileal tyrosine digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	79.9 ^{AB} (± 1.769)	80.1 ^{AB} (± 1.769)	80.0 ^{AB} (± 1.251)
LQ	78.7 ^{AB} (± 1.769)	79.5 ^{AB} (± 1.769)	79.1 ^{BC} (± 1.251)
HQ	83.5 ^{aA} (± 1.769)	82.3 ^{bA} (± 1.769)	82.9 ^A (± 1.251)
OP	76.7 ^B (± 1.769)	75.9 ^B (± 1.769)	76.3 ^C (± 1.251)
Mean	79.7 (± 0.884)	79.5 (± 0.884)	

^{A-C} Within each column, values within the same protease inclusion, between soybean meal (SBM) quality levels, and mean values, regardless of protease inclusion, without a common superscript differ significantly ($P < 0.05$).

^{a-b} Within each row, digestibility values of feeds with and without protease within the same level of quality of soybean meal (SBM) without a common superscript differ significantly ($P < 0.05$).

UP: under-processed SBM; LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

No significant differences ($P > 0.05$) in the ileal digestibility were observed for any of the quality levels of SBM, whether fed with or without protease, for the following AAs: isoleucine, phenylalanine, proline, threonine and valine as shown in Tables 4.16-4.24.

Table 4.16: Ileal arginine digestibility of feed (%; dry matter basis) with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	80.2 (± 1.149)	77.5 (± 1.149)	78.8 (± 0.812)
LQ	77.6 (± 1.149)	76.4 (± 1.149)	77.0 (± 0.812)
HQ	77.3 (± 1.149)	77.8 (± 1.149)	77.6 (± 0.812)
OP	77.9 (± 1.149)	78.9 (± 1.149)	78.4 (± 0.812)
Mean	78.3 (± 0.574)	77.7 (± 0.574)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.17: Ileal aspartic acid digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	74.1 (± 1.425)	72.1 (± 1.425)	73.1 (± 1.008)
LQ	72.0 (± 1.425)	73.6 (± 1.425)	72.8 (± 1.008)
HQ	71.0 (± 1.425)	72.4 (± 1.425)	71.7 (± 1.008)
OP	72.6 (± 1.425)	73.9 (± 1.425)	73.3 (± 1.008)
Mean	72.4 (± 0.713)	73.0 (± 0.713)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.18: Ileal glycine digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	69.4 (± 1.314)	67.9 (± 1.314)	68.6 (± 0.929)
LQ	69.5 (± 1.314)	67.6 (± 1.314)	68.5 (± 0.929)
HQ	68.7 (± 1.314)	68.3 (± 1.314)	68.5 (± 0.929)
OP	71.0 (± 1.314)	69.8 (± 1.314)	70.4 (± 0.929)
Mean	69.6 (± 0.675)	68.4 (± 0.657)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.19: Ileal glutamic acid digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	83.3 (± 1.045)	80.5 (± 1.045)	81.9 (± 0.739)
LQ	81.1 (± 1.045)	81.4 (± 1.045)	81.3 (± 0.739)
HQ	81.0 (± 1.045)	81.2 (± 1.045)	81.1 (± 0.739)
OP	82.8 (± 1.045)	82.1 (± 1.045)	82.4 (± 0.739)
Mean	82.1 (± 0.523)	81.3 (± 0.523)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.20: Ileal isoleucine digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	78.9 (± 1.041)	78.1 (± 1.041)	78.5 (± 0.736)
LQ	79.6 (± 1.041)	78.4 (± 1.041)	79.0 (± 0.736)
HQ	79.0 (± 1.041)	78.2 (± 1.041)	78.6 (± 0.736)
OP	78.8 (± 1.041)	78.6 (± 1.041)	78.7 (± 0.736)
Mean	79.1 (± 0.521)	78.3 (± 0.521)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.21: Ileal phenylalanine digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	80.1 (± 0.934)	79.1 (± 0.934)	79.6 (± 0.660)
LQ	79.6 (± 0.934)	79.0 (± 0.934)	79.3 (± 0.660)
HQ	80.0 (± 0.934)	79.7 (± 0.934)	79.8 (± 0.660)
OP	79.6 (± 0.934)	79.4 (± 0.934)	79.5 (± 0.660)
Mean	79.8 (± 0.467)	79.3 (± 0.467)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.22: Ileal proline digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	78.4 (± 0.904)	77.2 (± 0.904)	77.8 (± 0.639)
LQ	78.6 (± 0.904)	77.8 (± 0.904)	78.2 (± 0.639)
HQ	78.2 (± 0.904)	78.1 (± 0.904)	78.2 (± 0.639)
OP	78.7 (± 0.904)	78.8 (± 0.904)	78.8 (± 0.639)
Mean	78.5 (± 0.452)	78.0 (± 0.452)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.23: Ileal threonine digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	65.5 (± 1.546)	64.8 (± 1.546)	65.1 (± 1.093)
LQ	66.4 (± 1.546)	64.2 (± 1.546)	65.3 (± 1.093)
HQ	66.2 (± 1.546)	65.4 (± 1.546)	65.8 (± 1.093)
OP	68.7 (± 1.546)	67.3 (± 1.546)	68.0 (± 1.093)
Mean	66.7 (± 0.773)	65.4 (± 0.773)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

Table 4.24: Ileal valine digestibility (%; dry matter basis) of feed with and without protease with soybean meal of different quality (\pm standard error of the mean)

Soybean Meal Quality	With protease	Without protease	Mean
UP	76.3 (± 1.091)	76.1 (± 1.091)	76.2 (± 0.771)
LQ	76.4 (± 1.091)	76.8 (± 1.091)	76.5 (± 0.771)
HQ	77.3 (± 1.091)	76.9 (± 1.091)	77.1 (± 0.771)
OP	75.6 (± 1.091)	75.8 (± 1.091)	75.7 (± 0.771)
Mean	76.4 (± 0.545)	76.39 (± 0.545)	

UP: under-processed soybean meal (SBM); LQ: low quality SBM; HQ: high quality SBM; OP: over-processed SBM.

4.5 Discussion

The aim of the study was to examine how the quality of SBM affects the efficacy of protease in diets containing SBM. The AID of CP increased significantly when diets containing protease were compared to those without, across similar SBM qualities. However, no significant increases were observed in the AID of DM, although significant differences were noted for AID of CP across all qualities. Increases and reductions in AIAAD were observed in treatments containing protease, with some differences proving significant and others not. Both SBM quality and the addition of protease had significant effects ($P < 0.05$) on the AID of certain AAs, while others remained unaffected ($P > 0.05$).

Although increases in DM digestibility are generally expected due to the effectiveness of exogenous proteases in degrading plant storage proteins (Ojha, 2019), only a slight tendency towards increased ileal

DM digestibility was observed: 1.13% for UP, 1.5% for LQ, 0.93% for HQ, and 0.24% on average across all sources, which were not statistically significant ($P>0.05$). Apparent ileal CP digestibility showed more pronounced increases when diets were supplemented with protease: 3.07% for UP, 2.36% for LQ, 2.72% for HQ, and 1.22% for OP. The mean increase across all SBM qualities was 2.3%. This aligns with findings in literature for maize-soybean diets supplemented with protease, where increases in CP digestibility typically range from 1.8% to 5.9% (Freitas et al., 2011; Fru-Nji, 2011; Cowieson and Roos, 2014; Park, 2020). The effectiveness of protease activity appears to vary with CP content in diets. While previous studies have suggested that higher CP levels enhance the effect of protease (Angel et al., 2011; Freitas et al., 2011; Dessimoni et al., 2019;), this study found the greatest increase in AID of CP (3.07%) occurred in LQ, which had the lowest CP content (24.94%). Conversely, Treatment 7, which had the highest CP content (25.96%) and included OP SBM (47.90% CP), showed the smallest increase in AID of CP (1.22%). The study demonstrated that protease supplementation significantly improves CP digestibility in SBM-containing diets across different qualities of SBM. However, the relationship between CP content and the efficacy of protease was not entirely consistent with previous findings, highlighting the complex interactions involved in protease activity in different diet formulations.

In contrast to the overall increase in AID of CP across all treatments containing protease, significant ($P<0.05$) increases as well as reductions in AIAAD were observed for specific AAs in certain treatments. The treatment using UP SBM exhibited the most pronounced response to protease supplementation, with significant increases of 2.69% for arginine, 2.07% for aspartic acid, 1.48% for glycine, and 2.83% for glutamic acid. However, a significant reduction in AID of histidine by 2.44% was observed in the same treatment. For treatments containing HQ SBM, significant increases were noted in histidine (0.65%), methionine (5.96%), and tyrosine (1.2%). Conversely, the treatments using LQ and OPSBM showed the least number of AAs responding to protease supplementation, with significant increases of 4.4% and 9.36%, respectively. Previous studies by Angel *et al.* (2011) and Bertechini *et al.* (2020) reported similar findings, noting significant increases in ileal AA digestibility when protease was supplemented. Angel *et al.* (2011) observed significant increases in arginine, aspartic acid, methionine, isoleucine, lysine, threonine, histidine, cysteine, and serine digestibility, while Bertechini *et al.* (2020) found significant increases in arginine, cysteine, glycine, histidine, lysine, methionine, proline, threonine, tyrosine, and valine digestibility with protease supplementation. Although not statistically significant, a trend towards increased AIAAD was observed in most AAs when protease was included in the diets in this study, consistent with the observations of Angel *et al.* (2011) and Bertechini *et al.* (2020). Overall, these findings underscore the variable and specific effects of protease

supplementation on AA digestibility in SBM-containing diets, influenced by the quality of SBM and potentially other factors related to diet composition and protease activity.

In the current study, the mean increase in AIAAD across all AAs in all treatments was 2.79%. This value is lower compared to findings reported by others, such as 5.7% by Cowieson *et al.* (2020), 3.86% by Bertechini *et al.* (2020), and 4.98% by Angel *et al.* (2011). The lowest significant increase was observed for histidine in the treatment containing HQ SBM at 0.65%, while the highest increase of 9.36% was observed for methionine in the treatment containing OP SBM. Cowieson *et al.* (2020) reported increases ranging from 1.1% for methionine to 12.9% for aspartic acid, while Bertechini *et al.* (2020) observed increases ranging from 2.3% to 10.02%. The substantial increases observed for methionine in LQ (4.4%), HQ (5.96%), and OP (9.36%) SBM treatments in this study contrast with findings by Aderibigbe *et al.* (2020), who reported no significant effect of protease on the AID of methionine. This is consistent with previous studies (Ravindran *et al.*, 2007; Angel *et al.*, 2011; Cowieson and Roos, 2014), which have suggested that the lack of effect on methionine digestibility could be due to the already high ileal digestibility of methionine (90-95%) typically observed in maize-soybean based diets.

In treatments where protease was supplemented, the most notable effect observed was with UP SBM, showing significant increases in four AAs: arginine, aspartic acid, glycine, and glutamic acid. Low Quality and OP SBM treatments exhibited significant increases specifically in methionine, while HQ showed significant increases in histidine, methionine, and tyrosine. This indicates a potential interaction between SBM processing level and protease efficacy, suggesting that using protease in diets containing less thermally processed SBMs could enhance AA bioavailability. Regarding AID, significant differences in alanine were observed between SBM qualities in treatments without protease, whereas no significant differences were noted in treatments with protease. Similarly, while no significant differences in leucine AID were observed among SBM qualities without protease, significant differences were evident across all qualities in treatments containing protease. These findings suggest that protease supplementation may mitigate differences in AA digestibility among SBM qualities. However, these conclusions contrast with studies by Marsman *et al.* (1997) and Salazar-Villanea *et al.* (2022), which suggest that protease may not effectively compensate for inadequately processed SBMs due to interactions between thermal processing and SBM quality. Additionally, Cowieson *et al.* (2020) found no interaction between protease and SBM from different origins that were significantly different nutritionally. In the present study, no significant differences ($P > 0.05$) were found in DM and CP content and the concentration of most AAs between diets containing different SBM qualities, regardless of protease supplementation. This suggests that different processing techniques may not significantly alter

the final quality of SBM in terms of these nutritional components.

A significant decrease of 2.44% in the AID of histidine was observed in the treatment containing UP SBM and protease compared to the treatment without protease. This finding aligns with the observations of Ravindran et. al., (2014), who argued against the longstanding assumption that an increase in AIAAD would directly correlate with an increase in AID of CP. Therefore, the supplementation of protease may not necessarily lead to improved CP digestibility when relying solely on fixed matrix values recommended by the manufacturer. Moreover, it may not be accurate to assume a linear relationship between improvements in CP digestibility and the digestibility of individual AAs. This highlights the complexity and potential variability in the effects of protease supplementation on nutrient digestibility in SBM-containing diets.

4.6 Conclusion

In summary, the study found that the use of protease was ineffective in increasing the AID of DM. Additionally, no differences in the AID of DM were observed among SBM qualities (UP, LQ, HQ, OP) regardless of protease supplementation. However, protease was effective in increasing the AID of CP and some AAs, although the effectiveness varied. This variability suggests that the supplementation of protease to maize-soybean diets may be beneficial in some instances depending on SBM quality, while in other cases, it may not yield significant benefits. One of the factors that may contribute to this was the poor recovery of titanium dioxide, which will underestimate the effect of protease on nutrient digestibility. It was also observed that an increase in the digestibility of AA does not always correspond with an increase in CP digestibility, and an increase in CP digestibility is not guaranteed when proteases are used. This highlights the complex interactions and variability in the effects of protease supplementation on nutrient digestibility in SBM-based diets.

Moving forward, further research is needed to better understand the effects and interactions of proteases in soybean-maize diets. It will be important to investigate how proteases affect SBM of different compositions and qualities. This will provide insights into when and under what conditions protease addition in diets may be financially viable. Based on the findings, the study rejects the null hypothesis that the use of protease will universally increase the ileal digestibility of nutrients in soybean-maize diets, regardless of SBM quality. Instead, it supports the alternative hypothesis that the efficacy of protease in soybean-maize diets is influenced by the quality of SBM used in the diet.

CHAPTER 5

General Discussion

The establishment and growth of the soybean processing industry in SA have been facilitated by new technology and equipment, development of climate-suited cultivars, and the dedication of farmers, the private sector, and government institutions (RussellStone Group, 2017). An increase in the capacity for processing increased the demand for locally produced soybeans. An increasing number of farmers began to rotate between maize and soybean to satisfy this demand (Cilliers, 2023). This evolution has transformed the industry from a net importer status to exporter status (Reidy, 2023).but it now faces challenges such as maintaining consistent supply and dealing with significant variation in the nutritional composition and quality of SBM batches within and between processors. Local soybeans are being processed under conditions similar to those used by processors in other regions of the globe. This has yielded SBM of similar nutritional composition and quality, as well as significant degrees of variation in quality.

A comparison with results of other similar studies, which include data from SA, as well as data from private research institutions show that SBM being produced by processors in SA is comparable to the SBM being produced by three of the largest processors worldwide including Argentina, Brazil and the USA (Tangendjaja, 2020; Aguirre et al., 2022). The variation seen between processors that utilise similar processing methods may be attributed to or influenced by other factors which include production conditions such as climate, planting and harvesting techniques and storage time as well as cultivar and other inherent factors (García-Rebollar et al., 2016). The significant variability observed between processors indicates that ensuring consistency in SBM composition and quality poses difficulties. The variability also underlines the importance for nutritionists to adjust nutrient matrices according to the supplier and even for batches from the same supplier.

The use of protease to enhance nutrient digestibility in SBM may or may not be feasible due to its efficacy being influenced by variation in nutritional composition and quality of SBM. In this study, the use of a monocomponent exogenous protease resulted in significant increases in the AID of CP across all treatments, attributed to the reduction of ANFs. However, not all diets supplemented with protease showed increases in AID for all individual AAs. This suggests that assuming a linear relationship between CP digestibility and AIAAD is not valid. While feed formulation commonly assumes a linear relationship between digestible AA content and CP content (Stefanello, 2016), the contradictory results

in AA digestibility from this study imply that predicting the nutritional value of SBM and formulating diets based solely on CP content may be oversimplified (Dudley-Cash, 1998). Therefore, improvements in CP digestibility based on fixed matrix values recommended by manufacturers cannot reliably predict increases in the digestibility of individual AAs when diets are supplemented with protease. An interaction was noted between processing level or SBM quality and the potential for protease to increase the AID of certain AA's. The greatest significant ($P < 0.05$) increase in the AID of CP was observed in the treatment containing UP SBM, which contained the lowest CP content. Furthermore the smallest increase in the AID of CP was observed for the treatment containing OP SBM, which had the highest CP content of all treatment diets.

Soybean meal quality and protease supplementation both had significant and insignificant effects on the AID of some AAs. The mean increase in AIAAD was 2.79 %. Protease supplementation had the most significant increase on aspartic acid, glycine, and glutamic acid, in UP SBM. This suggests that protease supplementation may be effective in increasing the AIAAD of some AAs in SBMs that have not undergone sufficient thermal exposure. This may also suggest that it may not always be justified to use a protease to increase the AIAAD of nutritionally significant AAs, such as essential AAs, in diets containing SBM that contains insufficiently processed SBM. The least significant increase that protease had on AIAAD was on methionine for LQ and OP SBMs. Lastly a significant increase was observed for histidine, methionine, and tyrosine for the treatment containing HQ SBM. Overall, the varying effects of protease supplementation on AIAAD indicates a possible interaction between processing level and protease efficacy.

Lastly, for DM, CP and the concentration of most AAs, no significant ($P > 0.05$) differences were noted between diets containing different qualities of SBM, regardless whether protease was supplemented or not. This highlights that the processing technique used may not have a significant effect on the DM, CP and AA content of the SBM.

Further research is necessary to better understand and optimize the use of proteases in SBM-based diets, taking into account the variability in SBM quality and its impact on enzyme efficacy. This highlights the importance of considering SBM quality when evaluating the efficacy of protease and its impact on nutrient digestibility in practical diet formulations.

CHAPTER 6

General Conclusion and Recommendations

The processing industry in SA is relatively new, and thus it is crucial to continue gathering, consolidating, and analysing data on the nutritional composition and quality of SBM from processors across the country. This ongoing effort will provide nutritionists with a more accurate reference point for formulation. South African SBM has been found to be comparable to SBM from other origins in terms of its nutritional composition and quality. However, significant variation in nutritional composition and quality exists between processors, a characteristic also observed in SBM from other countries. The variability in SBM composition as received from South African processors contributes to unpredictable outcomes when using protease. Interestingly, no significant differences were found in CP and DM content among treatments with different SBM qualities, suggesting that variations in processing techniques may not significantly contribute to the observed differences between SBM samples from different processors.

It can be concluded that the use of protease in soybean-maize based diets may offer benefits in enhancing the utilization of the protein fraction, evidenced by improvements in AID of CP and some AAs. However, the economic justification for using protease may not always be clear. Further research is needed to better understand the mechanisms by which protease acts on SBM, especially considering its varied effects on SBM of differing qualities. It is important to note that assumptions about improving CP digestibility based on fixed matrix values recommended by manufacturers, as well as assuming a linear relationship between CP improvement and the digestibility of individual AAs, may not be accurate when diets are supplemented with protease. Therefore, a more nuanced approach is necessary in understanding and utilizing protease in SBM-based diets.

The following are recommended:

1. **Quality Control:** Implement stringent quality control measures for sourcing and evaluating South African SBM to ensure consistency in nutrient composition and minimize variability in anti-nutritional factors. This could involve working closely with suppliers and conducting regular analyses of SBM batches.
2. **Optimal Protease Supplementation:** Determine the optimal inclusion level of exogenous protease based on the quality of SBM used in broiler diets. Conduct further studies to refine dosage

recommendations under different SBM quality scenarios to maximize benefits while minimizing costs.

3. Feeding Strategies: Consider incorporating exogenous protease primarily in diets containing SBM of lower quality, where its impact on nutrient digestibility and broiler performance is most beneficial. This targeted approach can optimize feed formulation and improve overall profitability in poultry production.
4. Further Research: Continue exploring the interactions between SBM quality, exogenous enzymes, and broiler performance to deepen understanding and refine feeding strategies. Investigate additional parameters such as carcass quality, immune response, and long-term effects on gut health to comprehensively assess the benefits.

CHAPTER 7

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