Fermentation and germination of pearl millet and cowpea

– a review of nutritional quality, antinutritional compounds and functional properties

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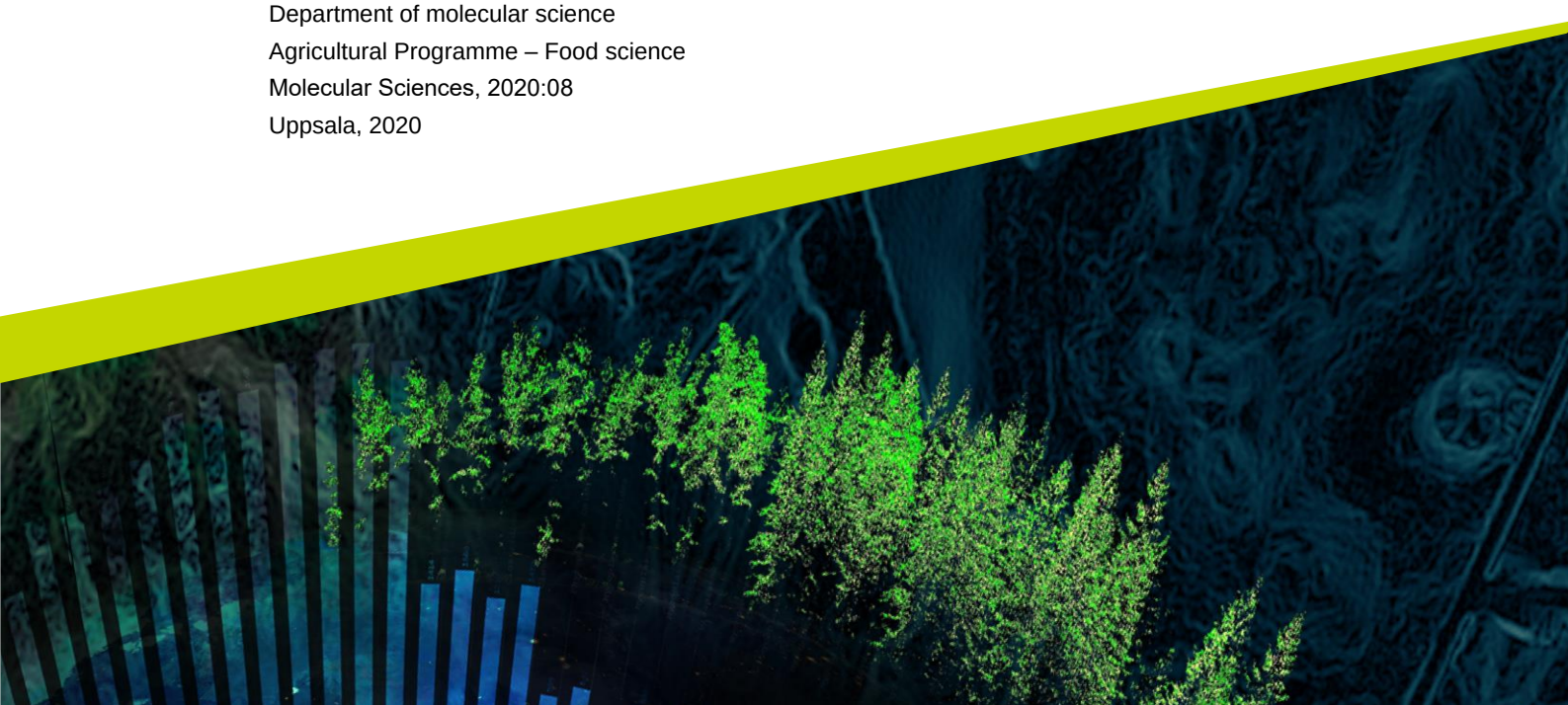

Fermentation and germination of pearl millet and cowpea - a review of nutritional qualities, antinutritional compounds and functional properties

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Abstract

Undernutrition is responsible for 45% of deaths of children under the age of 5 in low- and middle-income countries, where pearl millet (*Pennisetum glaucum*) is one of the staple cereals. Despite of pearl millets favorable chemical composition, challenges still lie in the utilization of them due to the presence of antinutritional compounds (ANCs). Furthermore, combining pearl millet with a legume, such as cowpea, can improve the nutritional composition in a foodstuff. However, ANCs are also prevalent in legumes. The main ANCs in pearl millet and legumes are phytate and polyphenols. Furthermore, legumes also contain flatulence producing oligosaccharides. The aim of this study is to investigate the effect of fermentation and germination, by a literature review, on nutritional qualities, functional properties and ANCs in pearl millet and cowpea to then further be able to assess their qualities as components of weaning foods. In general, fermentation has shown a decrease in the carbohydrate content, an increase in protein content and contradictory results in the fat content of pearl millet. Fermentation of cowpea generally showed a decrease in carbohydrate content, no significant changes in the protein content and contradictory results in the fat content. Germination generally showed a decrease in carbohydrate content, an increase in the protein content and a decrease in the fat content of pearl millet and cowpea. Overall, a reduction in the ANC content (phytic acid, polyphenols and raffinose family oligosaccharides) was shown after fermentation and germination. Germination and fermentation resulted in decreases in bulk density, increase in oil absorption capacity (OAC) and increase in water absorption capacity (WAC) of the millet samples. Furthermore, viscosity was decreased by germination and increased after fermentation of the millet samples. The evaluated results showed positive changes in the chemical composition, ANC content and functional properties with the intention of producing weaning food. Further studies are needed to optimize the processing techniques, evaluate the combinatory effect of fermentation and germination as well as investigating the ideal ratio of pearl millet and cowpea in weaning foods.

Keywords: pearl millet, cowpea, fermentation, germination, chemical composition, antinutritional compounds, functional properties

Table of contents

List of tables

Abbreviations

ANC	Antinutritional compound
B. subtilis	*Bacillus subtilis*
BU	Brabender units
C. perfringens	*Clostridium perfringens*
GOS	Galacto-oligosaccharides
IVPD	In vitro protein digestibility
L. brevis	*Lactobacillus brevis*
L. fermentum	*Lactobacillus fermentum*
L. plantarum	*Lactobacillus plantarum*
P. glaucum	*Pennisetum glaucum*
P. typhoideum	*Pennisetum typhoideum*
R. microspores	*Rhizopus microspores*
RFO	Raffinose family oligosaccharide
RVU	Rapid viscosity units
S. cerevisae	*Saccharomyces cerevisae*
S. diastaticus	*Saccharomyces diastaticus*
TPC	Total phenolic compounds
V. unguiculate	*Vigna unguiculate*
R. oligosporus	*Rhizopus oligosporus*
W. Beninensis	*Weissella Beninensis*

1. Introduction

1.1. Background

According to the World Health Organization (WHO) approximately 45% of deaths of children under the age of five in low and middle-income countries are caused by or related to undernutrition (World Health Organisation 2020). There are two forms of under nutrition which are protein-energy malnutrition and micronutrient deficiency. Protein-energy malnutrition constitutes a deficiency in proteins, carbohydrates and fats, whereas, micronutrient deficiency is a lack of micronutrients such as minerals or vitamins (Müller & Krawinkel 2005). Cereal based diets (wheat, rice, millet, etc.) are prevalent in many countries in Africa and Asia. A staple cereal in certain low-income regions, such as Africa and India, is pearl millet. Pearl millet, due to its nutrient profile with high protein, iron and zinc levels, could be beneficial in helping to tackle the problems of undernutrition.

However, pearl millet, as most cereals, is limited in essential amino acids, such as lysine (Dias-Martins *et al.* 2018), thus making their protein quality poorer. To enhance the amino acid profile, pearl millet can be combined with legumes (cowpea, pigeon pea, soybean etc.), creating an improved nutritional quality (Anitha *et al.* 2020). The bioavailability of these nutrients are inhibited by naturally occurring antinutritional compounds present in the pearl millet and legumes (Krishnan & Meera 2018). The most commonly discussed antinutritional compounds in pearl millet and legumes are phytate, phenolic compounds, tannins and enzyme inhibitors (Rani *et al.* 2018). Furthermore, legumes also contain flatulence producing oligo-saccharides (Ibrahim *et al.* 2002). To enhance the nutritional quality and decrease the antinutritional factors, processing of pearl millet and legumes has been suggested in scientific literature and implemented to some extent in product manufacturing. Therefore, to further advance the knowledge on nutritional profile of pearl millet and legumes as well as develop processing techniques to remove the problems of antinutritional compounds present in pearl millet and legumes, there is a need for evaluation of the currently available studies. Two commonly used processing techniques, fermentation and germination, are

reviewed in detail with focus on pearl millet and legumes, more specifically cowpea, to limit the extent of this review, with emphasis on the changes in macronutrients and ANCs. The ANCs discussed in this review are phytic acid, polyphenols and tannins regarding pearl millet as well as cowpea. Furthermore, the effect of processing on flatulence inducing oligosaccharides in cowpea is also discussed in this review. The fermentation process entails different changes in the composition of the substrate due to the activity of the present fermenting microorganisms and the enzymes produced by them during the process (Nkhata *et al.* 2018). Germination is a natural occurring development phase of grains and is used as a process where activation of endogenous enzymes is utilized by their ability to change the composition and enhance nutritional properties (Obadina *et al.* 2017).

1.2. Aim

The aim of this study is to conduct a comprehensive review of scientific articles on the subject of the effect of fermentation and germination on nutritional qualities functional properties and antinutritional compounds in pearl millet and cowpea. It has been suggested in scientific literature that pearl millet and cowpea can be complementary components in weaning food produced in Africa. Therefore, there is a need for evaluating the effect of simple and affordable processing methods on these crops.

1.3. Method

Literature for this project has been obtained through the use of different databases such as Primo, Web of Science and Google scholar. Review articles has mainly been used in the reports first part (2. Proximate composition and antinutritional compounds in pearl millet and cowpea.) to assist in getting a comprehensive overview on the subject. Whereas, research studies were more prioritized in the reports second and third parts (3. Fermentation and 4. Germination) for comparisons of results. More recent literature has been prioritized when possible. However, sometimes older literature has been used due to the lack of new studies on the subject.

Search words that has been used in different combinations are: "*Pearl millet"*, "*Pennisetum glaucum"*, *Ferment**, *Germinate**, "*Phytic acid"*, "*Phenolic compounds"*, *Tannins*, "*Antinutritional factors"*, *Nutrition*, *Composition*, "*Functional properties"*, "*Weaning foods"*, *Processing*, *Cowpea*, *"Vigna unguiculata"*, *Oligosaccharides*, *Flatulence*, "*Proximate composition"*

2. Proximate composition and antinutritional compounds in pearl millet and cowpea

2.1. Pearl Millet

Pearl Millet (*Pennisetum glaucum*) is a widely cultivated crop in countries with low rainfall and poor soils due to its short life cycle and deep root system (Jukanti *et al.* 2016). The cereal is counted to be the sixth most cultivated crop in the world and is mainly found in Asia and Africa (Boncompagni *et al.* 2018). Pearl millet is an annual crop which produce panicles that can generate between 500 – 2000 seeds per panicle. The seed have the shape of pearls hence the name of the plant (Dias-Martins *et al.* 2018). Pearl millet is, compared with other cereals, one of the cheapest food sources based on nutritional values. The nutritional quality together with the ability to grow in arid areas makes it an important staple food in countries where it is grown (Passot *et al.* 2016). Besides being an important food source, pearl millet is also used as animal feed, for ethanol production and in biofuel production (Jaiswal *et al.* 2018). The total production quantity of millet in the world was in 2018 approximately 31 million tons, whereas about 15.9 million tons was produced in Africa and 14.4 million tons in Asia (FAO 2019). The average yield of pearl millet in Africa is low compared to other crops due to lack of agricultural developments. A pervading poverty among farmers cultivating millet together with environmental factors makes it hard to raise the yield. Farmers not able to establish improvements together with a low budget regarding pearl millet breeding makes pearl millet one of the least developed crops compared to other crops such as wheat and maize. A yield increase has been seen in Asia where improved cultivars such as hybrids has been successfully adopted by both farmers and seed production companies. African countries have poor financing regarding seed production from the public sector, therefore, a market for improved cultivars has been hard to establish (FAO & ICRISAT 1996). Another factor that challenges the yield increase in Africa is the climate changes where the driest production areas are predicted to become even drier (Passot *et al.* 2016). According to FAO, the average yield in

2018 of millet in Africa was 7180 hg/ha and in Asia 13108 hg/ha which even now shows a difference in efficiency between production sites (FAO 2019).

2.1.1. Chemical composition and nutrient profile of pearl millet

The nutritional quality of Pearl millet can vary between plants due to growing site, genotype, soil, water accessibility and other growth limiting factors (Dias-Martins *et al.* 2018). Seen in table 1, the content of carbohydrates in two varieties, Ex-borno and HHB-67 is 69.44 % and 71.5 % respectively (Obadina *et al.* 2016; Siroha *et al.* 2016). A protein content between 8.38 % and 12.7 % has been seen in three different varieties (Ex-borno, HHB-67, HC-4) of pearl millet. Similar results in the protein content were observed in two separate reports by Chowdhury & Punia (1997) and Siroha *et al* (2016) both studying the variety HHB-67. A content of 10.3 % and 9.9 % was detected (ibid). The fat content ranged from 5.6-7.05 % (Chowdhury & Punia 1997; Obadina *et al.* 2016; Siroha *et al.* 2016). However, the variety HHB-67 resulted in different fat content in studies by Chowdhury & Punia (1997) and Siroha *et al* (2016), 5.6 % and 6.6 % respectively.

The amount of phytic acid was measured to be 786.2 mg/100 g in a composite population III variety (*Elyas et al*. 2002) and 1050 mg/100 g in a ugandi variety (Ahmed *et al.* 2010) (Table 1). The content of phenolic compounds was observed to be 764.45 mg/100 g in a HBB-50 variety, although a content of 347.26 mg/100 g was seen in a variety by the name Shambat (Archana *et al.* 1998; Abdelrahaman *et al.* 2007).

Table 1. Chemical composition and nutrient profile in pearl millet

Component	Content	Variety	Country of origin	References
Carbohydrates (% of dry matter)	69.44± 4.23	Ex-borno	Nigeria	(Obadina *et al.* 2016)
	71.5±0.29	HHB-67	India	(Siroha *et al.* 2016)
Protein (% of dry matter)	8.38± 0.33	Ex-borno	Nigeria	(Obadina *et al.* 2016)
	10.3	HHB-67	India	(Chowdhury & Punia 1997)
	12.7	HC-4	India	(Chowdhury & Punia 1997)
	9.9±0.30	HHB-67	India	(Siroha *et al.* 2016)
Fat (% of dry matter)	7.05 ± 0.67	Ex-borno	Nigeria	(Obadina *et al.* 2016)
	5.6	HHB-67	India	(Chowdhury & Punia 1997)
	5.9	HC-4	India	(Chowdhury & Punia 1997)
	6.6±0.21	HHB-67	India	(Siroha *et al.* 2016)
Phytic acid (mg/100g)	786.2±0.00	Composite Population III	Sudan	(Elyas *et al.* 2002)
	1050±15.0	Ugandi	Sudan	Ahmed *et al.* 2010)
Phenolic compounds (mg/100g)	764.45±0.94	HHB-50	India	(Archana *et al.* 1998)
	347.26±1.70	Shambat	Sudan	(Abdelrahaman *et al.* 2007)

2.2. Cowpea

Cowpea (*Vigna unguiculata*) is an annual plant mainly cultivated in regions with a dryer climate such as Africa, south Asia and Latin America. It is also known as black eye pea due to its appearance. The cowpea is a legume in the Fabacea family with the majority of production taking place in the sub-Saharan Africa, mainly West and Central Africa (Boukar et al. 2019). The total production of cowpeas (dry) in 2018 was about 7.2 million metric tons with approximately 6.1 million metric tons

having been produced in West Africa (FAO 2019). Cultivation of cowpea has shown both economic and environmental advantages because it can be grown in semi-arid regions, moreover, the required input for the cultivation is low. Furthermore, the entire plant, including leaves, green pods, green beans and mature beans, is utilized for different purposes (Gonçalves *et al.* 2016).

2.2.1. Chemical composition and nutrient profile of cowpea

The proximate composition and content of certain antinutritional compounds of different cowpea varieties has been analysed by several researchers (table 2). The percentage of carbohydrates in four cowpea varieties (Dan Borno, Kannonado, CS-88 and Cherodhi) ranged from 59.14 to 63.30 %, protein content was measured from 19.84 to 22.66 % and fat content ranged from 1.86 to 3.77 % (Punia 2000; Owolabi *et al.* 2012). The amount polyphenols in CS-88 and Cherodhi was measured at 809.45 and 866.48 mg/100 g respectively in a study by Punia (2000). Phytic acid content in the CS-88 variety was 927.45 mg/100 g, whereas, Cherodhi contained 940.15 mg/100 g (ibid).

The content of certain flatulence producing oligosaccharides (verbascose, sucrose, raffinose and stachyose) in cowpea has been studied by several researchers (table 2). Verbascose content in two undefined varieties ranged from 1.04 to 1.24 g/100 g (Tresina & Mohan 2011; Kalpanadevi & Mohan 2013). Raffinose content was studied in three varieties of cowpea, IT 84S-2246-4, VITA-4 and one undefined, with observed values ranging from 0.51 to 2.34 g/100 g (Nwinuka *et al.*; Egounlety & Aworh 2003; Tresina & Mohan 2011). Stachyose was also studied in the previously mentioned varieties with measured values ranging from 1.72 to 3.41 g/100 g (ibid).

Table 2. Chemical composition and nutrient profile of cowpea

Component	Content	Variety	Country of origin	References
Carbohydrates (% of dry matter)	60.06±1.05	Dan Borno	Nigeria	(Owolabi *et al.* 2012)
	63.30±0.33	Kannanado	Nigeria	(Owolabi *et al.* 2012)
	59.14±0.25	CS-88	India	(Punia 2000)
	61.32±0.10	Cherodhi	India	(Punia 2000)
Protein (% of dry matter)	22.13±0.41	Dan Borno	Nigeria	(Owolabi *et al.* 2012)
	19.84±0.18	Kannanado	Nigeria	(Owolabi *et al.* 2012)
	20.07±0.12	CS-88	India	(Punia 2000)
	22.66±1.06	Cherodhi	India	(Punia 2000)
Fat (% of dry matter)	3.77±0.32	Dan Borno	Nigeria	(Owolabi *et al.* 2012)
	3.46±0.05	Kannanado	Nigeria	(Owolabi *et al.* 2012)
	1.96±0.05	CS-88	India	(Punia 2000)
	1.86±0.05	Cherodhi	India	(Punia 2000)
Polyphenols (mg/100g)	809.45±1.57	CS-88	India	(Punia 2000)
	866.48±3.73	Cherodhi	India	(Punia 2000)
Phytic Acid (mg/100g)	927.85±2.27	CS-88	India	(Punia 2000)
	940.15±4.63	Cherodhi	India	(Punia 2000)
Verbascose (% of dry matter)	1.04±0.10	Not mentioned	India	(Tresina & Mohan 2011)
	1.24±0.06	Not mentioned	India	(Kalpanadevi & Mohan 2013)
Raffinose (% of dry matter)	1.22±0.02	IT 84S-2246-4	Nigeria	(Egounlety & Aworh 2003)
	0.51±0.04	Not mentioned	India	(Tresina & Mohan 2011)
	2.34±0.03	VITA-4	Nigeria	(Nwinuka *et al.1997*)
Stachyose (% of dry matter)	3.41±0.08	IT 84S-2246-4	Nigeria	(Egounlety & Aworh 2003)
	1.72±0.18	Not mentioned	India	(Tresina & Mohan 2011)
	3.04±0.04	VITA-4	Nigeria	(Nwinuka *et al.1997*)

2.3. Antinutritional Factors

2.3.1. Phytic Acid

Phytic acid is a storage form of phosphorus in plant tissue and is mainly found in legumes, cereals, nuts and oil seeds. The salt form of the compound is called phytate and due to its negatively charged phosphate groups at physiological pH (6-7) phytate can form complexes with minerals such as zinc, iron, calcium and magnesium. The chelating activity creates insoluble compounds and prevents gastrointestinal absorption of the minerals (Emanuelli *et al.* 2014). At a pH over 7, proteins can bind to the mineral in the mineral-phytate complex and create a ternary complex. If the pH is 5 or lower, proteins can bind directly to the negatively charged phytate molecule and create protein-phytate complex. These complexes can be formed in the gastrointestinal tract where hydrolyzation of the bound protein is obstructed which lowers the bioavailability of the proteins (Kies *et al.* 2006). Phytase is an enzyme able to degrade phytic acid and by this release inorganic phosphate from the molecule. The enzyme is found naturally in plants as an endogenous enzyme and by the release of inorganic phosphate stimulating germination and plant growth. Phytase also occur as an exogenous microbial enzyme (Ou *et al.* 2011).

2.3.2. Phenolic compounds

Polyphenolics, a second metabolite produced by plants, are a group of compounds consisting of one or more hydroxyl groups attached to one or several aromatic rings. Flavonoids, phenolic acids and tannins are example of subgroups to phenolic compounds. Tannins are compounds able to form strong complexes with macromolecules such as proteins and are classified as polymeric phenols created by condensation of flavanols (Eyzaguirre *et al.* 2006).

Phenols can inhibit intestinal absorption of dietary metal ions such as iron and zinc by complex formation. Protein and starch digestibility can also be affected by polyphenols by interfering with amylolytic and proteolytic enzymes (Abdelrahaman *et al.* 2007). Due to inhibiting the bio-accessibility of nutrients in cereals and legumes, phenolic compounds are often seen as antinutrients. However, phenolic compounds hold health promoting capacity (Adebiyi *et al.* 2017) such as antioxidant activity (Chandrasekara & Shahidi 2010).

2.3.3. Flatulence producing oligosaccharides

Galactose-oligosaccharides (GOS) are carbohydrates undigestible by human due to the lack of enzymes capable to hydrolyse the oligosaccharides. Undigested

carbohydrates that enters the colon undergo anaerobic fermentation by microorganisms, which results in gas formation. The flatulence inducing properties limits the acceptance of legumes, nonetheless, the flatulence producing process is harmless and health benefits from undigestible carbohydrates has been reported (Madodé *et al.* 2013). Verbascose, stachyose and raffinose are oligosaccharides from the raffinose family (RFOs), which are synthesized by combining galactose molecules with sucrose molecules. RFOs is suggested to act as osmoprotectants in seeds due to their accumulation during seed development and dehydration (Nishizawa *et al.* 2008).

2.4. Functional properties

Bulk density is a measurement of the total mass of a sample in relation to the total volume that is taken up by the same sample, where air and water is included. It is dependent on the container in which it is measured, therefore, it is not an intrinsic property (Qiu *et al.* 2015). Viscosity has been defined as the flow of a liquid in relation to the force that is applied to the liquid (Dikeman & Jr 2006). Water absorption capacity (WAC) and oil absorption capacity (OAC) are measurements indicating a material's ability to hold water or oil. These physiochemical properties are important to consider when producing weaning foods in low-income areas where protein-energy malnutrition is prevalent in the infant population. Mainly since high bulk density and viscosity can lead to complications in meeting energy requirements when additional water is needed to acquire a thin enough consistency, imperative for the ability of infants to ingest the weaning foods (Kulkarni *et al.* 1991).

3. Processing methods

3.1. Fermentation

Fermentation is a processing method traditionally used to preserve different food stuffs, with evidence found of its use as early as 6000 BC (Liptáková *et al.* 2017). The process entails different changes in the composition of the substrate due to the activity of the present fermenting microorganisms and the enzymes produced by them during the process (Nkhata *et al.* 2018). Fermented foods can acquire an enhanced flavor profile, texture and aroma as well as an enhanced nutrient profile through removal of ANCs during the fermentation and formation of biopeptides and proteins, essential fatty acids and vitamins. There will also be an added preservative effect by lactic acid, acetic acid, alcohol and alkaline fermentations (Kohajdová & Karovicová, 2007).

The effects of fermentation on nutritional and anti-nutritional factors in pearl millet was evaluated by several researchers and are shown in table 3 and 4 below.

3.1.1. Effect on nutritional qualities in pearl millet

Carbohydrates

Studies on fermentation of pearl millet have reported both significant decreases in the carbohydrate content of pearl millet during fermentation as well as significant increases, as shown in table 3. A study conducted by Akinola *et al* (2017) showed a decrease in the proximate composition of carbohydrates in the pearl millet from 75.75 to 73.76% after 72 hours of fermentation of pearl millet grains. Akinola *et al* (2017) discusses the fact that the reason for this decrease could be due to the microorganisms and enzymes using the sugars in the pearl millet for their metabolism during the fermentation. There have also been contradictory results on carbohydrate content in fermented pearl millet published. Adebiyi *et al* (2017) showed an increase in the carbohydrate content of pearl millet from 78.56 to 81.01% after 72 hours of fermentation of pearl millet grains. There are no clear differences in the method of determination or in the fermentation of the pearl millet

grains in the study conducted by Akinola *et al* (2017) and Adebiyi *et al* (2017) that could explain the differences in results. Furthermore, an explanation was lacking in the study by Adebiyi *et al* (2017) as to why this contradictory result were obtained.

In a study conducted by Osman (2011) looking at the fermentation of pearl millet flour with a 1:2 millet to water ratio the carbohydrate content was reduced from 72.63 to 70.97% after 24 hours of fermentation, with fluctuating levels of carbohydrate contents throughout the 24 hours. The fluctuating levels of carbohydrates during the fermentation have been attributed to the enzymatic activity of α- and ß-amylase from the fermenting organisms, which is high initially, although, the activity will be limited by low pH potentially causing an increase in carbohydrate content during the later stages of the fermentation when the pH is lowered (Osman 2011).

In a study observing the activity of different fermenting organisms (*Saccharomyces cerevisae*, *Saccharomyces diastaticus*, *Lactobacillus brevis* and *Lactobacillus fermentum*) on the carbohydrate content of pearl millet flour by Khetarpaul and Chauhan (1990) showed that all organisms used the starch as a food. However, there was a larger decrease in the starch content when the fermenting organism was a yeast. Khetarpaul and Chauhan (1990) also discussed that the increase of temperature combined with a moist environment might break down the starch, releasing soluble sugars, which initially increases the amount of soluble sugars. These soluble sugars could then be consumed by the fermenting organisms if the fermentation is continued leading to a lower level of soluble sugar in the fermented food (ibid).

Proteins

Several studies have shown an increase, of varying levels, in the proximate protein composition in pearl millet after fermentation, as shown in table 3 below. According to Akinola *et al* (2017) there was a significant increase in the protein content during spontaneous fermentation of pearl millet grains for 72 hours, more specifically from 10.99 to 13.65% of the dry weight. In a study of the fermentation of pearl millet performed by Adebiyi *et al* (2017), with a similar spontaneous fermentation of pearl millet grains for 72 hours, showed similar results with an increase in the protein content from 5.47 to 5.80% of the dry weight. The increase in the protein content could be attributed to the amino acids produced as well as the accumulation of proteins during the fermentation according to Adebiyi *et al* (2017).

In a study conducted by Osman (2011) the results reported showed no significant increase in the protein content of pearl millet during the fermentation (table 3). Although, this study was performed with a method differing quite a bit from the previously discussed since the fermentation time was 24 hours, therefore, significantly shorter, as well as fermenting pearl millet flour instead of pearl millet grains. This could explain the difference in the results compared to the studies on

pearl millet grains. Ali *et al* (2003) also studied the effects of fermentation on protein content of pearl millet flour from two pearl millet cultivars (*Madelkawya* and *Population 1/*Shambat) with similar results as the study by Osman (2011), showing no significant changes. In this study pearl millet flour with water (1:2 ratio) was fermented for 14 hours in room temperature (30±2 degrees Celsius) using previously fermented dough as a starter. Fermenting flour with water (1:2 weight ratio) showed no significant changes in protein content, whereas, both methods described by (Adebiyi *et al.* 2017; Akinola *et al.* 2017) discussed that fermented whole grains showed a significant increase in protein content (table 3). This unique feature may be possible only due to the different form or matrix of pearl millet used for fermentation.

Furthermore, the study by Ali *et al* (2003) also focused on the changes in the *in vitro* protein digestibility (IVPD) of the two pearl millet cultivars during fermentation. The results reported showed that the IVPD increased during fermentation of both samples. From 69.0 to 77.5% in the *Madelkawya* cultivar and from 76.9 to 86.8% in the *Population 1/*Shambat cultivar (Ali *et al.* 2003). Similar results have been reported by Elyas *et al* (2002) while studying the IVPD of the pearl millet cultivars composite pop III and baladi, with increases from 60.5 to 86% and 61.9 to 86.2% respectively. According to the authors, proteolytic enzymes produced by the fermenting organisms as well as the decrease of phytic acid could be contributing factors to the increase in IVPD of pearl millet after fermentation (ibid). Therefore, it might indicate that even though there was no significant increase in the protein content there will still be a nutritional benefit in fermenting pearl millet flour since it increases the IVPD.

Fats

There have been contradictory results reported from several studies on the effect of fermentation on the fat content in pearl millet, as depicted in table 3 below. Akinola *et al* (2017) reported an increase in the proximate composition of fat after 72 hours of spontaneous fermentation of pearl millet grains from 1.83 to 3.71%. Whereas, Adebiyi *et al* (2017) reported a decrease in the fat content from 2.25 to 1.70% in a study conducted with similar conditions of spontaneous fermentation, 72 hours in the form of pearl millet grains. According to Adebiyi *et al* (2017) the decrease in the fat content could be caused because lipids are being degraded during the fermentation process. As mentioned earlier, the differentiating points in the methods conducted by Akinola *et al* (2017) and Adebiyi *et al* (2017) are the weight to water ratio, 1:3 and 1:4 respectively, and the times and temperature of the drying, 50 degrees Celsius for four hours compared to 40 degrees Celsius for 24 hours. In the study by Osman (2011) no significant changes was shown in the fat content of fermented pearl millet flour. There were no explanations offered as to why no changes were observed in the fat content. However, Osman (2011) discussed the

need for further studies on the changes in the fat content of pearl millet due to its relatively high lipid content that could possibly affect the shelf life to a large extent.

Although, in a study on a different pearl millet species (*Pennisetum typhoideum*) Khetarpaul and Chauhan (1989) compared the effects of natural fermentation, where flour from untreated pearl millet grains were added to a mixture of autoclaved pearl millet flour, to the effects of single culture fermentation with *Saccharomyces cerevisae*, *Saccharomyces diastaticus*, *Lactobacillus brevis* and *Lactobacillus fermentum*. All of the fermentations were carried out for 72-hours. In the results Khetarpaul and Chauhan (1989) showed that the spontaneous fermentation significantly increased the amount of fat. Whereas the single culture fermentations, regardless of fermenting organism, either decreased or had no significant change on the fat content. According to the authors there are yeast strains that are fat producing, furthermore, their possible presence in the naturally fermented pearl millet flour could explain why the fat content increased in that sample. Even though this study is not conducted in the same manner as the previously discussed, it could perhaps still indicate that it is possible to get contradicting results of the effect of fermentation on fat content under similar conditions based on what fermenting organisms are available in the pearl millet.

Table 3. Nutritional qualities in pearl millet before and after natural fermentation

G/F	**Grain: Water (w/w)**	**Hours (h)**	**Temp (ºC)**	**Results (% of d.m)**						**References**
				Carbohydrate		Protein		Fat		
				Before	After	Before	After	Before	After	
G	1:3	72	RT	75.75±0.01	73.76±0.01	10.99±0.00	13.65±0.01	1.83±0.01	3.71±0.01	(Akinola *et al.* 2017)
G	1:4	72	28	78.56±1.00	81.01±1.34	5.47±0.06	5.80±0.09	2.25±0.35	1.70±0.28	(Adebiyi *et al.* 2017)
F	1:2	24	30	72.63±0.56	70.97±0.37	15.25±0.21 *	15.35±0.35 *	5.77±0.25 *	5.79±0.03 *	(Osman 2011)

Results are expressed as mean ± standard deviation
**insignificant change*
G/F – Grain/Flour, d.m – dry matter, RT – room temperature

3.1.2. Effect on antinutritional factors in pearl millet

Phytic acid

The studies shown in table 4 depicts a decrease in the phytic acid content, in varying levels, of the fermented pearl millet flour. In a study by Elyas *et al* (2002), where composite population III (a variety of *P. typhoidum*) flour was allowed to naturally ferment for 36 hours (results depicted in table are from 24 hours of fermentation) in water with a ratio of 1:2, there was a significant decrease of the phytic acid content. Elyas *et al* (2002) reported a decrease from 786 to 550 mg/100 g phytic acid in the fermented pearl millet flour. Similar findings have also been reported in studies by Osman (2011) and Eyzaguirre *et al* (2006), with reductions in the phytic acid content from 647 to 310 mg/100 g and 383 to 105 mg/100 g respectively. These studies were conducted in a similar way as the one by Elyas *et al* (2002), with the exceptions of using *Pennisetum glaucum* as well as adding starter from a previous fermentation in the beginning of the fermentation process. Furthermore, Eyzaguirre *et al* (2006) used a variety called full form IKMP-5, which is known for its low phytic acid and polyphenolic content. Therefore, the relatively low numbers of phytic acid before fermentation in that study was to be expected. According to the authors of the discussed studies, the decrease can be attributed to both endogenous phytase of the pearl millet as well as microbial phytase of the fermenting organisms (Elyas *et al.* 2002; Eyzaguirre *et al.* 2006; Osman 2011).

Studies by Ahmed *et al* (2010) and El Hag *et al* (2002), where the Ugandi variety of pearl millet was fermented for 14 hours with the addition of a starter in the beginning of the fermentation process, also showed a decrease in the phytic acid content after fermentation. Ahmed *et al* (2010) showed a decrease from 1050 to 887 mg/100 g after fermentation at 37 degrees Celsius for pearl millet grains kept in water (1:2 ratio of millet:water). The study by El Hag *et al* (2002) reported a decrease in the phytic acid content from 1076 to 550 mg/100 g after fermentation at 30 degrees Celsius for pearl millet grains kept in water (1:1 ratio of millet: water). The decrease in the phytic acid content could be connected to the phytase activity of microbial phytase as well as phytase already present in the pearl millet (El Hag *et al.* 2002; Ahmed *et al.* 2010), as the three previously mentioned studies also discussed. Furthermore, El Hag *et al* (2002) also mentions the possibility of the contributing element of the lowered pH during the fermentation, which is beneficial for the phytase activity.

Phenolic compounds

There have been contradictory results reported regarding the effect of fermentation on the amount of phenolic compounds in pearl millet. Elyas *et al* (2002) reported a

decrease in the polyphenol content of the composite population III variety of pearl millet flour from 318 to 196 mg/100 g after 24 hours of fermentation. The decrease of the polyphenolic content could be attributed to the effect of the activated enzyme polyphenol oxidase according to Elyas *et al* (2002). A study conducted by Eyzaguirre *et al* (2006) showed a contradictory effect on the polyphenolic content after fermentation in the IKMP-5 variety of pearl millet, where an increase from 2 to 3.93 OH-equivalents occurred. The difference in results, despite the similarity in fermentation conditions, in the two discussed studies could possibly be attributed to the different varieties in pearl millets as well as the difference in method of determination. Polyphenol content was determined by the Pression Blue spectrophotometric method in the study by Elyas *et al* (2002), whereas the Folin-Ciocalteu assay was used by Eyzaguirre *et al* (2006).

The reports by Ahmed *et al* (2010) and El Hag *et al* (2002) regarding the 14-hour fermentation of the Ugandi variety of pearl millet flour both showed a significant decrease in the polyphenolic content. Ahmed *et al* (2010) reported a decrease from 120 to 111 mg/100 g and El Hag *et al* (2002) from 444 to 306 mg/100 g. Ahmed *et al* (2010) discusses the possibility of leaching of polyphenols in the water during the acidic conditions of the fermentation. Whereas, El Hag *et al* (2002) postulates that the decrease could be attributed to the activity of the microorganisms during the fermentation. Although both studies report a significant decrease there is still quite a discrepancy in the size of the decrease even though the studies are performed under similar conditions with the same variety of pearl millet. However, the methods of determination were not performed in the same way, Ahmed *et al* (2010) used the Prussian Blue spectrophotometric method as opposed to El Hag *et al* (2002) that used the Folin-Denis method. This difference could possibly contribute to the explanation of the discrepancy.

Table 4. Antinutritional compounds in pearl millet before and after natural fermentation

G/F	Grain: Water (w/w)	Time (h)	Temp (ºC)	V	Results				References
					Phytic acid (mg/100 g)		Polyphenols (mg/100 g)		
					Before	After	Before	After	
F		24	30±2	Composite Population III	786.2±0.00	550.3±0.00	318.6±0.04	196.1±0.09	(Elyas *et al.* 2002)
F	1:2	24	30		647.00±27.01	310.95±7.00			(Osman 2011)
F		24	30	IKMP-5	383±45	105±19	2.01±0.17 (OH-eq)	3.93±0.25 (OH-eq)	(Eyzaguirre *et al.* 2006)
F		14	37	Ugandi	1050±15.0	887±7.0	120.43±4.07	111.08±2.54	(Ahmed *et al.* 2010)
F		14	30±2	Ugandi	1076±0.16	580±0.06	444±0.05	306±0.02	(El Hag *et al.* 2002)

Results are expressed as mean ± standard deviation
Blank space indicates not specified
G/F – Grain/Flour, V -variety, d.m – dry matter, RT – room temperature, oh-eq – OH equivalents (1 OH eq = 170 mg OH kg^{-1} d.m)

3.1.3. Effect on functional properties in pearl millet

Bulk density

The effects of fermentation on functional properties in pearl millet were evaluated by several researchers (table 5). In all of the studies, reviewed in table 5, pearl millet grains were fermented for 72 hours before grinding to a flour. Several pf the scientific studies showed that bulk density of the pearl millet flour decreases after fermentation. Akinola *et al* (2017) showed a significant decrease from 1.12 to 0.87 g/ml. The study by Adebiyi *et al* (2016) showed a nonsignificant decrease from 0.78 to 0.76 g/cm^3 and the report by Rathore and Singh (2018) showed a decrease from 0.61 to 0.56 g/ml, where no statistical analysis was conducted. According to Adebiyi *et al* (2016) the decrease could be due to the degradation of for instance carbohydrates and proteins to smaller molecules. Which, according to the authors will produce flours that are not as bulky, therefore, yielding a more nutrient dense flour (ibid).

Oil absorption capacity

The effect of fermentation of pearl millet grains on the oil absorption capacity (OAC) of the resulting pearl millet flour is presented in table 5, with all studies showing an increase in the OAC. Sade (2009) reported an increase from 150 to 166 %. The research conducted by Adebiyi *et al* (2016) showed an increase from 120 to 124 %, moreover, Rathore and Singh (2018) observed an increase from 161 to 175 %. According to Adebiyi *et al* (2016) the main contributing factors of OAC is the possibility of bonding between lipids and the nonpolar protein chain as well as trapping the lipids physically. Furthermore, Adebiyi *et al* (2016) discusses the possibility of higher amounts of nonpolar amino acids in the pearl millet flour after fermentation. This phenomenon would lead to a more lipophilic flour, hence, a higher OAC (ibid).

Water absorption capacity

The water absorption capacity (WAC) was shown to increase in three out of the four reviewed studies in table 5, with an insignificant decrease shown in the fourth. Sade (2009) reported an increase from 226 to 260 %, Adebiyi *et al* (2016) reported an increase from 1.32 to 1.66 g/g and Rathore and Singh (2018) reported an increase from 235 to 277 %. Adebiyi *et al* (2016) reasons that the increased WAC after fermentation of the samples reflects the breakdown of larger starch molecules to smaller constituents. Moreover, a larger amount of soluble sugars and proteins also contributes to the increase in the WAC (ibid). Akinola *et al* (2017)

reported a contradictory result in their study, with an insignificant decrease in WAC from 359 to 356 %.

Viscosity

The viscosity of pearl millet flour after fermentation of the pearl millet grains was evaluated by Sade (2009) and Akinola *et al* (2017), with an increase reported in both studies. In the study conducted by Sade (2009), an increase from 91 to 129 rapid viscosity units (RVU) was observed. Moreover, the research by Akinola *et al* (2017) reported an increase from 68 to 92 brabender units (BU). Akinola *et al* (2017) discusses the fact that the different pasting properties are in relation with the starch content of the flour used, showing that fermentation has had an effect on the starch content of the pearl millet. The viscosity as a measurement of stability of the starch granule is discussed by Sade (2009), mentioning that the increased viscosity of the fermented pearl millet flour will lead to a more stable flour when put through certain processing conditions.

Table 5. Functional properties in pearl millet before and after natural fermentation

G/F	Grain: Water (w/w)	Time (h)	Temp (ºC)	Results								References
				Bulk density (gml^{-1})		OAC (%)		WAC (%)		Viscosity (RVU, Bu)		
				Before	After	Before	After	Before	After	Before	After	
G		72				150±0.06	166.7±0.07	226±0.03	260±0.06	91.50 (RVU)	129.00 (RVU)	(Sade 2009)
G	1:3	72	RT	1.12±0.02	0.87±0.01			359.33±1.45*	356.67±0.33*	68.11±0.02 (Bu)	92.02±0.01 (Bu)	(Akinola *et al.* 2017)
G	1:4	72	28	0.78±0.01*	0.76±0.01*	1.20±0.02 (g/g)	1.24±0.02 (g/g)	1.32±0.02 (g/g)	1.66±0.16 (g/g)			(Adebiyi *et al.* 2016)
G		72	30	0.61	0.56	161±0.06	175.9±0.06	235±0.06	277±0.06			(Rathore & Singh 2018)

Results are expressed as mean ± standard deviation
**insignificant change*
Blank space indicates not specified/results not available
G/F – Grain/Flour, d.m – dry matter, RT – room temperature, Bu – Brabender units, RVU – Rapid Visco units, OAC – oil absorption capacity, WAC – water absorption capacity

3.1.4. Effect of fermentation on cowpea

Chemical composition

The effects of fermentation on the chemical composition in cowpea has been evaluated by several researchers (table 6). A decrease in the carbohydrate content was observed after natural fermentation of a cowpea slurry in studies by Akpapunam and Achinewu (1985) and Giami (1993), from 62.7 to 60.9 % and from 59.9 to 57.6 % respectively. No significant changes was shown in the protein content of cowpea after fermentation in the studies conducted by Akpapunam and Achinewu (1985) and Giami (1993). Akpapunam and Achinewu (1985) observed an insignificant decrease from 21.7 to 21.6 %, whereas Giami (1993) showed an insignificant increase from 24.0 to 24. 6%. The fat content of cowpea after fermentation showed a significant increase, from 1.5 to 3.2 %, in the research by Akpapunam and Achinewu (1985). Giami (1993) showed an insignificant increase in the fat percentage from 1.7 to 1.9 %. There were no explanations offered in the discussed studies as to what caused the changes in the chemical compositions.

Furthermore, studies have reported positive impacts in the digestibility of nutrients in cowpea (Kiers *et al.* 2000; Madodé *et al.* 2013). In the study by Kiers *et al* (2000) fermentation of cowpea with the mould *Rhizopus oryzae* and *Rhisopus microsporus* var *oligopsorus* was implemented after soaking and cooking to investigate the effects on *in vitro* digestibility. The results indicated an enhanced digestibility of 3 % after fermentation with the moulds after processing. However, the authors observed an increase in absorbability (nutrients that are absorbable without the need for hydrolysis by enzymes), arguing that fermentation with moulds could possibly benefit the uptake of nutrients in the gastrointestinal impaired (ibid). Digestion of carbohydrates in cowpea, related to flatulence inducing oligosaccharides will be discussed further in the section below.

Table 6. Nutritional qualities in cowpea before and after natural fermentation

S/F	**Grain: Water (w/w)**	**Time (h)**	**Temp (ºC)**	**Results (% of d.m)**						**References**
				Carbohydrate		Protein		Fat		
				Before	After	Before	After	Before	After	
F	1:1.5	72	30	62.7±0.6	60.9±0.8	21.7±1.2*	21.6±1.3*	1.5±0.2	3.2±0.3	(Akpapunam & Achinewhu 1985)
F	1:4	72	RT	59.9±0.7	57.6±0.4	24.0±04*	24.6±0.3*	1.7±0.1*	1.9±0.1*	(Giami 1993)

Results are expressed as mean ± standard deviation
**insignificant change*
Blank space indicates not specified/results not available
S/F – Seed/Flour, d.m – dry matter, RT – room temperature

Antinutritional factors

The effect of fermentation on certain antinutritional compounds in cowpea has been reported by several researchers (table 7). Studies conducted by Akpapunam and Achinewhu (1985) as well as Ibrahim *et al* (2002) reported a decrease in the phytic acid content of cowpea after fermentation. Akpapunam and Achinewhu (1985) studied the effect of natural fermentation for 72 hours on a milled cowpea slurry (1:1.5, weight:water) and observed a decrease in phytic acid from 5.9 to 1.8 mg/g. According to the authors, the observed decrease could be attributed to the breakdown of phytic acid by the fermenting microorganisms. Furthermore, in their study, Akpapunam and Achinewhu (1985) also observed a significant increase in the total amount of phosphorus in the cowpea. In a study by Ibrahim *et al* (2002), researching the effect of fermentation by a specific fermenting organism (*Rhizopus oligosporus* and *Lactobacillus plantarum*) on cowpea grains of the Dokki 311 variety, a significant decrease in phytic acid was reported. Fermentation with *R. oligosporus* for 48 hours lead to a decrease in phytic acid from 4.54 to 2.79 g/100 g, whereas, inoculation with *L. plantarum* and a 36-hour fermentation period showed a decrease from 4.54 to 2.39 g/100 g. The authors postulates that the decrease could be related to phytase of the fermenting organisms (Ibrahim *et al.* 2002).

Giami (1993) observed the effects on polyphenols after natural fermentation for 72 hours in a cowpea slurry (1:4, grain to water) and showed a decrease from 0.45 to 0.25 g/100 g. An explanation as to what caused the decrease was lacking in the study. However, contradictory results have also been reported. Gan *et al* (2016) conducted a study observing the effects of natural fermentation as well as fermentation with two bacterial cultures (*Lactobacillus paracasei* and *L. plantarum*) for 48 hours on polyphenols (in soluble and bound fractions) and antioxidant capacity of eight edible legumes, including cowpea, milled to a flour (1:5, flour to water). Their study showed an increase in total phenolic compounds (TPC) in cowpea soluble and bound fractions after natural fermentation as well as bacterial fermentation. However, bacterial fermentation showed a larger increase in the soluble fractions, whereas, natural fermentation impacted the bound fraction to a larger degree. Furthermore, Gan *et al* (2016) also studied the effect on specific phenolic compounds in the cowpea, showing that some phenolic compounds were increased (e.g. catechin) whereas protocatechuic acid was decreased after natural fermentation. The authors argue that fermenting microorganism are able to transform or metabolize phenolic compounds in the cowpea, postulating that the increase in catechin could be the results from these processes on proanthocyanidins (Gan *et al.* 2016). Gan *et al* (2016) also discusses the possibility of relating the increase in soluble TPC to bound phenolic compounds being released during the fermentation period.

Several researchers have evaluated the effects of fermentation on RFOs in cowpea (table 8). Raffinose and stachyose was reduced to non-detectable levels after fermentation of cowpea beans with *R. oligosporus* and *L. plantarum* for 48 and 36 hours respectively, in a study by Ibrahim *et al* (2002). Adewumi and Odunfa (2009) observed decreases in raffinose- and stachyose-content after 72 hours of fermentation of a cowpea slurry with *L. plantarum.* Raffinose content was reduced from 0.796 to 0.41 %, whereas, stachyose content was reduced from 1.38 to 0.63 % (ibid). After 24 hours of natural fermentation of a cowpea slurry Akinyele and Akinlosotu (1991) showed a reduction in stachyose and verbascose, from 3.56 to 3.35 % and from 4.03 to 0.82 % respectively. However, an increase, contradictory to commonly reported results, was shown in raffinose from 1.95 to 2.2 % with no explanation offered as to what caused it in the study (ibid). It has been argued that the decrease in RFOs in cowpea during fermentation can be attributed to the α-galoctosidase activity of microorganisms in possession of this enzyme (Akinyele & Akinlosotu 1991; Ibrahim *et al.* 2002; Adewumi & Odunfa 2009). Furthermore, Madodé *et al* (2013) studied the effect of traditional processing (removal of hulls, boiling and soaking) and fermentation by *Weissella beninensis (W. beninensis*), *Bacillus subtilis* (*B. subtilis*), *Rhizopus microspores* (*R.* microspores) and *Saccharomyces cerevisiae* (*S. cerevisiae*) on *in-vitro* fermentability index (gas production by *Clostridium perfringens (C. perfringens*)) and *in-vivo* fermentability index (hydrogen concentration of the breath after ingesting a cowpea-meal). Showing lower *in-vivo* fermentability indexes after fermentation when compared to the traditional processes. Moreover, arguing that their study indicates that the most preferable techniques for reducing the flatulence inducing factors is a combination of soaking and fermentation (Madodé *et al.* 2013).

Table 7. Antinutritional compounds in cowpea before and after fermentation

S/F	Grain: Water (w/w)	Time (h)	Temp (ºC)	M.O	V	Results				References
						Phytic acid		Polyphenols		
						Before	After	Before	After	
F	1:1.5	72	30	N	-	5.9±0.09 (mg/g)	1.8±0.10 (mg/g)			(Akpapunam & Achinewhu 1985)
F	1:4	72	RT	N	-			0.45±0.03 (g/100g)	0.25±0.02 (g/100g)	(Giami 1993)
S	1:10	48	30	*R. oligos porus*	Dokki 311	4.54 (g/100g)	2.79 (g/100g)			(Ibrahim *et al.* 2002)
S	1:10	36	30	*L. planta rum*	Dokki 311	4.54 (g/100g)	2.39 (g/100g)			(Ibrahim *et al.* 2002)

Results are expressed as mean ± standard deviation
Blank space indicates not specified/results not available
S/F – Seed/Flour, M.O – microorganism, V -variety, d.m – dry matter, N – natural fermentation, RT – room temperature

Table 8. RFOs in cowpea before and after fermentation

S/F	Grain: Water (w/w)	Time (h)	Temp (ºC)	M.O	V	Results (% of d.m)						References
						Raffinose		Stachyose		Verbascose		
						Before	After	Before	After	Before	After	
S	1:10	48	30	*R. oligosporus*	Dokki 311	0.52	0.00	2.04	0.00			(Ibrahim *et al.* 2002)
S	1:10	36	30	*L. plantarum*	Dokki 311	0.52	0.00	2.04	0.00			(Ibrahim *et al.* 2002)
F	1:1.5	24	30	N		1.95±0.00	2.2±0.9	3.56±0.2	3.35±3.0	4.03±1.5	0.82±0.3	(Akinyele & Akinlosotu 1991)
F	1:1.2	72	33±1	*L. plantarum*	Drum	0.796±0.0001	0.41±0.002	1.38±0.004	0.63±0.003			(Adewumi & Odunfa 2009)

Results are expressed as mean ± standard deviation
Blank space indicates not specified/results not available
S/F – Seed/Flour, M.O – microorganism, V -variety, d.m – dry matter, N – natural fermentation, RT – room temperature, RFO – Raffinose family oligosaccharides

3.2. Germination

In the lifecycle of plants, germination is the first phase of development in seeds which is followed by growth of the plant. The initiation phase of germination starts with uptake of water and is dependent on favourable conditions such as the quality of soil, light, temperature, access to water as well as internal factors. When the seed is fully hydrated, the water uptake becomes limited and phase two is initiated where cellular activity and metabolic events take place. The completion of germination correlates to an increased water uptake which typifies phase three of germination. (Wolny *et al.* 2018). The metabolic activity is due to mobilization of stored macromolecules for the seed to utilize until sufficient development of the plants is attained and absorption of nutrition is possible (Bewley 1997). Controlled germination, also known as malting, is a process applied to utilize endogenous enzymes within the grains to enhance nutritional properties. Malting is usually performed by monitored soaking followed by a germination phase where the grains are placed on a sterile cloth and kept on a specific temperature and humidity to optimize the germination. Activation of hydrolytic enzymes breaking down macromolecules such as starch, creates a higher accessibility of nutrition in the grains (Obadina *et al.* 2017).

3.2.1. Effect on nutritional qualities in pearl millet

Carbohydrates

Seen in table 9, studies by Obadina *et al* (2017), Obizoba and Atii (1994) and Sade (2009) have shown a decrease in carbohydrates after germination of pearl millet grains. According to Obadina *et al* (2017), the decrease of carbohydrates (calculated by difference) is linked to enzymatic activation during germination, where α- amylase and β-amylase break down starch molecules to simple sugars. An increase of simple sugars (glucose, fructose and sucrose) and a decrease of starch is shown in a study by Khetarpaul and Chauhan (1990) where germination of pearl millet (*Pennisetum typhoid*) was studied. Monosaccharides can then be utilized as energy during seed development (ibid) and a higher amount of simple sugars increase the digestibility of cereals (Zhang *et al.* 2015). However, in a study conducted by Adebiyi *et al* (2017), results reported an increase in carbohydrates from 78.56 to 80.66 % after 48 h of germination, an explanation as to what caused the increase was lacking in the study.

Protein

Scientific studies shown in table 9 reported an increase of protein content in pearl millet after germination. In a study by Obadina *et al* (2017), the protein content in

pearl millet flour increased from 7.52 to 8.83 % after germination for 48 h in 25 ºC. Studies by Obizoba and Atii (1994) and Sade (2009) also reported an increase of protein content in pearl millet flour from 7.0 to 9.8 % and 14.0 to 19.4 % respectively after germinating for 48 h. Adebiyi *et al* (2017) analyzed and compared nutritional quality in native, germinated and fermented pearl millet flour in a study which resulted in an increase in protein content from 5.47 to 6.69 % after 48 h of germination. According to Akinola (2017), the synthesis of proteins during germination can explain the increased protein content in the germinated grains. Proteins are synthesized to create cellular material making the seed able to sprout (ibid).

During germination, storage proteins are broken down by activated proteolytic enzymes and new proteins are synthesized due to cellular growth. According to Nkhata *et al* (2018), a decrease is protein content due to proteolytic active enzymes can give an increase in specific amino acids. However, the net protein content depends on the outcome of both breakdown and synthesis of proteins in the seed embryo (Nkhata *et al.* 2018).

Fat

Studies by Obadina *et al* (2017), Obizoba and Atii (1994), Adebiyi *et al* (2017) and Sade (2009) shown in table 9, present a decrease in fat content in germinated pearl millet flour. An insignificant decrease in fat content from 6.34 to 5.32 % was seen in pearl millet germinated for 48 h in a study investigating changes in nutritional changes and physico-chemical properties in an Ex-borno variety of *P. glaucum* conducted by Obadina (2017). Results from Obizobas and Atiis (1994) report evaluating processing techniques effecting nutrition in pearl millet showed a decrease from 11.1 % to 10.1 % in fat content after 48 h of germination in 25 ºC. A study by Adebiyi (2017) reported decrease of fat content from 2.25 to 1.99 % when pearl millet was germinated using a temperature of 28 ºC for 48 h. An insignificant decrease in fat content from 5.7 to 5.6 % was seen in pearl millet flour after 48 h of germination in 25 ºC in the study conducted by Sade (2009). According to Akinola *et al* (2017) and Traoré *et al* (2004), the decrease in fat content is due to the germination process. Fat molecules are degraded by enzymes into smaller fractions which can be utilized as energy for the embryo during germination (Inyang & Zakari 2008). A decrease in fat content gives a lower energy value, however, low levels of lipids increase the shelf life due to reduced risk of rancidity (Akinola *et al.* 2017).

Table 9. Nutritional qualities in pearl millet before and after germination

Germination time (h)	Temp (ºC)		Results (% of d.m)			References
			Carbohydrates	Protein	Fat	
48	25	Raw	74.14±1.34*	7.52±0.07	6.34±0.01*	(Obadina *et al.* 2017)
		Germinated	72.47±1.32*	8.83+0.13	5.32±0.96*	
48	25	Raw	72.8±0.4	7.0±0.12	11.1±0.06	(Obizoba & Atii 1994)
		Germinated	72.5±0.06	9.8±0.06	10.1±0.1	
48	28	Raw	78.56±1.00	5.47±0.06	2.25±0.35	(Adebiyi *et al.* 2017)
		Germinated	80.66±0.04	6.69±0.19	1.99±0.01	
48	25	Raw	76.3	14.0±1.16	5.7±0.34*	(Sade 2009)
		Germinated	71.1	19.4±1.54	5.6±0.23*	

Results expressed as mean value ± standard deviation
**insignificant change*
d.m – dry matter

3.2.2. Effect on antinutritional compounds in pearl millet

Phytic acid

A study investigating the impact of germination regarding mineral extractability and antinutritional content in cultivars of *P. glaucum* and *P. typhoideum* conducted by Abdelrahaman *et al* (2007) resulted in a decrease from 501.77 to 353.37 mg/100g of phytic acid in *P. glaucum* flour after 48 h of germination (table 10). Archanas *et al* (1998) also showed similar results in pearl millet flour, a decrease of phytic acid from 833.42 to 449.32 mg/100g. A variety of pearl millet called HBB-50 were used by Archanas *et al* (1998) where reduction of phytic acid and polyphenols by malting and blanching were studied. A decrease of phytic acid in pearl millet after 48 h of germination was also observed in a report by Badau *et al* (2005). The phytic acid content decreased from 3.16 to 0.501 % in an Ex-borno cultivar of pearl millet. Badau *et al* (2005) used a germination temperature of 32±3ºC (table 10).

According to Abdelrahaman *et al* (2007) and Archana *et al* (1998), a decrease in phytic acid is caused by both soaking and germination. Due to a concentration gradient, the phytic acid may have leached out into the soaking water which results in a decrease of phytic acid before germination of the grains. Nevertheless, activation of phytase during germination results in further decrease of phytic acid (Archana *et al.* 1998; Abdelrahaman *et al.* 2007). The level of phytic acid varies in pearl millet due to environmental factors such as growing site and fertilizer, however, genetics also influence the content. Abdelrahaman *et al* (2007) argued

that the phytate level relates to the amount of protein within the grains, a higher protein content results in a higher amount of phytate in pearl millet.

Phenolic compounds

Abdelrahaman *et al* (2007) and Archana *et al* (1998) both reported the decrease of phenolic compounds in pearl millet after 48 h of germination (table 10). In Abdelrahamans *et al* (2007) report, a decrease from 347.26 to 267.57 mg/100g was observed. Archanas *et al* (1998) study reported a decrease in phenolic compounds from 765.45 to 468.27 mg/100g. According to Archana *et al* (1998) polyphenols, similar to phytic acid, leach out during steeping and thus account to the loss of phenolic compounds. Obilana *et al* (2018) discussed the decrease of phenolic compounds to be related to the presence of polyphenol oxidase during germination. However, an increase of phenolic compounds from 70.34 to 74.05 mg GAE/g was reported in a study conducted by Adebiyi *et al* (2017) (table 10). An increase in phenolic compounds can be attributed to the formation of lignin during germination due to seedling growth (Opoku *et al.* 1981; Obilana *et al.* 2018).

Table 10. Antinutritional compounds in pearl millet before and after germination

Germination time (h)	**Temp (ºC)**	**Variety**		**Results (mg/100g)**		**References**
				Phytic acid	Polyphenols	
48	32±3	Shambat	Raw	501.77±16.19	347.26±26	(Abdelrahaman *et al.* 2007)
			Germinated	353.37±9.42	267.57±2.57	
48	25-30	HHB-50	Raw	833.42±0.68	765.45±0.94	(Archana *et al.* 1998)
			Germinated	449.32±0.11	468.27±0.36	
48	32±3	Ex-borno	Raw	3.16±0.27 (%)		(Badau *et al.* 2005)
			Germinated	0.501±0.301 (%)		
48	28		Raw		70.34±1.65	(Adebiyi *et al.* 2017)
			Germinated		74.05±1.07 (mg GAE/g)	

Results are expressed as mean ± standard deviation.
mg GAE/g – mg gallic acid equivalents/gram.
Blank space indicates not specified/results not available.

3.2.3. Effect on functional properties in pearl millet

Bulk density

A study by Adebiyi *et al* (2016) present a decrease in bulk density from 0.78 to 0.69 g/cm^3 in malted pearl millet flour after 62 hours of germination (table 11). However,

a study conducted by Akinola *et al* (2017), showed no significant change in the bulk density of pearl millet flour after 48 h of germination (table 11). Adebiyi *et al* (2016) suggested that the enzymatic activation during germination results in degradation of carbohydrates and protein into smaller fractions which results in decreased density of the flour. Lower bulk density gives a higher nutrition concentration in the flour (ibid).

Oil absorption capacity

In a report conducted by Sade (2009) where antinutritional factors and functional properties of processed pearl millet was studied, an increase of oil absorption capacity was observed in pearl millet flour from 150 to 180 % (table 11). An increase of OAC was also seen in a study by Obadina *et al* (2017) and Adebiyi *et al* (2016) after 48 and 62 h respectively of germination on pearl millet flour (table 11). In the study by Obadina *et al* (2017), an increase from 0.33 to 0.69 ml/g was reported and an increase from 1.20 to 1.29 g/g was seen in the study by Adebiyi *et al* (2016) (table 11). According to Adebiyi *et al* (2016), the capacity of absorption of fat in flour depends on the ability to bind fat molecules, the attributes that non-polar amino acids have. Therefore, enzymatic breakdown of macromolecules due to germination is discussed by Adebiyi *et al* (2016) to be accounted for an increase in amino acids and leading to binding with more fat.

Water absorption capacity

In a study by Sade (2009), the WAC increased from 226 to 270 % in pearl millet flour after germination for 48 h (table 11). An increase in WAC in pearl millet was also seen in a report by Adebiyi *et al* (2016), from 1.32 to 2.26 g/g after 62 h of germination (table 11). Adebiyi *et al* (2016) and Sade (2009) discuss the increase of WAC to be attributed to the enzymatic breakdown during germination where soluble sugars and proteins results in a higher WAC due to the affinity to water molecules. Nonetheless, a study conducted by Akinola *et al* (2017) presented a decrease in WAC from 359.3 to 319.0 % in pearl millet flour after a germination time of 48 h and no further explanation to why this occurred was offered in the report (table 11).

Viscosity

The viscosity of germinated pearl millet flour was shown to decrease in two studies as can be seen in table 11. Sade (2009) reported a decrease from 91.5 RVU to -10.17 RVU and Obadina *et al* (2017) reported a decrease from 1559 to 11.60 RVU, both using a germination time of 48 h. According to Sade (2009), low viscosity is desirable in formulas intended for infants. Obadina *et al* (2017) argues that the reduction in viscosity is due to the amylase activity during germination. The ability to form a paste after cooking is attributed to the quality of starch. An interference

by amylase in the starch structures results in a lower ability to form a paste, which decrease the final viscosity (Obadina *et al.* 2017).

Table 11. Functional properties in pearl millet before and after germination

Germination time (h)		Results				References
		Bulk density (g/ml)	OAC	WAC (%)	Viscosity (RVU)	
48	Raw Germinated		150±0.06 180±0.06 (%)	226±0.03 270±0.06	91.50 -10.17	(Sade 2009)
48	Raw Germinated	1.12±0.02* 1.15±0.02*		359.33±1.45 319.00±0.58		(Akinola *et al.* 2017)
48	Raw Germinated		0.33±0.58 0.69±0.03 (ml/g)		1559.0±23.43 11.60±1.52	(Obadina *et al.* 2017)
62	Raw Germinated	0.78±0.01 0.69±0.01 g/cm3	1.20±0.02 1.29±0.04 (g/g)	1.32±0.02 2.26±0.08 (g/g)		(Adebiyi *et al.* 2016)

Results are expressed as mean ± standard deviation.
**insignificant change*
RVU – Rapid Visco Units, OAC- Oil absorption capacity, WAC- Water absorption capacity,
Blank space indicates not specified/results not available.

3.2.4. Effect of germination in cowpea

Chemical composition

A significant decrease of carbohydrates in cowpea is seen in studies conducted by Devi *et al* (2015), Jirapa *et al* (2001) and Uwaegbute *et al* (2000) after germination (table 12). This is argued to be due to the enzymatic activation during germination where amylase starts to break down starch molecules to utilize as energy during seed development (Jirapa *et al.* 2001; Devi *et al.* 2015). Studies show a decrease in fat content after 24 and 48 h of germination from 2.64 to 1.99 % and 1.61 to 1.17 % respectively (ibid). The decrease is argued to occur due to breakdown of fat molecules during germination to generate energy (ibid). However, an increase in fat content from 3.5 to 6.5 % was seen in a report by Uwaegbute *et al* (2000), no further explanation in the report was offered as to why these results occur. Significant increase of protein content in cowpea was observed in studies by Devi

et al (2015) and Uwaegbute *et al* (2000) after germination for 24 h and 48 h. The loss of dry matter, more specific carbohydrates, during sprouting is discussed by Devi *et al* (2015) to increase the crude protein content in cowpea. Nonetheless, protein synthesis activated during germination results in an increase of protein content (ibid).

Table 12. Nutritional qualities in cowpea before and after germination

Germination time (h)	**Temp (ºC)**		**Results (% d.m)**			**References**
			Carbohydrates	Fat	Protein	
24	25	Raw	66.50±0.06	2.63±0.27	27.15±0.26	(Devi *et al.* 2015)
		Germinated	63.92±0.19	1.99±0.13	29.72±0.04	
48	25	Raw	63.62	1.61	22.44*	(Jirapa *et al.* 2001)
		Germinated	61.70	1.17	22.30*	
48	27	Raw	50.3±0.36	3.5±0.1	24.2±0.36	(Uwaegbute *et al.* 2000)
		Germinated	28.1±0.17	6.5±0.30	27.1±0.1	

Results are expressed as mean ± standard deviation.
**insignificant change.*
d.m – dry matter

Antinutritional compounds

Several studies shown in table 13 present a significant reduction of phytic acid in cowpea after germination (Ibrahim *et al.* 2002; Sinha & Kawatra 2003; Kalpanadevi & Mohan 2013; Devi *et al.* 2015). Raw cowpeas were found to contain 836.00 mg/100g phytic acid and were reduced to 620.00 mg/100g after 48 h of germination in a study by Sinha & Kawatra (2003). In a research by Devi *et al* (2015), a reduction in phytic acid from 308.83 to 19.07 mg/100g was observed after 24 h of germination. In a report conducted by Ibrahim *et al* (2002), raw cowpeas showed a content of 4.54 % phytic acid which decreased to 3.50 % after 48 h of germination. A reduction of 79 % in phytic acid was seen in in cowpea after 48 h of germination in a research conducted by Kalpanadevi and Mohan (2013). Sinha and Kawatra (2003) argue that the decrease of phytic acid is due to the activation of phytase during sprouting. A decrease in phytic acid is seen over time during spouting in the study by Sinha and Kawatra (2003), where 24, 48, 60 and 72 hours was used as germination time. Leaching of phytate ions during soaking is also discussed to contribute to the reduction of phytic acid (Devi *et al.* 2015). A decrease of polyphenols from 517.0 to 422.80 mg/100g and from 1210 to 360 mg/100 g was observed in studies by Sinha & Kawatra (2003) and Kaloanadevi and Mohan (2013) respectively. Activated polyphenol oxidase during sprouting is discussed by Sinha

and Kawatra (2003) to be accounted for the reduction of polyphenols during germination. However, as phytic acid, polyphenols are argued to leak out into the soaking water before germination (ibid). Germination of cowpeas resulted in a significant decrease of RFOs (Ibrahim *et al.* 2002; Kalpanadevi & Mohan 2013). Levels of raffinose and stachyose was reduced to non-detectable concentrations in cowpea after 48 h of germination in a study by Ibrahim *et al* (2002), who explain the reduction to occur due to activation of α-galactosidase during germination. A significant reduction of raffinose, stachyose and verbascose was observed (54 %, 89% and 87% respectively) in cowpea after 48 h of germination in a report conducted by Kalpanadevi and Mohan (2013). The greatest reduction was seen in verbascose followed by stachyose which is argued to be due to the activity of selectively attacking verbascose first, thenceforth stachyose and at last, raffinose (Kalpanadevi & Mohan 2013).

Table 13. Antinutritional compounds in cowpea before and after germination

Germination time (h)		**Results**					**References**
		Phytic acid (mg/100g)	Polyphenols (mg/100g)	Raffinose (g/100g)	Stachyose (g/100g)	Verbascose (g/100g)	
48	Raw	836.00±2.30	517±1.41				(Sinha & Kawatra 2003)
	Germinated	620.00±2.30	422.80±1.53				
24	Raw	308.83±5.21					(Devi *et al.* 2015)
	Germinated	19.07±3.06					
48	Raw	4.54%		0.52	2.04		(Ibrahim *et al.* 2002)
	Germinated	3.50 %		0.00	0.00		
48	Raw	398.28±1.12	1210±0.06	0.68±0.04	1.94±0.07	1.24±0.06	(Kalpanadevi & Mohan 2013)
	Germinated	82.50±0.11	360±0.01	0.31±0.01	0.21±0.01	0.16±0.01	

Blank space indicates not specified/results not available.

4. Conclusion

The aim of this study was to evaluate scientific articles regarding the effect of processing techniques on nutritional composition, antinutritional compounds as well as functional properties in pearl millet and cowpea. More specifically, the traditional and affordable processing techniques fermentation and germination were studied. Pearl millet and cowpea both have good nutritional contents, however there are issues related to their consumption. Prevalence of antinutritional compounds, forming complexes with macro- and micronutrients, inhibiting their bioavailability is a major issue. Fermentation and germination are techniques addressing this.

Both fermentation and germination affected the nutritional qualities in pearl millet and cowpea. In fermentation microbes play an essential role, where their metabolism and enzymes lead to changes in the fermenting medium. In germination, the change in macronutrients is attributed to the activation of endogenous enzymes due to the increased energy requirement during seedling growth. Generally, fermentation and germination results in degradation of macronutrients increasing soluble molecules, hence an enhanced digestibility. Furthermore, the change in protein content after germination is due to both enzymatic degradation of storage proteins as well as synthesis of proteins attributed to the growth of the seeds. In fermentation there were tendencies indicating a disparity in protein content based on the matrix of the fermented crop, where fermented grains and flour gave different results.

Fermentation and germination have proved to be suitable techniques for decreasing the ANC content in pearl millet and cowpea. However, comparative studies on polyphenols in pearl millet and cowpea was difficult to obtain due to the extent of compounds included in the group, leading to a diverse range of studies analyzing different sub-groups of phenolic compounds. Furthermore, due to the ambivalence of the health impact of this group, with positive as well as negative effects being reported, difficulties arise in the search for information.

Changes in functional properties in pearl millet was shown after fermentation and germination, where these changes were mainly positive for the purpose of producing weaning foods, where high energy density and low viscosity are desirable.

In conclusion, germination and fermentation have shown several positive impacts on the nutritional qualities, ANCs and functional properties in relation of production of weaning foods. However, there is a need for further studies to acquire a better understanding in order to optimize these simple processes. Furthermore, studies on the combinatory effects of fermentation and germination would be interesting to evaluate and further study. To tackle the problems with undernutrition, the most favourable nutritional composition is of interest. Therefore, further studies on combining pearl millet and cowpea, moreover, investigating and finding the optimal ratio, would be of immense interest.

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