NUTRITIONAL, PHYSICO-CHEMICAL AND SENSORY CHARACTERISTICS OF A PEARL MILLET-BASED INSTANT BEVERAGE POWDER

BY

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REFERENCE DECLARATION

I, Mr. A.O. Obilana – 20722333, Prof Bharti Odhav and Prof Victoria Jideani do hereby declare that in respect of the following dissertation:

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AUTHORS DECLARATION

This study presents original work by the author. It has not been submitted in any form to another academic institution. Where use was made of the work of others, it has been duly acknowledged in the text. The research described in this dissertation was carried out in the Department of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology and Cape Peninsula University of Technology, Cape Town South Africa, under the supervision of **Prof Bharti Odhav** and **Prof Victoria Jideani**.

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LIST OF ABBREVIATIONS

AA	Amino Acid
AgG	AgriGreen
Ala	Alanine
Arg	Argenine
Asp	Aspergine
Ba	Babala
BV	Breakdown Viscosity
EPMM	Extruded Pearl Millet Malt
ExPM	Extruded Pearl Millet
ExPMMx	Extruded Pearl Millet Mix
FV	Final Viscosity
Glu	Glutamic acid
Gly	Glycine
His	Histadine
HS	Holding Strength
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
MPM	Malted Pearl Millet
Phe	phenylalanine
PMF	Pearl Millet Flour
PMIBP	Pearl Millet Instant Beverage Powder
PT	Peak Time
PV	Peak Viscosity
RPM	Raw Pearl Millet
RVA	Rapid Visco Analyser
Ser	Serine
SV	Setback Viscosity
TDF	Total Dietary Fibre
Thr	Threonine
TPC	Total Phenolic Content
Tyr	Tyrosine
Val	Valine
WAI	Water Absorption Capacity
WHC	Water Holding Capacity
WSI	Water Solubility Index

ABSTRACT

A pearl millet (*Pennisetum glaucum*) based instant beverage powder (PMIBP) was prepared from two different varieties of pearl millet (Agrigreen (AgG) and Babala (Ba)) by a combination of malting and extrusion cooking. The millet grains were germinated (30°C and 98% RH for 36 h), kilned (50°C for 48 h), cooled to room temperature, ground and stored in a chiller at 5°C until used. The raw and malted pearl millet grains were extruded under different parameters to accommodate the types of pre-treatment applied to the pearl millets. Combination processing of the pearl millet grain was achieved by extrusion of malted pearl millet of both varieties individually, and as a mixture of raw and malted pearl millet (50:50). The effect of the processing methods on the physical, functional, nutritional and biochemical properties of the raw and processed pearl millets varieties were evaluated. Combination processing led to a significant ($p \leq 0.05$) decrease in total fat and total dietary fibre (TDF) (3.85 and 22.99 g/100 g, respectively) content of AgriGreen (AgG) extruded malted pearl millet (EMPM); a decrease in TDF (18.12 g/100 g) content of AgG extruded raw pearl millet-malted pearl millet mix (ERPMMPM). Combination processing led to a decrease in ash, total fat, total dietary fibre, Fe and Zn (1.76, 3.48, 14.26 g/100 g, 7.78 and 4.74 mg/100 g, respectively) content of Babala (Ba) EMPM. It also led to a significant ($p \leq 0.05$) decrease in ash, total fat, TDF, Fe and Zn (1.88, 4.22, 21.71 g/100 g, 7.24 and 4.14 mg/100 g, respectively) content of Ba ERPMMPM. Regardless of the pearl millet variety, malting led to a significant ($p \leq 0.05$) decrease in moisture, total, saturated, mono- and polyunsaturated fats, total dietary fibre iron, zinc and protein digestibility; a significant ($p \leq 0.05$) increase in total carbohydrates, total phenolic content, antioxidant activity (TEAC) and water solubility index. Extrusion however, led to a significant ($p \leq 0.05$) decrease in total dietary fibre, zinc, total phenolic content and protein digestibility; a significant ($p \leq 0.05$) increase in ash, total, saturated and monounsaturated fats, total carbohydrates, iron, starch digestibility water

absorption index (WAI) and water solubility index (WSI). The beverages of 10% total solids (8% pearl millet + 2% sugar for taste) prepared from the processed pearl millet were offered to an untrained consumer panel consisting of students and staff of the Cape Peninsula University of Technology, under similar sets of conditions in a sensory evaluation room at the Food Technology Department. The following characteristics of the products were rated and scored on a 9-point hedonic scale (1 – like extremely and 9 – dislike extremely): appearance, colour, aroma, flavour, mouth-feel and overall acceptability. In general, Ba RPM was rated 4 - like slightly, and AgG malted pearl millet (MPM) was rated 6 - dislike slightly and all other pearl millet samples from both varieties were rated 5 - neither like nor dislike.

CHAPTER 1: INTRODUCTION

Cereal grains are the most important source of the world's food and have a significant role in human diet throughout the world. As one of the most important drought-resistant crops, millets are widely grown in the semiarid tropics of Africa and Asia. It serves as a major food component in various traditional foods and beverages such as bread, porridges and snack foods, specifically among the non-affluent segments in their respective societies (Chandrasekara and Shahidi, 2011). It constitutes a major source of carbohydrates and proteins as well as other important phytonutrients for people living in these areas (Li *et al.*, 2008). Pearl millet may be the best cereal grain of the future in semi-arid regions of the world. This hardy plant can survive drought and flooding and give higher yields than maize and sorghum under similar conditions (Kumar, 1989).

Utilisation of millet in food and beverage products also affords the consumers of these products potential health benefits which include; preventing cancer and cardiovascular diseases, reducing the incidence of tumours, lowering blood pressure, lowering cholesterol and rate of fat absorption, delaying gastric emptying, and supplying gastrointestinal bulk (Gupta *et al.*, 2012; Truswell, 2002).

Food products (stiff porridge) from millet, sorghum, rice or maize grains, and in some areas also from starchy roots or tubers (Kim and Henrik, 1998), known by various names in different parts of the continent (Inyang and Zakari, 2008) are the main complementary foods for infants and young children (Complementary foods are any non-breast milk foods or nutritive liquids that are given to young children to augment their needs for more vitamins, minerals, proteins and carbohydrates than are generally available from breast milk (Brown *et al.*, 1998)). These food products are usually prepared by simple traditional household technologies applied to the cereal in order to improve the edible, nutritional, and sensory properties (Nout, 1992a;b). These processes include roasting, germination, and fermentation,

cooking and soaking, grinding/milling, which cause changes in the physicochemical characteristics of the components of the millet.

Achieving a thin drinkable consistency from the thick porridges requires addition of copious amounts of water during preparation (Stephenson *et al.*, 1994), lowering the energy density. In addition, producing an instant powder from the cereal grains, could possibly require combination processing involving malting and then extrusion cooking of the malted millet grains.

Malting is a three-stage process, namely; (i) steeping (soaking of grains in water at ambient temperature for a period); (ii) germination (25 to 30°C, ~98% relative humidity (RH) for 3 to 5 days); and (iii) kilning/drying (50°C for 24 to 48 hours). During the steeping and germination of the grains, endogenous enzymes start to modify the grains constituents (Katina *et al.*, 2007) causing changes in soluble sugars, protein and fats. The kilning step is usually applied to dry germinated grains and develop malt flavour.

Germination has a profound effect on the nutritional quality of the cereal (Chavan and Kadam, 1989b). Consequently, starch (amylose and amylopectin) is degraded, resulting in significant reduction of the viscosity and an increase in protein bioavailability when preparing porridge. Flour from germinated cereals can be added to ordinary flour to initiate starch degradation and thereby reduce viscosity (Kim and Henrik, 1998).

The extrusion cooking of the raw and malted grains involves exposing the milled grains to high temperatures and pressures for short periods in an enclosed barrel. The process leads to cooking expansion (puffing) and partial drying of the milled grains.

Due to the high nutritional properties of pearl millet, food and beverage products made from them will possess a high percentage of a full protein and amino acid profile. Such beverages will provide an easy way to improve nutritional wellbeing of the consumer. The high amylose content of pearl millet will confer the beneficial effect on the proposed beverage

having a slow digestible property resulting in low glycaemic load when consumed. The beverage is expected to provide known functional property of dietary fibre to the consumers because of the high polysaccharide content of the grain. Consequent to the high fat content and inherent high levels of vitamins A and E, it is expected that beverages made from whole pearl millet grain will possess high antioxidant properties.

In producing the pearl millet instant beverage powder, decortication of the millet grain is strongly discouraged. Rather, the whole grain is used to produce whole meal flour, which is further processed to produce the beverage in whatever form. This is to ensure that most if not all the essential fatty acids, which are located in the outer layers (normally removed during decortication) are retained during the process. However, processing steps to reduce lipid oxidation would need to be incorporated into the manufacturing process.

Pearl millet instant beverage powder from whole pearl millet flour contains phenolic compounds which confer antioxidant properties. These are known to play a role in preventing and managing various diseases associated with oxidative stress. Furthermore, the bioavailability of nutrients in the beverages will be improved by malting and/or addition of phenolic enzymes and pasteurization.

1.1 Problem Statement

In spite of the nutritive value and potential health benefits of millet grains which are comparable to major cereals such as wheat, rice, maize; and the different processing technologies used to improve their edible and nutritional characteristics, utilization of millet grains as food is still mainly limited to populations in rural areas at the household level. This is due to lack of innovative millet processing technologies to provide easy-to-handle, ready-to-cook or ready-to-eat, and safe food and beverage products at a commercial scale that can be used to feed large populations in urban areas (Ushakumari *et al.*, 2004). However, with

an increasing population and thus increasing demands for food, feed, and fuel, society will be pressed to increase agricultural production—whether by increasing yields on already cultivated lands or by cultivating currently natural areas—or to change current crop consumption patterns (Licker *et al.*, 2010). Moreover, diversification of food production must be encouraged both at national and household levels in tandem with increasing yields. Providing more healthy and traditional whole-grain and multi-grain substitutes for refined carbohydrates can be one important aspect of therapeutic dietary modification and promoting utilization of minor-grain foods (Singh and Raghuvanshi, 2012). Novel processing and preparation methods are needed to enhance the bioavailability of the micronutrients and to improve the quality of millet diets. Research is also needed to determine the bioavailability, metabolism, and health contribution of millet grains and their different fractions in humans. Manufacturing millet food and beverage products that deliver convenience, taste, texture, colour and shelf-stability at economical cost for poor people is needed. In addition, for promoting utilization of millet grains in urban areas to open new markets for farmers to improve their income, developing highly improved products from millet is needed.

Maize is the staple food crop in South Africa and the use of pearl millet (commercially or otherwise) despite all its nutritional advantages, is virtually non-existent. Ready-To-Eat (RTE) pearl millet flour products are substantially less common than enriched flours, probably because the technology is more sophisticated and they are more expensive with a much smaller market (Taylor *et al.*, 2010). Nevertheless, because of some of its nutritional properties, and the fact that it is "gluten–free", pearl millet makes for an ideal alternate cereal grain for individuals suffering from or are at risk of suffering from coeliac disease (gluten intolerance).

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1.2 Aim

1.2.1 Overall aim

The primary aim of this research project was to develop a nutrient dense pearl millet instant beverage powder (PMIBP) which is acceptable to consumers, using a combination of malting and extrusion cooking.

1.2.2 Specific objectives

The specific objectives of the research were to:

1. determine an ideal germination time as measured by the α-amylase activity during a germination period of 72 h;

2. determine the effect of the processing methods (malting, extrusion and a combination thereof) on the nutritional profile [macro (protein, fats and carbohydrates) and micro (Ca, Fe and Zn) nutrients] and the biochemical properties [protein and starch digestibility, total phenolic content (TPC) and antioxidant activity] of the pearl millet flour (PMF), PMIBP and their beverage;

3. determine the effects of the processing methods (malting, extrusion, and a combination thereof) on the functional/physical properties (expansion ratio, water solubility and water absorption indices, pasting properties TPC colour) of the PMF, PMIBP and their beverages

4. Evaluate sensory characteristics of the processed PMF and PMIBP beverage.

1.2.3 Hypotheses

1. An instant beverage powder produced by malting followed by extrusion of pearl millet will have better nutritional qualities than the corresponding beverage powder produced

by extrusion alone. This is because the malting would break down some of the available starch to dextrins allowing for a less viscous drink whilst the extrusion step will gelatinize intact starch. It would be possible to increase the total solid content hence the nutritional value of the beverage without affecting viscosity.

2. The instant beverage powder produced by malting followed by extrusion of pearl millet will have a lower viscosity than the corresponding beverage powder produced by extrusion alone.

3. The instant beverage powder produced by malting followed by extrusion of pearl millet will be more acceptable to the consumers than that produced by extrusion alone.

1.1.4   Importance of the Study

The success of this project will lead to the availability of a new pearl millet based instant beverage powder. This could have far-reaching positive consequences on several aspects of the socio-economic landscape; e.g. (i) Use of otherwise unproductive or fallow land to cultivate millet for the manufacture of the food product, due to the minimal requirements for pearl millet cultivation; (ii) job creation within the millet producing communities, leading to improvements of these communities; and (iii) implementation of supply chains to ensure the continuous availability of the raw material and product.

CHAPTER 2:   LITERATURE REVIEW

This review examines the research on several aspects of pearl millet (*Pennisetum glaucum* (L.) R. Br.) grains as applicable in their use in traditional and conventional food systems. It highlights studies on the grain structure, biochemical, physico-chemical aspects and sensory acceptability of products thereof, as well as effects of processing (malting and extrusion especially) on these aspects.   It further examines their health benefits, and technologies applicable for commercialisation.

2.1   Millets and Pearl Millet

Millets are not a single species, or even different species within a single genus. They are simply cultivated grasses (cereals) that have small kernels and they are grouped together solely on this basis (Taylor and Emmambux, 2008). The word millet is derived from the French word "mille" meaning thousand, implying that a handful of millet contains thousands of grains (Taylor and Emmambux, 2008). The study of millet literature is problematical because different common names are used for the same species and even different proper species names are in widespread use (Taylor and Emmambux, 2008).

Millets are small-seeded with different varieties such as pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), kodo millet (*Setara italic*), little millet (*Panicum sumatrense*), and barnyard millet (*Echinochloautilis*). Pearl millet (*Pennisetum glaucum*) is by far the most important millet, accounting for about half of total millet production (Taylor *et al.*, 2010). Collectively millets are known as course cereals together with maize (*Zea mays*), sorghum (*Sorgum bicolor*), oats (*Avena sativaa*), and barley (*Hordeum vulgare*) (Bouis, 2000; Kaur *et al.*, 2012).

Millets are collectively the world's sixth most important cereal, with an annual production of around 30 million tons (Taylor and Emmambux, 2008). Pearl millet is one of

the most important drought-tolerant crops of the tropical and subtropical regions of the world; it is able to produce good yields of grain under conditions unfavourable to most other cereals (Abdalla *et al.*, 1998a). In addition to their agronomic advantages, millets are found to have high nutritive value and are comparable to that of major cereals such as wheat and rice (Parameswaran and Sadasivam, 1994).

The presence of most the required nutrients in millets makes them suitable for large-scale utilization in the manufacture of food products such as baby foods, snack foods, and dietary food. Increasingly, more millet products have entered into the daily diets of people, including millet porridge, millet wine, and millet nutrition powder (Liu *et al.*, 2012; Subramanian and Viswanathan, 2007).

2.1.1 Structure of Pearl Millet

The millet grain is about one-third the size of wheat, however, despite its small grain size, pearl millet has relatively good nutritional value compared to other cereal grains. This is because of its proportionally large germ compared to endosperm (Taylor *et al.*, 2010). Pearl millet grains are shaped like a liquid drop (Jain and Bal, 1997) (**Figure 1**). They can be up to 2 mm in length and their weight ranges between 3 mg and 15 mg. This is a small grain in comparison with other tropical cereal grains such as maize and sorghum. Pearl millet grains pack closely together, leaving little airspace compared to other cereal grains such as maize. Consequently, pearl millet grain has a density of approximately 1.6 g/cm^3 (Jain and Bal, 1997; Serna-Saldivar and Rooney, 1995), which is significantly higher than that of wheat (1.39 g/cm^3), maize (1.39 g/cm^3), rice (1.24 g/cm^3) and sorghum (1.24 g/cm^3) grains (Serna-Saldivar and Rooney, 1995).

The overall structure of the pearl millet kernel (Figure 1) is similar to that of sorghum, except that the pearl millet kernel is smaller in size and has a relatively smaller endosperm and proportionally larger germ than sorghum (Abdelrahman and Hoseney, 1984).

The pearl millet grain comprises about 8% pericarp, 17% germ (which is proportionally large) and 75% endosperm (Serna-Saldivar and Rooney, 1995), similar to values of 7.2 to 10.6%, 15.5 to 21% and 71 to 76%, for pericarp, the germ (embryo) and the endosperm, respectively (Abdelrahman and Hoseney, 1984). In contrast, Zeleznak and Varriano-Marston (1982) estimated that the germ forms about a third of the kernel, and the endosperm between 50 to 60% of the kernel. The observed differences may be because of differences in the varieties of pearl millets studied.

A thin waxy cutin layer which helps decrease the effects of weathering, covers the surface of the pericarp (Taylor, 2004b). The pericarp consists of an epicarp, a mesocarp (containing parenchyma cells) and an endocarp, which is characterized by the presence of cross and tube cells (Zeleznak and Varriano-Marston, 1982). The epicarp, which consists of 1 to 2 or 2 to 4 cell layers, may contain considerable amounts of pigments in some varieties. The presence of these pigments influences the colour of the kernel (McDonough and Rooney, 1989). Several rows of collapsed cells comprising the mesocarp layer are found under the epicarp. These layers are often indistinguishable from the cross and tube cells that form the endocarp. The mesocarp layer may vary across genotypes and is believed to play a role in pearl millet resistance to mould attack (McDonough and Rooney, 1989). The endocarp is the innermost section of the pericarp. Beneath the pericarp, is a thin layer of seed coat, and then a single aleurone layer (one cell thick) (Taylor, 2004b).

Style
Pericarp
Seedcoat
Aleurone layer
Corneous endosperm
Peripheral endosperm
Floury endosperm
Scutellar epithelium
Scutellum
Embryonic axis
Hilum
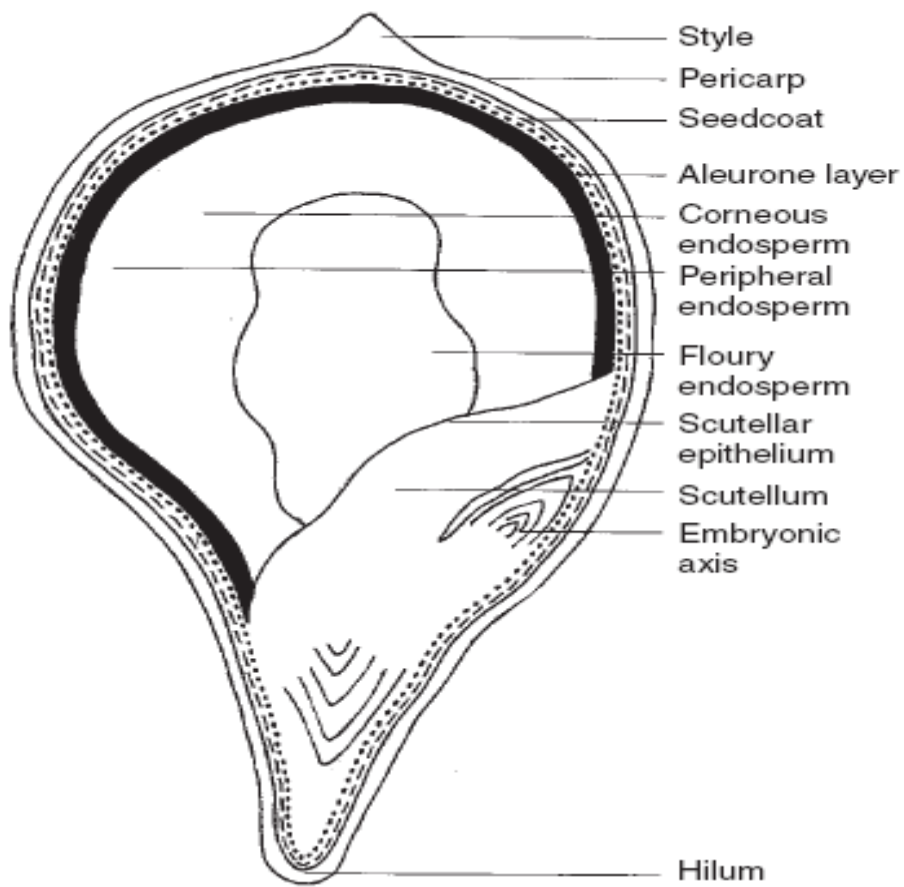
Figure 1   Longitudinal section through a pearl millet grain (Barrion, 2008)

The colour of pearl millet grains varies from pearly white to yellow, grey, brown and purple (Taylor, 2004b); even individual grains may not have a uniform colour. This is due to the presence of a thick pericarp in some grain varieties, which can mask the presence of pigments in the aleurone layer, while the pigments present in the aleurone and other endosperm layers are clearly visible in grains with a thin pericarp. If there are no pigments present in the kernel and the pericarp is thin, the resulting colour of the kernel is white. According to McDonough and Rooney (1989), it is easier to obtain an acceptable colour of food product when the pigmentation is primarily concentrated in the pericarp as the pigments can easily be removed during decortication. Below the endocarp is a thin single-layer and sometimes pigmented testa (seed coat) similar to that found in some sorghum (McDonough and Rooney, 1989).

Beneath the seed coat lies a single layer of aleurone cells that forms the first layer of the endosperm (Zeleznak and Varriano-Marston, 1982). These cells, which have thick cell walls, contain protein bodies and oval lipid bodies in their cytoplasm. Pearl millet starchy endosperm is classified according to the relative proportions of corneous and floury components. The corneous component is hard and vitreous-like, while the floury component is soft and floury. The former is found in greater proportion in the outer layer, while the latter predominates near the centre of the endosperm. The corneous and floury endosperm comprises the bulk of the starchy endosperm (McDonough *et al.*, 1986).

The pearl millet starchy endosperm has three distinctive areas namely: peripheral, corneous and floury endosperms. The peripheral endosperm varies between one and three cell layers thick and contains a thick protein matrix made up of a large number of different proteins (McDonough and Rooney, 1989). It is characterized by the presence of small polygonal starch granules embedded in the protein matrix (Badi *et al.*, 1976). The continuity of the protein matrix and the physical contact between the starch granules and storage proteins

are believed to be responsible for the hard texture of the endosperm (Hadimani *et al.*, 2001). The contents of the peripheral endosperm cells are so tightly packed together that the protein bodies leave distinct indentations in the starch granules (McDonough and Rooney, 1989). The corneous endosperm contains fewer polygonal starch granules and protein bodies enmeshed in the protein matrix, while its cells are loosely packed resulting in fewer indentations of the starch granules. The relative sizes of the starch granules and protein bodies in the corneous endosperm are 10 μm and 1.5 μm, respectively (Badi *et al.*, 1976).

Compared to both the peripheral and corneous endosperms, the floury endosperm contains larger and rounder starch granules that are embedded with fewer protein bodies (McDonough and Rooney, 1989). These protein bodies are loosely packed with many air voids between the starch granules. These air voids give the floury endosperm a chalky appearance. The pearl millet floury endosperm is, also characterized by the presence of a discontinuous protein matrix.

The last major structural component of the pearl millet kernel is the germ (embryo). The pearl millet germ to endosperm ratio is higher than that of other cereals (Serna-Saldivar and Rooney, 1995). The germ comprises two major parts: the scutellum cells and embryonic axis. The scutellum serves as a storage body for lipids, protein, enzymes and minerals (Serna-Saldivar and Rooney, 1995) and acts as a transport system organ. Scutellum cells have a smooth round appearance and are between 25.0 to 35.0 μm in diameter (McDonough *et al.*, 1986).

2.2   Nutritional Composition of Pearl Millet

The average protein and fat contents of millet at 12% moisture are 11.8% and 4.8% respectively (FAO, 1995). These are comparable to values for maize (9.2% protein, 4.6% fat) and sorghum (10.4% protein, 3.1% fat). Millet protein is a good source of essential amino

acids except lysine and threonine but is relatively high in methionine. Millets are also a rich source of phytochemicals and micronutrients (Mal *et al.*, 2010), and have been found to be significantly rich in resistant starch, soluble and insoluble dietary fibres, minerals, and antioxidants (Ragaee *et al.*, 2006), and contain about 92.5% dry matter ash 2.1%, 2.8%; crude fibre, 7.8% crude fat, 13.6% crude protein, and 63.2% starch (Ali *et al.*, 2003). Foxtail millet protein characterization showed that its protein is a potential functional food ingredient and the essential amino acid pattern suggests possible use as a supplementary protein source to most cereals because it is rich in lysine (Mohamed *et al.*, 2009). Finger millet, is known to have several potential health benefits and some of the health benefits are attributed to its polyphenol contents (Chethan and Malleshi, 2007). It has a carbohydrate content of 81.5%, protein 9.8%, crude fibre 4.3%, and mineral 2.7% that is comparable to other cereals and millets. Its crude fibre and mineral contents are markedly higher than those of wheat (1.2% fibre, 1.5% minerals) and rice (0.2% fibre, 0.6% minerals); its protein is relatively better balanced; it contains more lysine, threonine, and valine than other millets (Ravindran, 1991; Sripriya *et al.*, 1997). Black finger millet contains 8.71 mg/g dry weight fatty acid and 8.47 g/g dry weight protein (Glew *et al.*, 2008). Kodo millet and little millet were reported to have 37% to 38% of dietary fibre, which is the highest among the cereals; and the fat has higher polyunsaturated fatty acids (Hegde and Chandra, 2005). The protein content of proso millet (11.6% of dry matter) was found to be comparable with that of wheat, as well as being significantly richer in essential amino acids (leucine, isoleucine, and methionin) than wheat protein (Kalinova and Moudry, 2006). In developing countries, pearl millet is recognized as an important crop, which helps with food shortages and meeting the nutritional demands of an increasing population. It constitutes an important source of dietary calories and protein in the daily diet of a large segment of the poor population. Although pearl millet is consumed as a major staple food, the nutrient availability to the human gut is constrained by certain inherent

antinutritional factors (polyphenols and phytic acid). Similar to other cereals, pearl millet also contains phytic acid in the germ and polyphenols in peripheral areas of the seed (Simwemba *et al.*, 1984).

Investigations by Abdalla *et al.* (1998a) on 10 genotypes of pearl millet, showed that pearl millet contained 88-91% dry matter, l.6-2.4% ash, 2.6-4.0% crude fibre, 2.7-7.1% oil, 8.5-15.1% crude protein, 58-70% starch and 354-796mg g^{-1} phytic acid. Mineral contents were l0-80, 180-270 and 450-990 mg g^{-1} for Ca, Mg and P, respectively, and 70-110, 4-13, 53-70, 18-23, l0-18 and 70-180 μg g^{-1} for K, Na, Zn, Mn, Cu and Fe, respectively.

The nutrient composition of pearl millet in comparison to other cereal grains (Table 1) shows it has a relatively high gross energy content of approximately 363 Kcal/100 g. This high-energy content is due to the high fat content of the grain, which is related to the large germ size.

Pearl millet is a good source of minerals, containing appreciable amounts of calcium, phosphorus, magnesium, and iron, and is a good source of thiamine and possibly other B vitamins (Burton *et al.*, 1992). However, the grain also contains flavones and thiocyanate, which may be goitrogenic (Akingbala *et al.*, 2002; Osman and Fatah, 1981). The presence of these compounds however, should not deter the food uses of pearl millet since the reduction of these antinutritional constituents by various processing methods is widely known. Hopefully, malting and extrusion processes as will be applied in this research, will render the instant beverage low in these substances and therefore suitable for human consumption.

The nutritional quality of food is a key element in maintaining human overall physical well-being because nutritional well-being is a sustainable force for health and development and maximization of human genetic potential. Therefore, for solving the problem of deep-rooted food insecurity and malnutrition, dietary quality should be taken into consideration (Singh and Raghuvanshi, 2012).

2.2.1   Pearl Millet Proteins

Pearl millet grain has higher protein and oil content than wheat, rice, maize and sorghum
(Burton *et al.*, 1992).   The limiting amino acid is lysine, though, under similar conditions of
cultivation, pearl millet contains higher concentrations of lysine and other essential amino
acids than other cereal grains such as sorghum, maize, wheat and rice (Badi *et al.*, 1976).

Compared with other cereal grains, millets in particular, pearl millet (*Pennisetum
glaucum*, syn. *P. americanum*, *P. typhoideum*) is high in protein (Serna-Saldivar and Rooney,
1995) (Table 1), is considered to have one of the best protein quality or amino acid scores
(Almeida-Dominguez *et al.*, 1993).   The nutritional value of pearl millet is greatly enhanced
when mixed with legumes because the latter complement its profile of essential amino acids
(Serna-Saldivar *et al.*, 1988a).

The crude protein content of most pearl millet cultivars ranges between 10 and 15%
(Chowdhury and Punia, 1997; Elyas *et al.*, 2002; Hoover *et al.*, 1996; McDonough *et al.*,
1986).   This is a typical range, even if there is a wide variability in the protein contents of
different pearl millet genotypes (Hulse *et al.*, 1980).

Pearl millet protein can be categorised into prolamins, albumins, globulins and
glutelins (Serna-Saldivar and Rooney 1995).   According to Dahiya and Kapoor (1983), pearl
millet prolamins and glutelins comprise 63% of the total protein, and thus, are responsible for
a major share of the grains' amino acid content.

Table 1: Nutrient composition of sorghum, millets and other cereals (per 100 g edible portion; 12 percent moisture)

Food	Protein (g)	Fat (g)	Ash (g)	Crude fibre (g)	CHO (g)	Energy (kcal)	Ca (mg)	Fe (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)
Rice (brown)	7.9	2.7	1.3	1.0	76.0	362	33	1.8	0.41	0.04	4.3
Wheat	11.6	2.0	1.6	2.0	71.0	348	30	3.5	0.41	0.10	5.1
Maize	9.2	4.6	1.2	2.8	73.0	358	26	2.7	0.38	0.20	3.6
Sorghum	10.4	3.1	1.6	2.0	70.7	329	25	5.4	0.38	0.15	4.3
Pearl millet	11.8	4.8	2.2	2.3	67.0	363	42	11.0	0.38	0.21	2.8
Finger millet	7.7	1.5	2.6	3.6	72.6	336	350	3.9	0.42	0.19	1.1
Foxtail millet	11.2	4.0	3.3	6.7	63.2	351	31	2.8	0.59	0.11	3.2
Common millet	12.5	3.5	3.1	5.2	63.8	364	8	2.9	0.41	0.28	4.5
Little millet	9.7	5.2	5.4	7.6	60.9	329	17	9.3	0.30	0.09	5.2
Barnyard millet	11.0	3.9	4.5	13.6	55.0	300	22	18.6	0.33	0.10	4.2
Kodo	9.8	3.6	3.3	5.2	66.6	353	35	1.7	0.15	0.09	2.0

(FAO, 1995)

Taylor (2004b) indicated that prolamins may comprise 31 to 41% of the total protein in pearl millet. The albumins and globulins account for 25% of the total protein content (Hadimani *et al.*, 2001).

The prolamin content of pearl millet is about 31−34%, which is lower than that in other millets. This is related to the large germ, which is rich in albumins and globulin type proteins. The types of protein in pearl millet affect the amino acid composition. Because of a higher globulin and albumin content, the essential amino acid lysine is slightly higher (Table 1). Related to this, the true protein digestibility of pearl millet in rats has been reported to be high, at 94−97% (Singh *et al.*, 1987).

Pearl millet is considered to have one of the best protein qualities in terms of amino acid score (Almeida-Dominguez *et al.*, 1993). It has a higher protein content and amino acid score than sorghum (Serna-Saldivar and Rooney, 1995) because, quantitatively, pearl millet has much higher amounts of lysine, asparagine and methionine than sorghum (Serna-Saldivar and Rooney, 1995). Pearl millet has higher arginine, threonine, valine, isoleucine and lysine values (Adeola and Orban, 1995) than that of maize. However, Barragán Delgado and Serna-Saldivar (2000) reported that lysine remains the most limiting essential amino acid of pearl millets. Badi *et al.* (1976) reported an amount of 3.6 g lysine /16 g N in pearl millet. The lysine is found in high amounts in albumins and globulins located in the pearl millet germ (Taylor, 2004b). Significant variation exists among lysine values claimed for pearl millet grain. It most likely depends on the structure and relative proportion of the germ to endosperm.

The highest amount of protein is found in the peripheral endosperm, decreasing from the exterior to the interior of the kernel (M[c]Donough and Rooney, 1989). The endosperm protein exists as a continuous matrix in the peripheral and corneous endosperms. Protein

bodies embedded in these matrices are spherical and roughly uniform in size, regardless of their location in the endosperm.

Hadimani *et al.* (2001) observed that the protein constituents contribute to the corneous endosperm texture, which facilitates good milling properties. Relatively few protein bodies are found in the floury endosperm, with McDonough and Rooney (1989) reporting that a considerable amount of the pearl millet protein is present in the germ.

2.2.2   Pearl Millet Carbohydrates

The major nutritional component of cereal grains, including pearl millet is starch (Table 1). Starch content of pearl millet varies from about 50 to 75% of the grain composition (Hadimani *et al.*, 2001; Hoover *et al.*, 1996; Oshodi *et al.*, 1999; Shahidi and Naczk, 2003b), and is similar to the starch composition of sorghum. Aside from starch, the carbohydrates in pearl millet grain include free sugars and non-starch polysaccharides (Hadimani *et al.*, 2001).

Pearl millet starch has a lower amylose content than sorghum (Serna-Saldivar and Rooney, 1995). The amylose content in pearl millet grain ranges between 17.0 to 21.5% (Taylor 2004). Hoover *et al.* (1996) and Hadimani *et al.* (2001) showed that the starch amylose content may be as high as 28.8 to 38% in some varieties, an indication of great variability in amylose content in pearl millet starch. Pearl millet starch gelatinizes at 61 to 69ºC (Serna-Saldivar and Rooney, 1995). The starch granules in pearl millet are present in the endosperm and appear smaller but otherwise similar to maize and sorghum starch (Hoover et al. 1996).

Similar amounts of total soluble sugars can be found in sorghum and pearl millet, 2.3 and 2.6%, respectively (Serna-Saldivar and Rooney, 1995); these free sugars include glucose, fructose, raffinose and xylose (Hadimani et al. 2001) and range in content from 1.2 to 2.5%. The amount of sucrose found in pearl millet and sorghum is essentially the same. Pearl

millet, however, has higher raffinose content than sorghum, with a value of 0.71% compared to 0.23% found in sorghum (Serna-Saldivar and Rooney 1995).

Research on the dietary fibre content of pearl millet is limited. The dietary fibre content in pearl millet ranges between 8 to 9% (Taylor 2004). Glucose, arabinose and xylose were the major non-starch polysaccharide fractions of pearl millet (Hadimani *et al.*, 2001), an indication that the pentosans are composed predominantly of arabinose and xylose; whereas the glucose comes mainly from cellulose. Serna-Saldivar and Rooney (1995) observed that the dietary fibre components such as cellulose, hemicellulose, lignin, pectins and gums in cereals are found in the pericarp and endosperm cell walls.

2.2.3   Pearl Millet Fats

Typically, pearl millet grain has a fat content of approximately 5.1% (Taylor, 2004b), which is higher than what is typically found in sorghum of 3.2% (Serna-Saldivar and Rooney, 1995), but lower than the fat content of maize (Taylor 2004). This is in contrast to values reported by FAO (1995) of 3.1%, 4.6% and 4.8% fat content for sorghum, maize and pearl millet respectively. Unsaturated fatty acids, such as palmitoleic acid (16:1), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) account for approximately 75% of the total fatty acids in pearl millet (Serna-Saldivar and Rooney 1995). The saturated fatty acids, which include palmitic acid (16:0) and stearic acid (18:0), account for approximately 25% of the total fats. The degree of unsaturation of fatty acids contributes to the development of objectionable odours and flavours after the grinding of pearl millet (Kaced *et al.*, 1984).

The high oil content of pearl millet is related to the relatively large germ size in relation to the endosperm (Adeola and Orban, 1995). This relatively high fat content can be reduced by decortication, as reported by Hadimani *et al.* (2001), observing a reduction of 1.2% in pearl millet fat content. Lipid oxidation is prevented and shelf life is substantially

improved in pearl millet flour by the removal of the pearl millet germ, which is rich in unsaturated fatty acids (Slavin *et al.*, 2001).

2.2.4   Pearl Millet Minerals and Vitamins

Millets are important sources of vitamins and minerals (Table 2), which are found in the pericarp, aleurone layer and germ, which are rich sources of ash; therefore, refining which involves removing some or sometimes all of these parts of the millet, result in the loss of some of these important nutrients (Chowdhury and Punia, 1997; Serna-Saldivar and Rooney, 1995; Shahidi and Naczk, 2003a).

Humans require more than 22 mineral elements, which can all be supplied by an appropriate diet (White and Broadley, 2005). Some are required in large amounts, but others, such as Fe, Zn, Cu, I and Se, are required in trace amounts because higher concentrations can be harmful (Welch and Graham, 2004). Mineral elements, which are required in low concentrations, are known as trace elements, microelements or micro minerals, and are considered essential and beneficial to the human body. However, the diets of populations subsisting on cereals, or inhabiting regions where soil mineral imbalances occur, often lack Fe, Zn, Ca, Mg, Cu, I or Se (White and Broadley 2005). Iron is the most abundant microelement in the human (Carpenter and Mahoney, 1992). It should be noted that the body absorbs not all of the minerals in all foods in general, millet included after their consumption. When dealing with the absorption of minerals, their bioavailability must be considered. This is defined as the proportion of the mineral in the diet or the food that can be used by the organism (Fairweather-Tait, 1992).

Table 2:   Ca, Fe and Zn content of eight unprocessed cereal grains (100-gram samples)

	Wheat	Maize	Rice	Barley	Sorghum	Oats	Rye	Millet
Calcium, mg	29.0	7.0	23.0	33.0	28.0	53.9	33.0	8.0
	(4%)	(1%)	(3%)	(4%)	(4%)	(7%)	(4%)	(1%)
Iron, mg	3.19	2.71	1.47	3.60	4.40	4.72	2.67	3.01
	(21%)	(18%)	(10%)	(24%)	(29%)	(31%)	(18%)	(20%)
Zinc, mg	2.65	2.21	2.02	2.77	n.a	3.97	3.7	1.68
	(22%)	(18%)	(17%)	(23%)	(n.a.)	(33%)	(31%)	(14%)

Values in (parentheses) represent RDA%, n.a. = Not Available, Adapted from (Cordain, 1999)

Factors affecting the bioavailability of trace minerals to humans and/or animals in individual food and feeds have been the subject of numerous research reports (House, 1999). Foods in the various studies included mixed diets as well as individual cereals, fruits, leafy vegetables, legumes and animal products. Data from many of these studies have been summarized and presented in recent review articles. Some of these reviews included several elements and covered various aspects of trace element bioavailability in humans (Fairweather-Tait, 1992; 1996; Godber, 1990; Lonnerdal, 1989). It is well recognized that several trace elements are essential constituents of enzymes and play a vital role in human metabolism (Singh and Garg, 2006). All the nutrient elements are primarily supplied through diet. For all the elements essential for metabolism, there exists a range of intake over which their supply is adequate for the body (Singh and Garg 2006).

In all cereal grains, phosphorus is the mineral found in greatest amounts and its availability is negatively related to the amount bound by phytates and good sources of dietary calcium, except for finger millet and teff (Serna-Saldivar and Rooney 1995). At a range of 450 to 990 mg/g and 180 to 270 mg/g, respectively phosphorus and magnesium constitute the major minerals of pearl millet (Abdalla *et al.*, 1998b), although, the total phosphorus content appears to be influenced by the nature of the soil and applied fertilizers. As with the other cereals, the vitamin and mineral contents of pearl millet are concentrated in the pericarp, aleurone layer and germ (Taylor 2004).

Total mineral (ash) content of all millets is often higher than that of sorghum and other cereals (Klopfenstein and Hoseney, 1995). Values for individual minerals in pearl millet vary widely and depend largely on the mineral composition of the soils in the growing areas (Klopfenstein and Hoseney 1995), as well as the variety of the pearl millet in question.

Minerals such as calcium, phosphorus, zinc, magnesium and manganese are found in almost identical amounts in pearl millet and sorghum (Serna-Saldivar and Rooney 1995). The

iron and copper content is apparently slightly higher in sorghum than in pearl millet. Zinc, on the other hand, is the only major component that is significantly higher in pearl millet than sorghum. Khalil and Sawaya (1984) reported that calcium content of whole pearl millet flour is 22 mg/100 g (wet basis).

Like other cereal grains, pearl millet grain is an important source of thiamine, niacin and riboflavin (Taylor 2004). Riboflavin has, however, been implicated in lipid deterioration in the presence of light (Hamilton, 1999). Thus, it may also be a potential enhancer of the deterioration of pearl millet triglycerides. Due to its high oil content, pearl millet is also a good source of lipid-soluble vitamin E. Its content in pearl millet is about 23 mg/100 g (Taylor 2004). Besides its nutritional role in foods, vitamin E is also known for its antioxidant activity in the form of tocopherols (Bramley *et al.*, 2000; Huang *et al.*, 1995). Its presence could be of importance to pearl millet as an antioxidant that may curb triglyceride deterioration. Moreover, pearl millet is also a good source of the lipid-soluble vitamin A. Vitamin A content for pearl millet is typically about 24 Retinol Equivalents (Taylor 2004). These lipid-soluble vitamins are mainly located in the germ.

In order to retain all benefits offered by using pearl millet as far as its vitamins and minerals content is concerned; decortication of the grain is again strongly discouraged. Pearl millet is a good source of minerals if whole grain is consumed, but decortication can decrease total iron content by 30% (Lestienne *et al.*, 2007).

2.2.5   Pearl Millet total polyphenols and antioxidant activity

The total polyphenol content (TPC) of pearl millet varies due to genetic and environmental effects. Furthermore, the measured total phenolic content varies depending on the methods of analysis used. Chowdhury and Punia (1997) reported 714 mg/100 g of TPC in pearl millet grains. In contrast Elyas *et al.* (2002) who reported lower values ranging between 294 and

319 mg/100 g. These differences could be due to varietal differences, cultivation conditions, , method of analyses used, or any combination of these factors.

Phenolics which include phenolic acids, flavonoids and tannins are characterised by the presence of an aromatic ring with one or more hydroxyl groups (Naczk and M.Shahidi, 2004). Pearl millet phenolic acids include ferulic, coumaric, gentisic, cinnamic, caffeic, vanillic, protochatechuic, ρ-OH benzoic, syringic and sinapic acids (McDonough and Rooney, 1989). Other phenolic compounds reported to occur in pearl millet are flavonoids and these are antioxidants (Kaur and Kapoor, 2001; Pretorius, 2003; Rice-Evans, 2001) and may prevent various diseases associated with oxidative stress such as cancers, cardiovascular disease and inflammation. Phenolic compounds and phytic acid also possess antinutritional properties.

Polyphenol and phytic acid contents in millets have been reported to be higher than rice (Ravindran, 1991). Many processing methods are reported to reduce the level of these compounds. Thus polyphenols and phytic acids, which constitute a major portion of antinutritional compounds in cereals and pulses, could be appreciably reduced by following common methods of malting and blanching. Malting (72 hours) was found to be most effective in lowering the antinutritional content of pearl millet. The use of malting and blanching processing techniques, raise the nutritional quality of cereal grains (Archana and Kawatra, 1998).

2.2.6 Cereal grain foods and beverages

Pearl millet has antinutrients (phytic acid and polyphenols) present in considerable amounts (Mahajan and Chauhan, 1987) which limit protein and starch digestibility (Carnovale *et al.*, 1988; Thorne *et al.*, 1983); hinder mineral bioavailability, (Harland and Oberlease, 1987); and inhibit proteolytic and amylolytic enzymes (Sharma *et al.,* (1978). Despite these factors, itis

still considered highly palatable, a good source of protein, minerals and energy and contributes a great part of dietary nutrients for large segments of people in Africa and Asia.

There are several traditional millet foods and beverages, which can be categorized as whole-grain foods, foods made from meal/flour, and non-alcoholic and alcoholic beverages. These traditional products are consumed in Africa, the Indian subcontinent and East Asia (Taylor and Emmambux, 2008).

Pearl millets are largely used to prepare traditional, thick or thin, fermented or unfermented porridges in Africa (**Table 3**). The second major use in Africa is malting for the brewing of traditional beers and wines (Gomez and Gupta, 2003). For production of most of these foods, the grains are decorticated using mortar and pestle or mechanical dehullers. Germination and/or fermentation enhance the nutritional value of sorghum and millets by causing significant changes in chemical composition and elimination of antinutritional factors (Chavan and Kadam, 1989a; Chavan and Kadam, 1989b).

However, the small sizes of the grain as well as an inadequate supply of grains of suitable quality for processing (Gomez and Gupta, 2003; Taylor *et al.*, 2010) are some of the disadvantages in large-scale commercialization endeavours.

Table 3:   Most common indigenous millet-based fermented foods and beverages

Product	Microorganisms	Nature of use	Regions
Bagni	Unknown	Liquid drink	Caucasus
Boza	*Lactobacillus, Saccharomyces cerevisiae, Leuconostoc*	Thick, sweet slightly sour beverage	Albania, Turkey, Bulgaria, Romania
Busa	*Lactobacillus, Saccharomyces*	Liquid drink	Syria, Egypt, Turkestan
Chikokivana	*Saccharomyces cerevisiae*	Alcoholic beverage	Zimbabwe
Dalaki	Unknown	Thick Porridge	Nigeria
Darassum	Unknown	Liquid drink	Mongolia
Doro	Yeast and bacteria	Colloidal, thick alcoholic drink	Zimbabwe
Jaanr	*Hansenula anomala, Mucor rouxianus*	Alcoholic paste mixed with water	India, Himalaya
Kunu-zaki	LAB, Yeasts	Liquid drink	Nigeria
Merissa	*Saccharomyces*	Alcoholic drink	Sudan
Munkoyo	Unknown	Liquid drink	Africa
Ogi	*Lactobacillus plantarum, Saccharomyces cerevisiae, Candida mycoderma, corynebacterium, Aerobacter, Rhodotorula, Cephalosporium, Fusarium, Aspergillus and Penicillium*	Paste as staple for breakfast or weaning food for babies	Nigeria, West Africa
Thumba	*Endomycopsin fibuliger*	Liquid drink	Eastern India
Uji	*Leuconostoc mesenteroides, Lactobacillus plantarum*	Porridge as a staple	Kenya, Uganda, Tangayika
Vada	*Pediococcus, Streptococcus, Leuconostoc*	Breakfast or snack food	India

Adapted and modified from Blandino *et al.*, (2003).

2.2.7   Indigenous millet based beverages

Although millets do not contain gluten-forming proteins, they have the potential to add variety to our diet and may have useful health promoting properties, particularly antioxidant activity (Taylor and Emmambux, 2008).   In the developing world, millets have generally retained their popularity, even as people are becoming increasingly urbanized (Taylor and Emmambux, 2008).   There are a plethora of traditional beverages produced using a variety of traditional methods, that are widely consumed worldwide.   These traditional products are consumed in Africa, the Indian subcontinent and East Asia (Taylor and Emmambux, 2008). For the production of most of these foods and beverages, the grains can either be decorticated using mortars and pestles or mechanical dehullers, or germinated and/or fermented to enhance the nutritional value by causing significant changes in chemical composition and elimination of antinutritional factors (Chavan and Kadam, 1989a; Chavan and Kadam, 1989b). Traditional beverages produced from cereal grains can be classified as either alcoholic or non-alcoholic, depending on the process used.   Most are produced by crude/traditional fermentation processes.

These beverages can and do have various purposes in the diets of the consumers which include but are not restricted to supplementing dietary needs as well as alleviating malnutrition in children and adults.   These traditional beverages are rarely mentioned, and are not classified with other beverages as far as beverage reviews are concerned.   Further studies into the processing technologies and subsequent commercialization of these beverages are necessitated by the paucity of information relating to the commercialization of some of these products.

In West African countries, e.g., Senegal, millet is used for making couscous, pap and fritters.   In Cameroon, pearl millet-based gruels and steamed cakes are prepared for feeding infants and preschool children (Gomez and Gupta, 2003).   Oskikundu is a very popular pearl

millet beverage in Namibia (Taylor and Emmambux, 2008), a lactic acid fermented product made from cooked pearl millet flour with added sorghum malt flour (Taylor, 2004a). A similar product, kunun tsamiya, which literally means, "Gruel of tamarind available in Nigeria is produced from extrusion cooked instant flour of pearl millet." The product is flavoured with tamarind oil, another example of an acid flavoured African pearl millet food (Taylor *et al.*, 2010). Similar products called togwa, made from maize meal and finger millet malt (Oi and Kitabataki, 2003), and kunun zaki , which may be made from pearl millet, sorghum and white fonio, are produced in Tanzania and Nigeria, respectively. In Zimbabwe, a traditional fermented beverage combines milk and finger millet to give a highly nutritious product (Mugocha *et al.*, 2000).

Malted pearl millet in combination with legumes has been used to prepare malted weaning foods. Pearl millet can be malted and used wholly or partially in place of sorghum malt in the traditional or industrial brewing of opaque beer (Gomez and Gupta, 2003). Malted finger millet powder, which is mixed with hot milk or water to make a beverage, is a popular commercial product in India (Malleshi and Hadimani, 1994).

''Mageu,'' also commonly known as ''amahewu'' or ''magou,'' is a southern African maize-based fermented beverage or gruel (Taylor, 2004b). It is produced in the home and in institutions such as hospitals and mine hostels. It is also produced on a large industrial scale in South Africa and Botswana. Industrially produced mageu has a solids content of ~8% with an energy content of 1595 kJ and a pH of approximately 3.5. Mageu is regarded as a nutritious, energy food, ideal for those doing hard manual work (Taylor, 2004b).

It should be noted that fermentation seems to be the preferred method of processing these nonalcoholic beverage products. Very little malting is employed for the production of most of the non-alcoholic beverages, however, cereal grain malting is carried out on very large scales in several sub-Saharan African countries for the production of traditional beers.

The majority of the non-alcoholic beverages produced from cereals are either extruded or gun puffed in order to pre-gelatinize the starch present in the cereal (commercial cereal grain beverages) or fermented using microorganisms, preserving the final product by reduction in pH as a result of the production of acids (traditional cereal grain beverages). Due to problems with dispersibility of the malted millet powder, many of the products on the market contain less than 10% millet malt (Taylor and Emmambux, 2008).

2.3   Potential Health Benefits of Millet Grains and their Fractions

Millets should provide several potential health benefits such as preventing cancer and cardiovascular diseases, reducing tumour incidence, lowering blood pressure, risk of heart disease, cholesterol, and rate of fat absorption, speed up gastric emptying, and supplying gastrointestinal bulk required for human health (Gupta *et al.*, 2012; Truswell, 2002). Recently, the U.S. Dept. of Agriculture's nutritional guidelines put grains and/or grain products at the base of the food guide pyramid to emphasize grains or grain product consumption as part of a normal diet for optimal health (Ahmed and Blumberg, 2009; Dietary-Guidelines-Advisory-Committee, 2005).

Evidence from research studies has shown that diets rich in plant foods are protective against several degenerative diseases such as cancer, cardiovascular ailments, diabetes, metabolic syndrome and Parkinson's disease (Chandrasekara and Shahidi, 2012; Manach *et al.*, 2005; Scalbert *et al.*, 2005). In addition, there is strong evidence that whole-grain cereals protect the body against age-related diseases such as diabetes, cardiovascular diseases and some cancers (Fardet *et al.*, 2008). For years, the vitamins, minerals, essential fatty acids and fibre in whole grains were believed to be responsible for their health benefits, but recent research suggests that the combination of these other bioactive substances work to exert aforementioned positive effects. These bioactive substances include resistant starch,

oligosaccharides, lipids, antioxidants (phenolic acids, avenanthramides, and flavonoids), hormonally active compounds (lignans and phytosterols), and antinutrients (phytic acid and tannins) (Miller, 2001; Edge *et al.*, 2005).

2.4 Traditional Beverage Preparation Processes and their Effects on the Antinutrient and Physico-Chemical Properties of Cereal Grains

Processing methods used in the manufacture of traditional beverages include hydration/soaking, decortication/dehulling, fermentation, roasting and cooking. These traditional food-processing methods have been observed to cause appreciable changes in the chemical composition, as well as in the nutritional and functional properties of cereal grains in general, and pearl millet specifically. These processes and their impact on nutritional and some functional properties of cereal grains and their products are reviewed.

2.4.1 Hydration / Soaking

Soaking is a simple technological treatment that is often used by mothers to prepare complementary foods at home. It can be a simple prolongation of the obligatory washing of the seeds and can have other advantages, such as facilitating dehulling or swelling of seeds (Lestienne *et al.*, 2005). Previous studies have shown that a long soaking period before fermentation or germination, leads to a reduction in phytate content and to an enhancement of mineral HCl-extractability, used to estimate mineral bioavailability (Sandberg and Svanberg, 1991; Duhan *et al.*, 2002). The extent of phytate reduction depends on the species, pH, length and conditions of soaking. A simple soaking procedure appropriate for rural subsistence households has been developed that can reportedly reduce the phytate content of unrefined maize flour by 50% (Hotz and Gibson 2001). Davidsson *et al.* (2004) have reported improvements in the absorption of iron, zinc, and calcium in cereal-based foods prepared with reduced phytate content.

Soaking cereal and most legume flours (but not whole grains or seeds) in water can result in passive diffusion of water-soluble Na, K, or Mg phytate, which can then be removed by decanting the water (Hotz and Gibson, 2001; Perlas and Gibson, 2002). Some polyphenols and oxalates that inhibit iron and calcium absorption, respectively, may also be lost by soaking (Erdman and Pneros-Schneier, 1994). Lestienne *et al.* (2005) observed that depending on the botanical origin of the seeds, a significant reduction in phytate content (between 17% and 28%) was obtained by soaking whole seeds for 24 h at 30°C. Two groups were distinguished: millet, maize, rice and soybean, which showed a significant reduction in phytate content; and sorghum, cowpea and mung bean, which showed no significant reduction.

2.4.2 Decortication / Dehulling and Milling

Decortication by pestle and mortar or with abrasive dehullers removes the grains' outer layers; therefore, decorticated or 'pearled' kernels have reduced levels of fibre, ash, and fat. Sorghum and millets are usually decorticated to remove between 12 and 30% of the grain. Increased decortication causes increased losses of fibre, ash, and fat. Decortication slightly reduces protein and lysine content due to partial degermination (Serna-Saldivar and Rooney 1995).

Decortication of brown sorghum significantly reduces the amount of condensed tannins and presumably can overcome their deleterious effect on the nutritional quality of the product (Serna-Saldivar and Rooney 1995). Nwasaru *et al.* (1988) observed that decortication removed 37% of the original grain weight and reduced tannins from 4.5 to 0.2 catechin equivalents.

Serna-Saldivar et al. (1988a; 1988b) observed that nutrient digestibilities of decorticated sorghums were slightly higher than those of parent whole grain, however,

nitrogen retention and protein efficiency ratios were lower due to removal of the germ. Dehulling (decorticating) has been reported to lead to loss of protein, insoluble dietary fibre, fat, ash, lysine and other amino acids (Obilana and Manyasa, 2002).

The purpose of milling is first to reduce the grain in a dry or semi-wet state in such a way that the different botanical components can be separated as cleanly as possible by sifting. The separated fractions such as the endosperm, if too coarse, can then be finely milled in a simple processing step without the need for a subsequent sophisticated separation process. The mechanical forces are applied either by accelerating the grain against a metal surface (impact) or by utilizing the squeezing and shearing forces of rollers or rotating disks or pressurized cylinders (attrition), where not only are there grinding effects due to metal-to-seed action but also the seed surfaces rub against each other. By introducing millstone elements or sieves (abrasion) as well as brushes, the friction forces are considerably increased, leading to special effects. Even when milling machinery is referred to as exploiting just one of these mechanical principles, in practice there are several principles working together in the milling process (Munck, 1995).

The grain milling process can be carried out, by either hand or machine, under dry; semi-wet or wet conditions, depending on the moisture content of the grain prior to milling. The effect of the milling process on the nutritive and anti-nutritive values of the ensuing flour is determined by the condition under which the grain is milled. Generally, flour products from mechanically milled processes are acceptable and have improved shelf life, whereas the traditionally milled products retain more nutrients (Obilana and Manyasa 2002).

A sound, dry uniform grain, uninfected by moulds and insects, of a defined variety and quality is the prerequisite for obtaining high-quality products by milling. As with wheat and maize, it is often advantageous to condition sorghum and millet seeds by adding water, administered in a mixing screw, followed by a resting period in a bin before milling.

Conditioning toughens the outer bran parts of the seed and makes them less prone to fine disintegration in milling, facilitating separation of the coarse bran particles from the endosperm, which due to its crystallinity, is more finely milled. While in traditional industrial wheat milling about 2-5% of water is added in the conditioning step, local hand pounding of sorghum and millets often uses up to 20% (Munck, 1995).

The key raw material characters for grain milling quality are the size, form, and structure of the kernel, including the development of its outer (bran) layers and the endosperm hardness. Also of great importance for quality is the distribution of the antinutritional factors as well as of outer pigments among the botanical tissue components that are removed in the milling and separation processes. Millet species in general have much more nutritionally favourable amino acid composition than sorghum, including a relatively high level of lysine. Due to their small grain size, milling is often more complex than with sorghum (Munck 1995).

The pestle and mortar or stone mill is used in Africa for further reduction of decorticated grain to flour. The flour reduction is a more laborious and time-consuming process than decortication. In the pestle and mortar system, the decorticated grain is placed in the mortar and pounded continuously with the pestle. The flour or semolina thus obtained is sieved, and the overs are returned to the mortar for further pounding. Locally fabricated sieves of various mesh sizes are used to obtain the flour particle size appropriate to the food product desired. The flour product is usually sun-dried and cooked preferably within 48 hr. Its high moisture content may allow partial fermentation during storage (Murty and Kumar, 1995).

The stone mill consists of a base plate and a roller. The base plate is placed on three levelling stones so that it slopes away from the operator, who sits, squats, or kneels behind the mill. A dish or a cloth is placed in front of the base plate to receive the ground material. The

roller stone is oval or round in cross section but becomes flattened after some use (Murty and Kumar, 1995).

One characteristic of milled pearl millet flour is its organoleptic implications resulting from the mousy odour exuded after brief storage in the presence of some moisture. This odour has been found to be due to enzymatic action and not to be associated with the rancidity of grain lipids, also a common trait in pearl millet flour due to its high fat content (Obilana and Manyasa, 2002). The enzymatic action responsible for the off odour has been determined to be on the flavone portion of one of the C-glycosyl flavones in the grain (Hoseney *et al.*, 1992).

Hulse *et al.* (1980) reviewed the information on the nutritional status of milling products obtained from processing sorghum and millets by traditional versus mechanical methods. In general, the traditionally milled products retained relatively more nutrients. However, the flour products from mechanical methods were more acceptable and had an improved shelf life.

2.4.3   Fermentation

Fermentation by lactic bacteria and yeast of cereals for complementary food has many potentially positive effects. Fermentation causes degradation of grain components, especially starch into soluble sugars, by enzymes from both the grain and fermentation media (Chavan and Kadam, 1989a). Fermentation improves the nutritional quality of the porridge. Protein digestibility might be enhanced. The effect on the viscosity of the prepared porridge is only marginal (Kouakou *et al.*, 2008).

Fermentation is widely used throughout rural Africa where modern food preservation methods are still not common. It helps to preserve many food products, provides a wide variety of flavours, and significantly improves the nutritional properties of the raw material.

Fermented foods are also produced and consumed worldwide (Gotcheva *et al.*, 2001; Mugocha *et al.*, 2000). Due to the fact that the chemical compositions of millet grains and their food products are modified by fermentation, they are used to produce different kinds of traditional fermented foods in developing countries in Africa and Asia. Fermentation has been reported to decrease the levels of anti-nutrients in food grains and increase the protein availability during digestion, and nutritive value of the millet based foods. Fermentation of pearl millet grains caused significant reduction in anti-nutritional factors, which was accompanied by significant improvement in the in-vitro protein digestibility (IVPD) (Hassan *et al.*, 2006). This improvement in the IVPD caused by fermentation could be attributed to the partial degradation of complex storage proteins to more simple and soluble products (Chavan *et al.*, 1988).

Due to the presence of anti-nutritional factors, including polyphenols and phytic acid, the availability of minerals from pearl millet may be low. Natural fermentation, sprouting and damirga (an Egyptian fermented millet food product consisting of a fine sour and white flour obtained traditionally from pearl millet grains, which is used to make Asedat-damirga (stiff, white porridge), Nasha-beida (white nasha) or Kisra-beida (white kisra) preparation were previously reported to decrease phytic acid (Abdalla *et al.*, 1998a; Abdalla *et al.*, 1998b; Khetarpaul and Chauhan, 1989; Kumar and Chauhan, 1993). Fermentation, dehulling and germination reduced polyphenols content of pearl millet (Khetarpaul and Chauhan, 1990).

Fermentation and sprouting were observed by Ahmed *et al.* (2010) to significantly reduce total phosphorus (P) content, while damirga preparation tremendously reduced it by 60.5%. The three traditional processes studied brought about a significant enhancement in non-phytate P and inorganic P with a corresponding decline in phytate P content of the two cultivars. The polyphenol content of fermented dough and damirga flour were found to be significantly lower compared to the whole flour. In contrast, sprouting significantly raised the

polyphenol content of the two cultivars. Damirga preparation, sprouting and fermentation significantly reduced the phytic acid content of the two cultivars by 10.60-68.50% (Ahmed et al. 2010). They also observed changes in the chemical composition viz. moisture, ash, fibre, fat and protein of the two cultivars of pearl millet tested. Moisture and oil contents were significantly reduced due to fermentation, damirga preparation and sprouting. Fermentation significantly increased the ash and protein content, but significantly, reduced the fibre content. Damirga preparation significantly lowered ash, protein and fibre content. On the other hand, sprouting significantly enhanced the fibre and protein content, but the mineral contents of the two cultivars of pearl millet were markedly reduced during the processing.

The chemical components of ogi, with the exception of starch, were reduced when the millet grain used was fermented into the ogi. The availability of starch and protein for digestion was higher in the ogi than in the grain. Grain protein quality was marginally improved by the increase in lysine and tryptophan contents due to fermentation only, but greatly improved when the grain was germinated before fermentation. Vitamin B2 content was increased while vitamin A and flavanol contents and paste viscosity were reduced by the conversion of grain into ogi. It may be concluded that the conversion of millet into ogi improves its nutritive value (Akingbala *et al.*, 2002).

According to Osman (2004), enzyme inhibitory activities were significantly decreased during a 24 h fermentation period of three different sorghum varieties used in his experiment. Trypsin inhibitory activity was reduced by 58%, 43% and 31% in Hamra, Shahla and Baidha, respectively, whereas amylase inhibitory activity was reduced by 74, 75 and 78% in the three varieties after a 24 h fermentation. Phytic acid contents of the three varieties were markedly reduced because of fermentation. The tannin content of Hamra, Shahla and Baidha were significantly reduced by, respectively, 31%, 15% and 35% after fermentation. Fermentation significantly improved the in vitro digestibility of sorghum proteins.

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2.4.4   Cooking and other processing methods

Technological treatments such as autoclaving, germination, soaking, and cooking (Fernandez *et al.*, 1996; Urbano *et al.*, 1999; Urbano *et al.*, 1995; Vidal-Valverde *et al.*, 1994) have been applied to reduce the level of antinutritional factors present in legumes and to improve their nutritional value.   Autoclaving has been used to destroy the heat labile antinutrients present in legumes and to increase the digestive use of protein from this foodstuff (Siddhuraju and Becker, 2001).   However extreme heating conditions may cause irreversible damage to protein and decrease the nutritive value of heat-treated foods (O'Brien and Morrisey, 1989).

Food processing procedures such as heat treatment (cooking), fermentation, germination, malting and soaking, as well as treatment with phytase, generally increase Zn bioavailability in foods by reducing the amount of dietary phytate or its lesser phosphorylated derivatives (Lei *et al.*, 1993; Sandstrom *et al.*, 1987; Schlemmer *et al.*, 1995; Svanberg *et al.*, 1993).   Moreover, microbial phytase added to a high-Zn diet that contained phytate did not affect the apparent absorption of Zn by rats (Rimbach *et al.*, 1995).   Generally, even limited dephosphorylation of inositol hexaphosphate markedly improves Zn bioavailability (Lonnerdal *et al.*, 1989).

Many biochemical changes affecting nutritional quality as well as texture and flavour of wheat flour occur during sourdough baking, as reviewed by Katina *et al.* (2005).   Levels of folate and easily extractable phenolic compounds have been shown to increase (Kariluoto *et al.*, 2004), whereas levels of phytate (Frolich *et al.*, 1986; Larsson and Sandberg, 1991), alkylresorcinols (ARs) (Verdeal and Lorenz, 1977) and tocopherols (Piironen *et al.*, 1987) have been reported to decrease during the sourdough baking process.

During soaking, germination, malting and bread making endogenous phytases activate and hydrolyse IP6 (Inositol hexaphosphate) (Beal and Mehta, 1985; Eskin and Wiebe, 1983;

Larsson and Sandberg, 1995; Larsson and Sandberg, 1991; Turk and Sandberg, 1992). The highest activity of phytase was found in wheat, rye, and barley (Konietzny *et al.*, 1995; Sandberg and Svanberg, 1991) among cereal phytases, and also legume phytases seem to have a broad activity (Eskin and Johnson, 1987; Han and Gallagher, 1987). The use of specific enzymes such as phytase produced by microorganisms and cereals may result in extensive phytate degradation (Beers and Jongbloed, 1992; Gustafsson and Sandberg, 1995). Beers and Jongbloed (1992) observed an improvement in the performance of piglets and apparent digestibility of phosphorus by the addition of phytase from *Aspergillus niger* in piglet diets.

Finger millet is the richest source of Ca among cereals (340 mg/100 mg) of which only 162 mg/100 mg is bioavailable in the raw grain. Processing improved its bioavailability up to 227 mg/100 mg. Iron availability improved from 0.34 to 1.4 mg/100 mg due to processing (Indumadhavi and Agte, 1992).

Hurrell *et al.* (2003), observed that the mean iron absorption in non-anaemic adult humans (with a wide range of iron stores) from precooked, roller-dried, cereal-based complementary foods reconstituted with water was very low, and ranged from 0.33% to 1.80%; similar findings were reported by Cook *et al.* (1997). Hurrell *et al.* (2003) also observed that when the phytic acid in their study sample was almost completely degraded by adding a commercial phytase during manufacture, iron absorption increased 2–12-fold.

Phytic acid degradation of low-tannin sorghum porridges increased iron absorption only 2-fold, and no improvement in iron absorption was observed on dephytinisation of high-tannin sorghum (absorption ratio: 1.34) (Hurrell *et al.*, 2003).

Press drying is a practical technique to process millet and cowpeas into dry flakes with acceptable physicochemical, nutritional, and storage characteristics. Presumably, other cereals and grain legumes might also be press-dried. Utilization of simple utensils, such as a pot for boiling, a griddle for drying, and a spoon for spreading, makes this process suitable for

low-income families living in rural areas in developing countries. However, press-drying, like many other home processes, involves tedious work that users need to learn (Almeida-Dominguez et al. 1993). Roasting is a dry heating of the cereal that can improve protein digestibility if it is not excess, but has little or no effect during further preparation of the cereal (Nout, 1992a).

2.5   Technologies that could be Utilised in the Commercialization of Traditional Beverages

The traditional methods described earlier would lead to products that though acceptable to consumers, invariably had short shelf lives because of several inherent and intrinsic properties of the ensuing products. Incorporating more modern or alternative technologies into the process of manufacturing some of the previously discussed traditional foods and beverages, could lead to beneficial results which would include but are not restricted to; increased shelf-life, better overall acceptability of product as a result of better aesthetic qualities, better marketability of resulting product, and others. This could also have far-reaching positive effects on down-stream, as far as the farmer is concerned and up-stream as far as the manufacturer and distributors are concerned. The more modern or alternative technologies that will be considered in this article are extrusion cooking and malting.

2.5.1   Extrusion processing

Extrusion cooking is a relatively modern, high temperature, short-time processing technology that was invented in 1940s to manufacture snack foods (Athar *et al.*, 2006). This technique has gained ground in human food and animal feed industries world-wide, primarily for the processing of cereal grains (Athar *et al.*, 2006). The best-known products are low-density foamed corn and rice breakfast and snack foods that are widely available. Similar products made from legumes are available in Asian countries.

Extrusion is one of the most versatile and well-established industrial processes used in the food industry today. It is being increasingly applied worldwide to materials including snacks, cereals, pastas, textured vegetable proteins, pet foods, animal feeds, instant beverage products, and meat analogues and extenders. The process generally involves the conversion of a plasticized biopolymer-based formulation into a uniformly processed visco-elastic mass that is suitable for forming or shaping into products by a die. The mechanical and/or thermal energy used to transport the material through rotating helical screws and the die brings about physical and chemical changes in the feedstock (Rizvi et al. 1995).

Extrusion cooking, particularly in the snack food industry, is a complex process that differs from conventional processing by using high shear rates and high temperatures (>150°C) for very short periods (seconds) (Athar *et al.*, 2006). A wide range of thermo-mechanical and thermo-chemical processes are involved during extrusion cooking, including shear, Maillard reactions, protein denaturation and hydrolysis. These processes result in the physical, chemical and nutritional modification of food constituents (Linko *et al.*, 1981).

The extrusion process can be divided into two general types. First, non-cooking or forming extrusion (also referred to as cold extrusion), which transforms the feed into a homogeneous cohesive extrudates without cooking. The pressure generated by the screws or piston forces the material through the die. Second, extrusion cooking which, as the name implies, involves the raw ingredients being cooked by the combined action of heat, mechanical shearing and pressure (up to 250 °C and 25 MPa), and is akin to a continuous chemical reactor process operating at high temperature and pressure. The resulting water-plasticized biopolymer melt may be homogeneous or heterophase (e.g. thermodynamically incompatible proteins and carbohydrates), and is subsequently fixed by rapid conversion, as it exits through a forming die, from a flowable state to a rubber-like state and finally to a shelf-stable glassy state on cooling and/or drying. Simultaneously, swelling on exiting the die, due

to the viscoelastic properties of the melt and moisture flash-off, cause the extrudates to expand anisotropically (i.e. to a different extent in different directions), imparting a porous structure to the product (Rizvi *et al.*, 1995). Extrusion cooking processes are further sub-divided based on thermodynamic considerations, pressure development or shear intensity (Hauck and Huber, 1989).

It is already known that extrusion cooking is used to produce expanded snacks and shaped foods. This cooking processing carried out using high temperatures and short time of treatment, gives finished product with high quality (high digestibility and nutritional value) and reduces degradation reactions that occur during thermal processing (for example, loss of nutrients). In the extruders, the components are mixed, sheared and subjected to elevated temperatures and pressures. So that the dough shows a plastic consistency that favours the expansion of the product at the exit of the die (De Pilli *et al.*, 2007).

Onyango *et al.* (2004a) observed in the studies of digestibility and antinutrient properties of acidified and extruded maize–finger millet blend in the production of uji that *in vitro* starch digestibility significantly improved after the raw blend was extruded, and all the extrudates did not differ significantly from each other. Other changes that occur in the starch granules and contribute to improved digestibility are hydration, loss of structural integrity and partial solubilisation of starch molecules (Bjork and Asp, 1983; Dahlin and Lorenz, 1993; Garcia-Alonso *et al.*, 1999). Starch needs to be gelatinized for efficient hydrolysis since gelatinized starch is more susceptible to enzymatic attack (Akdogan, 1999).

Extrusion denatures proteins by opening up their quaternary and tertiary structures, thus inducing polymerization, cross-linking and reorientation to fibrous insoluble structures (Akdogan 1999). High temperatures, intense mechanical shear (as are encountered during extrusion cooking) and low pH further promote structural changes and denaturation of storage proteins and increase their accessibility to proteolytic enzymes (Onyango et al. 2004a).

Thermal inactivation of protease inhibitors and anti-physiological factors such as polyphenols also contribute to improved protein digestibility (Bjork and Asp 1983).

The effect of extrusion cooking on phytic acid has not been clearly elucidated (Onyango *et al.*, 2005). Ummadi *et al.* (1995) and Gualberto *et al.* (1997) reported no change whereas Le Francois (1988) reported a decrease in phytic acid content in extruded products. El Hady and Habiba (2003) reported significant reduction in tannin content after extruding legume seeds at different moisture contents. Onyango *et al.* (2005) observed that tannin content decreased after extrusion of the unfermented blend (maize-finger millet) with further reduction after fermentation and extrusion.

Alonso *et al.* (1998) observed that extrusion was the most effective method for reducing trypsin inhibitor activity (TIA) when compared with the other treatments. About 95% reduction was caused by extrusion processing this may be due to reactions involving deamidation splitting of covalent bonds, such as hydrolysis of peptide bonds at aspartic acid residues, and interchange or destruction of disulphide bonds because of the high temperatures and pressure to which the proteins are exposed (Adams, 1991).

Fapojuwo *et al.* (1987), observed in their studies using two low-tannin sorghum varieties that extrusion improved the digestibility of one variety from 45.9 to 74.6% and of the other from 43.9 to 68.2%. The cooking temperature was the variable that most influenced digestibility. So far, sorghum and millet extrusion products have not yet been produced on a commercial scale.

2.5.2   Germination and Malting

Plant foods can be improved as sources of essential micronutrients either by increasing the concentrations of nutrients in the food, increasing the bioavailability of micronutrients in the food, or both of these. Quantities of minerals in edible portions of crops are influenced by

numerous complexes, dynamic and interacting factors, including plant genotype, soil properties, environmental conditions and nutrient interactions. Similarly, numerous dietary and host factors interact to affect the bioavailability of mineral nutrients in plant foods. Micronutrient bioavailability apparently can be improved by either increasing the quantity of substances within plant foods that enhance the absorption and utilization of micronutrients or by decreasing the quantity of dietary antinutrients that inhibit micronutrient absorption. However, processes that control and regulate the bioavailability of trace elements in plant foods consumed in mixed diets are not fully understood (House, 1999). The changes required to improve nutrient bioavailability in plant foods can be achieved through malting.

Malting is the germination of cereal grains in moist air under controlled conditions. The primary objective being to promote the development of hydrolytic enzymes, which are not present (or are present in limited amounts) in un-germinated grains. The main enzymes produced during germination that intervene in the hydrolysis of starch are α- and ß-amylases (Palmer, 1989). The α-amylases are liquefying enzymes that convert starch into soluble sugars (Traore *et al.*, 2004), while β amylases are saccarfying enzmes that release soluble sugars. Alpha amylase activity has been observed to increase during germination of cereals, especially sorghum and millet. This enzyme hydrolyses amylase and amylopectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Gibson and Ferguson, 1998).

Malting of cereals is a processing procedure traditionally used in many African countries for the manufacture of alcoholic drinks (Dewar *et al.*, 1997; Taylor and Dewar, 2001) like opaque beers; weaning foods, and other traditional dishes (Serna-Saldivar and Rooney 1995). The malting process can be divided into three physically distinct operations, i.e. steeping, germination (sprouting) and drying (Dewar *et al.*, 1997). Malting causes up to

30 % dry matter loss (Chavan and Kadam, 1989b); decreased levels of prolamine, fat, tannins and starch; and increased levels of free amino acids, albumins, lysine, reducing sugars, and most vitamins including synthesis of vitamin B-12 and C (Almeida-Dominguez *et al.*, 1993; Chavan *et al.*, 1981; Okoh *et al.*, 1989; Osuntogun *et al.*, 1989). The activation of intrinsic amylases, proteases, phytases, and fibre degrading enzymes disrupts protein bodies (Taylor *et al.*, 1985). Pelembe (2001) observed that malting (germination) significantly reduced the mousy odour, characteristic of ground pearl millet meals when stored.

Germination has been reported to improve the nutritional quality of seeds by increasing the contents and availability of essential nutrients, by lowering the levels of antinutrients (Chavan and Kadam 1989b).

Different traditional processes used in cereal malting were characterized and some biochemical modifications occurring in seeds were studied to examine the possibility of using malted cereal flours to reduce the viscosity of gruels (Traore *et al.* 2004). During the malting of a variety of cereal grains, a significant increase in sucrose, glucose and fructose content was noted, whilst a decrease in phytate content was more obvious in millet seeds than in red sorghum and maize seeds. Increase α-amylase activity was observed in all 3 types of cereals, but more in red sorghum seeds than in millet and maize seeds. Flours from malted red sorghum or millet seeds presented useful characteristics (α-amylase activity and nutrient contents) for incorporation into infant flours to improve the energy and nutrient density of gruels.

Mbithi-Mwikya *et al.* (2000) observed that germination was effective in increasing the HCl extractability of minerals. Calcium and iron extractability increased from 76.9% and 18.1% in the raw grain to 90.2% and 37.3%, respectively. Extractability of Zn, a trace element, increased from 65.3% to 85.8% at 96 h germination. Phytate content decreased from 0.36 g to 0.02 g per 100 g dry matter. Phytates bind with minerals forming insoluble

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complexes, which are not extractable in 0.03 mol/l HCl. These increases in the HCl extractability of minerals could be explained by the observed decrease in phytate content. Similar findings have been observed in faba beans, where phytate levels decreased by up to 77% during a 10 day germination period (Eskin and Wiebe, 1983).

The increase in HCI extractable minerals may be attributed to a reduction in phytate and the presence of enhancers such as organic acids and ascorbic acid (Indumadhavi and Agte, 1992). Sripriya *et al.* (1997), found germination was effective in increasing the extractability of the trace elements like Cu, Zn and Mn from 0.32, 1.28, 4.27 in the raw grain to 0.45, 1.57, 4.69 (mg/100 mg) which further increased to 0.62, 1.73 and 5.20 (mg/100 mg), respectively, on fermentation (48 h).

Badau *et al.* (2005) observed in their studies on pearl millet that the Ca content of the un-malted grains varied from 53.6 to 122 mg/100 g on dry matter basis and the HCl extractability of Ca varied from 42.3 to 45.3%. HCl extractability of Ca increased progressively from 0 to 72 h of germination and remained almost constant ($P > 0.05$) up to 96 h of germination for each of the cultivars tested. The Fe content of the grains varied from 16.3 to 18.3 mg/100 g on dry matter basis and the HCl extractability varied from 18.5% to 20.7%. There was no significant difference between the Fe extracted from un-malted and steeped grains. However, HCl extractability of iron increased rapidly from the beginning of the germination. There were no significant differences between the cultivars at various levels of germination until 72 h of germination. The Zn extracted from un-malted and steeped grains did not differ significantly. The total Zn content of un-malted grains varied from 2.82 to 3.24 mg/100 g on dry matter basis and the HCl extractability varied from 50.5% to 61.5%. They concluded from their studies that the germination of various pearl millet cultivars increased the HCl extractable parts of Ca, Fe, Zn, P, I, Cu and Mn, and also reduced the phytic acid content of the pearl millet cultivars significantly.

Phytase (an enzyme which hydrolyses phytate to phosphate and myoinositol phosphates) activity was also observed to increase during germination of wheat, barley, rye and (Larsson and Sandberg, 1992). These findings are in agreement with those of Badau *et al.* (2005) who observed, in their studies on pearl millet, that germination significantly reduced the phytic acid content of the grains, and Archana and Kawatra, (1998) who observed that the destruction of polyphenols (38 to 48%) and phytic acid (46 to 50%) was significantly higher in grains subjected to malting than blanching. The overall results suggested that malting with 72 hours of germination was most effective in reducing the antinutrient levels of pearl millet grains. Sripriya *et al.* (1997) also found that total phenols decreased on germination from 1.43 to 1.28 g/100 mg and increased on fermentation to 1.86 g/100 mg. Khetarpaul and Chauhan (1991) reported a similar increase in polyphenols during fermentation of pearl millet flour due to microbial enzyme activity, which may hydrolyse the condensed tannins to lower molecular weight phenols.

Opoku *et al.* (1981) reported that total oxalate in pearl millet decreased from 0.619 to 0.433% when the grain was malted for 84h. Perhaps a more significant observation from a health standpoint was that soluble oxalate decreased from 0.520 to 0.068% with malting (Klopfenstein and Hoseney, 1995). Different procedures have been proposed to eliminate or reduce antinutritional factors in legumes. Home practices such as soaking, dehulling and cooking effectively improve the nutritional value of legumes (Egounlety and Aworh, 2003). Malting significantly increase phosphorus availability in sorghum and millets due to increased phytase activity. These processes considerably improve bioavailability of other minerals as well (Serna-Saldivar and Rooney, 1995).

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CHAPTER 3: **INFLUENCE OF GERMINATION TIME ON THE NUTRITIONAL COMPOSITION AND AMYLASE ACTIVITY OF PEARL MILLET VARIETIES**

3.1 Abstract

This study was undertaken to determine the effects of germination time on the nutritional and enzymatic (α-amylase activity) properties of two varieties of pearl millet (*Pennisetum glaucum*) [Hybrid babala (Agrigreen – (AgG)) and Babala – (Ba)]. The two pearl millet varieties, AgG and Ba, were cleaned steeped and germinated at $30^{o}C$ for 72 h. Samples were withdrawn every 12h and dried at $50^{o}C$ for 48h. The un-malted and malted pearl millet varieties were analysed for germinative energy, malting loss, proximate content and amylase activity. The germinative energy (GE) of AgG, ranging from 87.33% to 95.33% at 72 h of germination, was significantly ($p \leq 0.05$) higher than the GE of Ba, which ranged from 50% to 82% at 72 h of germination, an indication of AgG's better suitability for malting. Malting led to significant increases in protein content (11.70 to 14.66 mg/100 g for AgG and 11.39 to 12.85 mg/100 g for Ba) and amylase activity (0.92 to 2.67 μg maltose for AgG and 1.04 to 2.60 μg maltose for Ba), but led to significant decreases in the moisture, fat and ash content of both varieties. The increase in amylase activity may lead to increased breakdown of starch to dextrins, causing a decrease in viscosity in the final product, which is a desirable outcome for a beverage product.

3.2 Introduction

The malting process can be divided into three physically distinct operations, steeping, germination and drying (Dewar *et al.*, 1997). The primary objective of malting is to promote the development of hydrolytic enzymes, which are not present in the un-germinated grain. The main enzymes produced during germination that hydrolyse starch are α- and ß-amylases (Palmer, 1989). According to Traore *et al.* (2004), the α-amylase is a liquefying enzyme which has been observed to increase significantly together with a significant increase in some

nutrients and a significant decrease in phytate content during malting of red sorghum, millet and maize seeds. This enzyme hydrolyses amylose and amylopectin into dextrins and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Gibson and Ferguson, 1998).

The objective of this experiment was to identify the germination time (h) of highest α-amylase activity for two different varieties of pearl millet [Agrigreen (AgG) and Babala (Ba)] germinated at 30°C and relative humidity (RH) of ~ 98% during a period of 72 h. The identified time would be subsequently used during the malting process, in the production of a pearl millet instant beverage powder (PMIBP).

3.3   Materials and Methods

3.3.1   Source of pearl millet grains and chemicals:

Two different varieties of pearl millet (*Pennisetum glaucum*) Babala (Ba) and hybrid Babala (Agrigreen (AgG)) were obtained from Agricol Pty. Ltd. Cape Town, South Africa. All chemical reagents were obtained from Sigma-Aldrich South Africa.

3.3.2   Cleaning

The pearl millet grains were placed in a tray and the chaff and damaged grains as well as stones/pebbles together with all other extraneous matter were removed by hand and discarded.

3.3.3   Germination

The method of Pelembe *et al.* (2002a) with some modifications was used to determine the optimum germination time of the grains as determined by α-amylase activity. Cleaned grains

(2500 g each) of both pearl millet varieties were steeped in 3 L 3% NaOH at room temperature (23 – 26°C) for 3 h in 10 L container. After steeping, the grains were drained using cheesecloths and washed thoroughly under running tap water, then allowed to drain off excess water. The grains were divided in to five (5) lots of 500 g each and placed into 1 L plastic buckets, which were then put in to a proofing oven/germination chamber (Snijders Scientific, Holland) at 30°C for 72 h. Every 12 h during the germination period, the millet was rinsed under running tap water, then drained properly and returned to the proofing oven/germination chamber. At the same time, one plastic container was withdrawn from the samples, contents spread out evenly on a stainless steel tray, which was placed in a forced air drier at 50°C for 48 h. After drying, the malted grains were vacuum-packed and stored in the cold room at ~5°C until analysed. This process was repeated for 72 h. Malting loss and GE were determined on the raw pearl millet (RPM) grains, whilst proximate content, fibre, vitamin C and amylase activity were determined on the RPM grains and malted pearl millet (MPM), which had been milled to pass through a 2 mm screen (Perten Instruments, Huddinge Sweden).

3.3.4   Germinative energy (GE)

The GE of both varieties was determined according to the method described by Gomez *et al.* (1997), with some modifications. Pearl millet grains (100 of each variety) were counted, in triplicate, into petri dishes lined with two filter papers that had been moistened with 10 ml of distilled water. The petri dishes were covered and placed in a proofing chamber (used as a germination chamber) at 28°C for 72 h. Germinated kernels after 24, 48 and 72 h were counted and counts recorded. At each time interval, percentage germinated grains was calculated. Duplicate determinations should not differ by more than +/- 5 grains, for example

first determination 95%, second determination 90%, or 100%. GE is the mean of the duplicate determinations, expressed as a whole number.

3.3.5 Malting loss

Total malting loss was calculated according to the method described by Gomez *et al.* (1997).

$$Malting\ Loss\ (\%) = \frac{(Initial\ grain\ dry\ weight - Dry\ malt\ weight) \times 100}{Initial\ grain\ dry\ weight}$$

3.3.6 Proximate content

The moisture content of the unprocessed pearl millet grains was determined using the air oven method (934.01) (AOAC, 2005). The protein content was estimated from the crude nitrogen content of the sample determined by the Kjeldahl method (N $\times$ 6.25) (920.53) (AOAC, 2005). Measurement of the total fat content was carried out using a Buchi B815 and B820 (Labortechnik, Switzerland) extraction and analysis unit, following the method (996.06) detailed by AOAC (2005). Measurement of the total ash content was carried out using a muffle furnace, following the method (923.03) detailed by AOAC (2000).

3.3.7 Amylase activity

Determination of amylase activity in the grains, involved two stages, namely extraction (crude extract) and quantification of the amylase activity. For the extraction stage, the Megazyme method was used and for the quantification stage, a method as described by Sigma Aldrich was used.

Firstly, a stock solution of the extraction buffer was prepared by adding 134.1 g malic acid, 58.4 g NaCl and 70 g NaOH to 800 ml of distilled water, the solution was allowed to cool to room temperature and then 5.9 g $CaCl_2$ was added. The pH of the solution was adjusted to 5.4 by drop-wise addition of NaOH (4 M) or HCl (4 M). Then 1.0 g sodium azide (NaN_3) was added, and the volume of the solution made up to 1 L and stored at room temperature. A working extraction buffer was prepared by diluting 50 ml of the stock solution of the extraction buffer to 1 L with distilled water and the pH adjusted to 5.4 if necessary.

Two different protocols were utilised for the extraction of enzymes from both the malted and un-malted pearl millet meals. Samples of the malted and un-malted meal, were ground to pass through 0.5 mm screen in a bench top Falling Number mill (Perten, Laboratory Mill 3100, Finland). For the un-malted meal, 3 g was accurately weighed into a 50 ml flask to which 20 ml of the extraction buffer was added and contents stirred vigorously. The enzyme was allowed to extract over 20 min at $40^{o}C$, with occasional mixing. The solution was then centrifuged at 1000 g for 10 min and the supernatant assayed within two hours.

For the malted meal, 0.5 g of meal was accurately weighed into a 100 ml volumetric flask, to which a solution of 50 ml of extraction buffer (1% NaCl, 0.02% $CaCl_2$ and 0.02% NaN_3) was added. The mixture was then adjusted to volume with distilled water. The enzyme was allowed to extract for 20 min at room temperature with occasional stirring. The solution was then centrifuged at 1000 g for 10 min. Supernatant (0.5 ml) was then diluted with 9.5 ml extraction buffer, 1 ml of this was transferred into a cuvette and absorbance read at 540 nm in a spectrophotometer (Ultrospec 1000 Pharmacia Biotech, Cambridge, England). The αamylase activity was recorded as mg maltose liberated per g starch per h of digestion – (mg/g starch/h).

3.3.8   Data Analyses

All data were collected in triplicate.   The data were subjected to a multivariate analysis to establish mean differences between treatments.   The Duncan multiple range test was used to separate means where differences existed.   Optimal Scaling Principal Component Analysis (CATPCA) was used to determine the relationships between proximate, physical and functional characteristics.   All data analyses were carried out using IBM SPSS Statistics version 21, 2012.

3.4   Results and Discussion

3.4.1   Effect of germination time on nutritional properties

The effect of malting on the proximate composition, energy and physical appearance of the AgG at 12 h intervals germination of the grain is detailed in Table 4 and **Figure 2**, respectively.   The effect of malting on the proximate composition and physical appearance of Ba is shown in Table 5 and **Figure 3**, respectively.   Figures 2 and 3 shows a progressive increase in the roots and shoots of the germinating pearl millet with time up to 72 h.   The increasing length in roots and shoots could be because of the conversion of stored seed energy into structural components during the germinating process.

The moisture content of AgG decreased from 10.93 g/100 g to 6.10 g/100 g, and that of Ba fluctuated significantly ($p \leq 0.05$) between 9.05 g/100 g and 6.60 g/100 g at 60 h of germination.   These observations are in agreement with Opoku *et al.* (1981), who postulated that the decrease in moisture content was the result of the kilning of germinated grains.

Table 4: Nutritional properties of pearl millet (Agrigreen) as affected by germination time at 30°C and 98% humidity (dry weight basis (d.b.))

Time (h)	Moisture Content (g/100 g)	Ash (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Carbohydrates (g/100 g)	Energy (KJ/100 g)
0	10.94 ± 0.18^{a1}	1.90 ± 0.07^{a}	11.70 ± 0.51^{a}	5.41 ± 0.47^{a}	73.37 ± 0.98^{a}	1642.41 ± 14.54^{a}
12	7.81 ± 0.17^{b}	1.83 ± 0.00^{b}	11.86 ± 1.20^{a}	4.56 ± 0.70^{b}	76.65 ± 0.33^{b}	1673.03 ± 22.22^{b}
24	8.44 ± 0.18^{c}	1.57 ± 0.00^{c}	12.01 ± 0.28^{a}	3.80 ± 0.58^{c}	76.75 ± 0.63^{b}	1638.08 ± 23.11^{a}
36	8.34 ± 0.20^{c}	1.63 ± 0.01^{d}	11.87 ± 0.12^{a}	3.48 ± 0.61^{c}	77.26 ± 0.51^{b}	1627.76 ± 25.16^{a}
48	6.84 ± 0.04^{d}	1.68 ± 0.02^{e}	12.50 ± 0.15^{a}	2.71 ± 1.43^{c}	78.66 ± 1.54^{c}	1627.17 ± 49.80^{a}
60	8.28 ± 0.19^{c}	1.70 ± 0.05^{e}	13.56 ± 0.99^{b}	2.60 ± 0.11^{d}	76.53 ± 1.19^{b}	1596.91 ± 0.40^{a}
72	6.10 ± 0.20^{e}	1.92 ± 0.01^{a}	14.66 ± 0.20^{c}	1.98 ± 0.81^{d}	77.88 ± 0.41^{b}	1610.30 ± 32.12^{a}
OTE[2]	8.11 ± 1.45	1.75 ± 0.13	12.59 ± 1.18	3.51 ± 1.31	76.73 ± 1.75	1630.81 ± 32.75

[1]Values are mean ± standard deviation. Different superscripts in columns differ significantly (p ≤ 0.05)
[2]Overall treatment effect
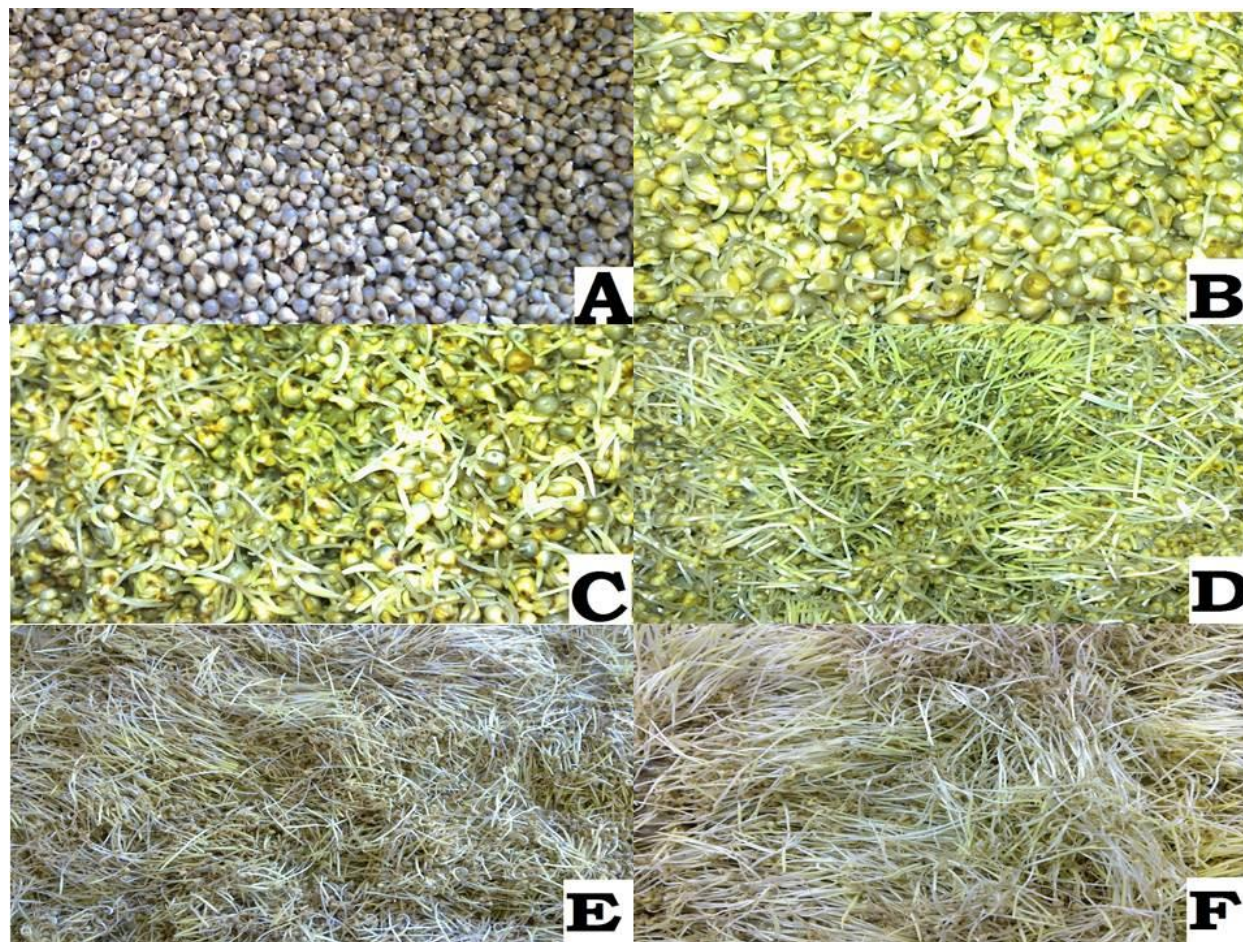
Figure 2: Agrigreen germination over a 72 h period (A = 12 h, B = 24 h, C = 36 h, D = 48 h, E = 60 h and F = 72 h)

Table 5: **Nutritional and some physicochemical properties of pearl millet (Babala) as affected by germination at 30°C and 98% humidity (d.b.)**

Time (h)	Moisture Content (g/100 g)	Ash (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Carbohydrates (g/100 g)	Energy (KJ/100 g)
0	11.03 ± 0.56[a1]	1.68 ± 0.01[a]	11.39 ± 0.26[a]	5.93 ± 0.26[a]	73.16 ± 0.33[a]	1662.21 ± 13.92[a]
12	7.97 ± 0.13[b]	1.60 ± 0.02[b]	12.19 ± 0.05[b]	5.57 ± 0.44[a]	75.32 ± 0.49[b]	1710.52 ± 15.21[b]
24	9.05 ± 0.05[c]	1.58 ± 0.00[c]	12.58 ± 0.16[b]	5.88 ± 0.16[a]	73.78 ± 0.20[a]	1701.13 ± 6.09[b]
36	8.31 ± 0.12[d]	1.57 ± 0.01[c]	12.50 ± 0.22[b]	5.14 ± 0.29[b]	75.16 ± 0.27[b]	1688.66 ± 10.17[c]
48	7.54 ± 0.84[b]	1.71 ± 0.09[a]	12.50 ± 1.55[a]	4.90 ± 0.19[c]	77.86 ± 0.55[c]	1692.21 ± 10.69[c]
60	6.60 ± 0.18[e]	1.62 ± 0.06[b]	12.85 ± 0.31[c]	4.83 ± 0.60[c]	76.53 ± 0.69[d]	1709.61 ± 26.36[b]
72	7.67 ± 0.24[b]	1.73 ± 0.02[a]	12.56 ± 0.24[b]	4.40 ± 0.32[d]	76.29 ± 0.31[d]	1671.45 ± 12.52[a]
OTE[2]	8.31 ± 1.38	1.64 ± 0.07	12.08 ± 0.95	5.23 ± 0.62	75.44 ± 1.58	1690.83 ± 21.46

[1]Values are mean ± standard deviation. Different superscripts in columns differ significantly (p ≤ 0.05)
[2]Overall treatment effect
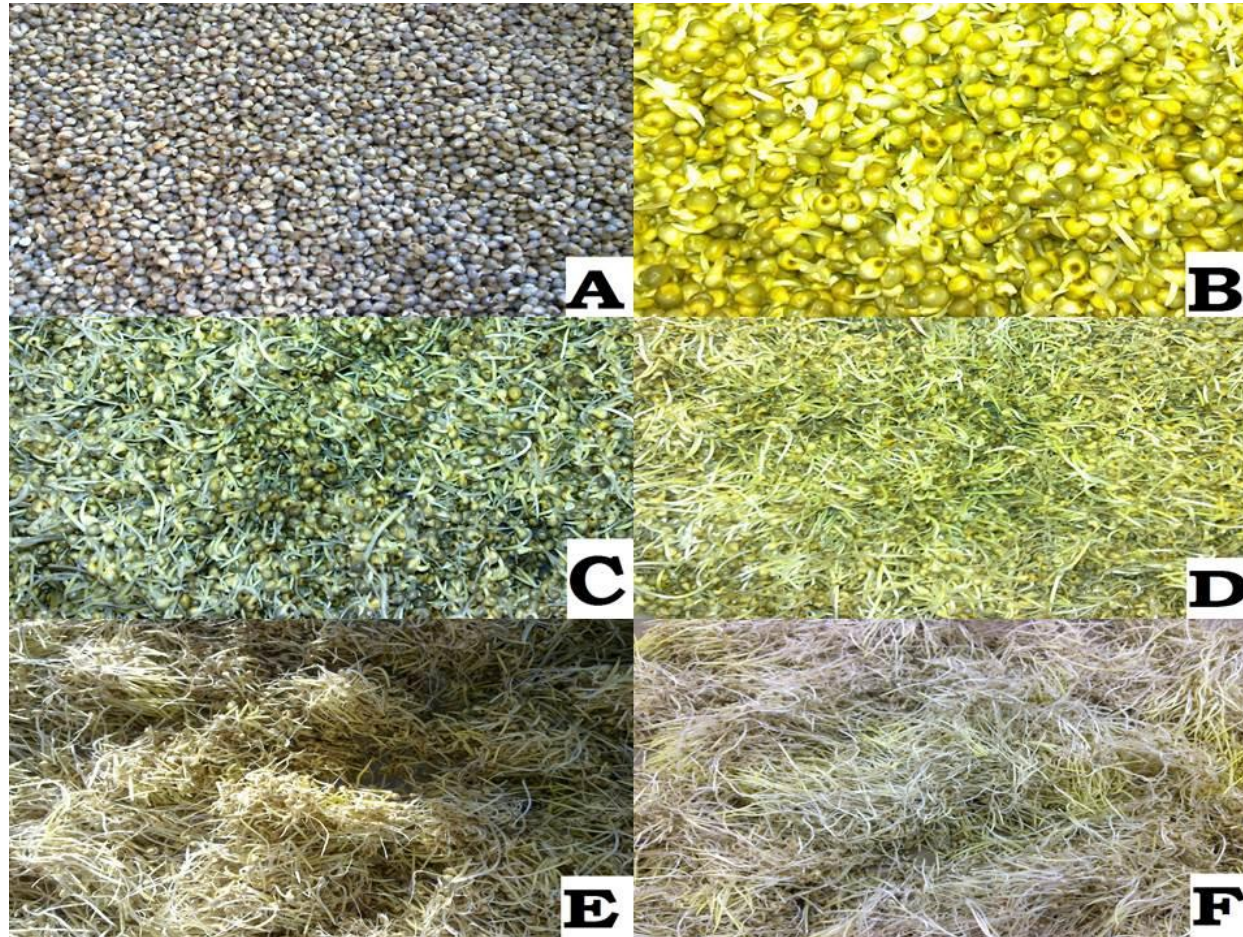
Figure 3: Babala germination at 12 h intervals over a 72 h period (A = 12 h, B = 24 h, C = 36 h, D = 48 h, E = 60 h and F = 72 h)

The ash content of AgG fluctuated significantly ($p \leq 0.05$) between 1.90 g/100 g and 1.57 g/100 g, whilst the ash content of Ba decreased significantly ($p \leq 0.05$) in the first 36 h of germination, then increased significantly up to 72 h of germination. There was an initial decrease in the ash content of AgG within the first 24 h of germination, then a subsequent increase up to 72 h of germination; this trend was also noticed in Ba. Overall, there was no significant ($p \leq 0.05$) change in ash content up to 72 h of germination in the AgG, whilst there was a significant ($p \leq 0.05$) increase in ash content up to 72 h of germination in the Babala. This is in agreement with Ahmed *et al.* (2009), and Malleshi and Desikachar (1986), but contradicts observations made by Dendy (1995). There was no significant change in protein content of AgG (11.7 − 12.5 g/100 g) up to 48 h of germination, this however increased significantly ($p \leq 0.05$) at 60 h (13.5 g/100 g) and 72 h (14.7 g/100 g) of germination. Meanwhile, the protein content of Ba fluctuated significantly ($p \leq 0.05$) during the 72 h of germination. This contradicts observations of slightly increased protein content (11%, 7% and 2%, respectively for red sorghum, millet and maize) made by Traore *et al.* (2004), with Shayo et al. (1998) also observing, an increase in protein content of 5% after 48 h of germination at 30°C in 2 varieties of millet from Tanzania.

Whilst the increase in protein content in these experiments was attributed to a passive variation due to a decrease in the carbohydrate compounds used for respiration (Opoku *et al.*, 1981), the lack of change in protein content in this particular experiment could be attributed to the difference in varieties and/or the equipment used for the germination and kilning process. The significant ($p \leq 0.05$) increase in protein content noted in both varieties of pearl millet was in agreement with the significant ($p \leq 0.05$) increases in protein content observed by Ahmed *et al.* (2009), Dendy (1995), Traore *et al.* (2004), and Shayo *et al.* (1998) as a result of malting. This increase in protein content can be attributed to a passive variation due to a

decrease in the carbohydrate content (Opoku *et al.*, 1981), as well as fat content used for respiration, during germination.

According to Chavan and Kadam (1989b), a considerable portion of endosperm carbohydrates decrease during germination causing apparent increase in protein and fibre contents of cereals, this could be the reason for no marked changes in the endosperm protein content of sorghum and pearl millet, although the rootlets separated from them contained substantial levels of protein.

The fat content of AgG decreased significantly ($p \leq 0.05$) from 5.42 g/100 g to 1.98 g/100 g at 72 h of germination. The fat content of Ba decreased significantly ($p \leq 0.05$) from 5.93 g/100 g to 4.40 g/100 g at 72 h of germination. Sprouting led to a significant ($p \leq 0.05$) decrease in the fat content of the two varieties of pearl millet studied. This is in agreement with observations made by Ahmed *et al.* (2009) of a reduction in fat to 3.67 and 3.74% in the two varieties of pearl millet (Ugandi and Dembi yellow, respectively) used in their experiments; as well as Dendy (1995) observing that sprouting decreased the oil content of pearl millet from 7.5 to 2.5%. Similarly, Elmaki *et al.* (1999), observed that steeping and germination of 2 varieties of sorghum from Sudan were followed by a significant decrease in lipid content. This decrease could be explained by the fact that lipids are used to produce the necessary energy for the biochemical and physiological modifications that occur in the seed during germination (Ahmed *et al.*, 2009; Elmaki *et al.*, 1999).

The effect of sprouting (germination) on the chemical composition of sorghum and millets has been reviewed by Chavan and Kadam (1989b). When grains are hydrated in ambient conditions, endogenous enzymes start to modify the grains constituents in particular, changes in soluble sugars, protein and activities in enzymes (Katina *et al.*, 2007). Subsequent germination of the grains had a significant effect on the nutritional quality of the cereal (Chavan and Kadam, 1989b).

According to Malleshi and Klopfenstein (1998), germination causes several biochemical, textural and physiological transformations in the seeds. The growing root and shoot mainly derive nutrients from the embryo, scutellum and the endosperm and this result in loss of protein, carbohydrates and minerals from the seed. Consequently, the proportion of some of these nutrients in the malt will be altered. Leaching of water-soluble compounds and metabolism of carbohydrates during germination also contribute for dry matter loss of seeds.

Irrespective of variety, germination time had the following overall effect on the proximate compositions of the two pearl millet varieties: Moisture content showed a general decrease from 9.83 g/100 g to 6.89 mg/100 g at 72 h. Ash content showed a general decrease from 1.75 g/100 g to 1.57 g/100 g at 24 h then an increase to 1.82 g/100 g at 72 h. Protein content, remained unchanged (11.49 − 12.29 g/100 g) through the first 48 h of germination, but then changed (13.21 − 13.61 g/100 g) significantly ($p \leq 0.05$) at 60 to 72 h, with no significant ($p \geq 0.05$) difference between 60 and 72 h. Fat content showed a general significant ($p \leq 0.05$) decrease from 5.58 g/100 g to 3.19 g/100 g at 72 h. The carbohydrate content, fluctuated (rising and falling) significantly during the germination period.

3.4.2   Effect of germination time on α-amylase activity

There was a significant ($p \leq 0.05$) increase in the α-amylase activity (**Figure 4**) from 0.92 µg maltose/h/g starch to 2.68 µg maltose/h/g starch at 36 h, following which there was no significant change through to 72 h of germination. The main enzymes produced during germination that hydrolyse starch are α- and ß-amylases (Palmer 1989).
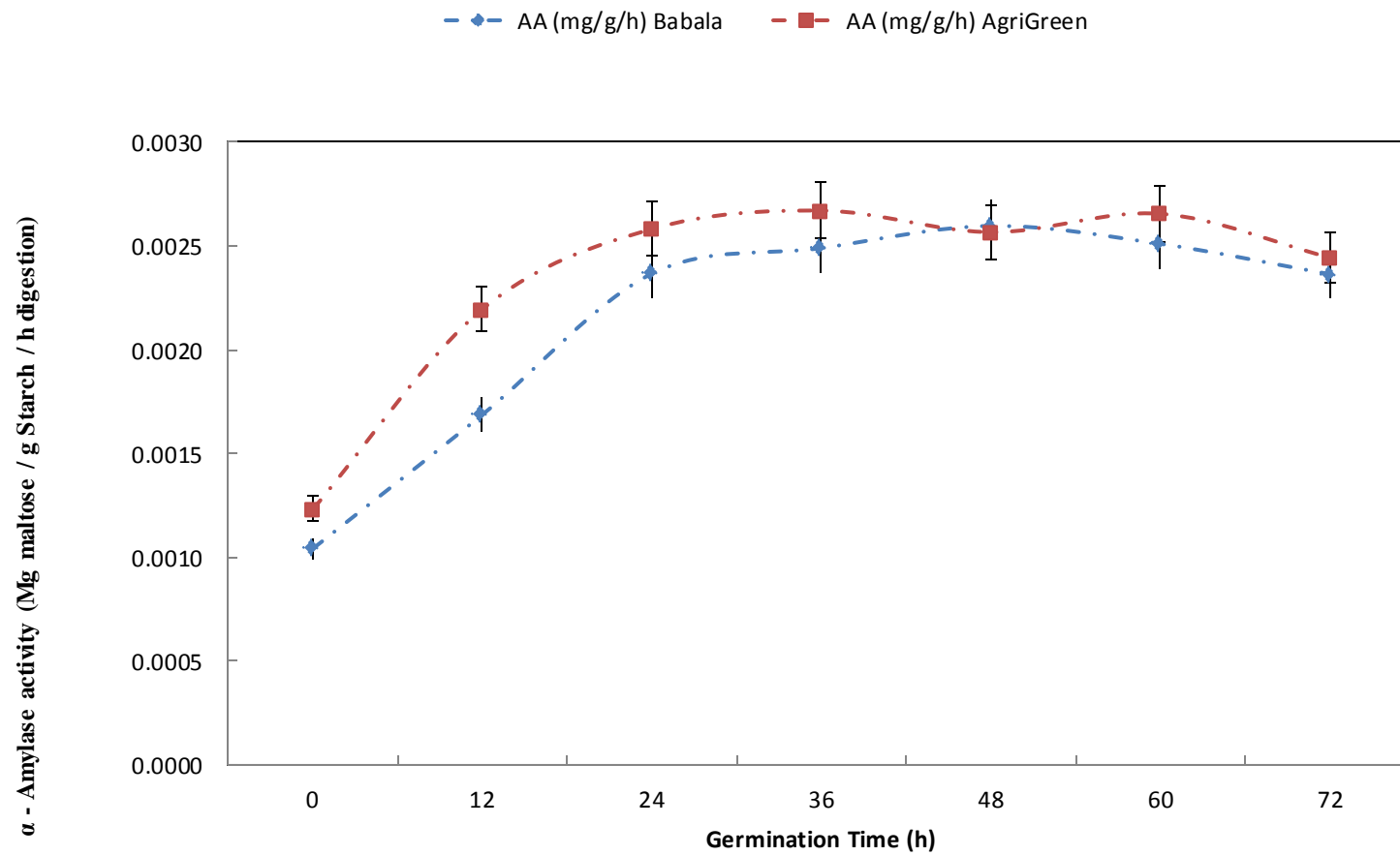
Figure 4: **Alpha amylase activity of two varieties of pearl millet over time**

The α-amylases are liquefying enzymes Traore *et al.* (2004) that hydrolyse amylose and amylopectin to dextrins and maltose (Gibson *et al.*, 1998), which have been observed to increase during germination of cereals, especially sorghum and millet. Similar trends were observed for both varieties of millet used in this experiment (**Figure 4**).

Amylases are hydrolytic enzymes, which depolymerise starch according to a classic acid-base mechanism, breaking them down to dextrins (Dicko *et al.*, 2006). α-Amylases are endo-enzymes that randomly split α-(1-4)-linkages in starch with retention of anomeric configuration of glucose residues, whilst β-Amylase is an exogenous enzyme acting from the non-reducing end, releasing β-maltose units from starch, hence the name β-amylase (Kaplan and Guy, 2004).The β-maltoses released undergo mutarotation into α-maltose (Dicko *et al.*, 2000). Both α-amylase and β-amylase cannot split α-(1-6)-linkages in amylopectin, therefore, the degradation of starch by these enzymes is incomplete. In addition, plant amylases scarcely hydrolyse raw starch: their action is lower than 5% hydrolysis (Dicko *et al.*, 1999).

Adewale *et al.* (2006) observed in their study on maize, sorghum and millet, that the activity of α-amylases in un-malted samples was negligible, was similar to results obtained during this experiment, with low levels of activity at time 0 h for both varieties of pearl millet studied.

Overall, irrespective of variety, germination over time led to a significant ($p \leq 0.05$) increase in α-amylase activity, from 0.9 µg maltose/h/g starch to 2.58 µg maltose/h/g starch at 36 h and remained stable through 60 h (2.58 µg maltose/h/g starch) and then decreased to 1.94 µg maltose/h/g starch at 72 h.

The initial increase in α-amylase activity during germination is an indication of increased breakdown of starch to dextrins during the process. This would inevitably lead to a lower viscosity of the beverage, with an increase in solid content, to a certain limit, giving a

beverage with a higher nutrient density than beverages made from un-malted pearl millet grains.

3.4.3   Effect of germination time on malting loss and germinative energy of pearl millet grains

Malting loss (**Figure 5**) increased significantly (p ≤ 0.05) to 15.13% after 72 h of germination. Germinative energy (**Figure 6**) increased significantly (p ≤ 0.05) from 87.3% to 95.3% after 60 h and remained constant thereafter. The malting loss of 15.61% for AgG and 14.76% for Ba (**Figure 5**), is in agreement with observations made by (Almeida-Dominguez *et al.*, 1993; Chavan *et al.*, 1981; Okoh *et al.*, 1989; Osuntogun *et al.*, 1989) of 8 to 30% dry matter loss as a result of malting. Malting loss increased significantly (p ≤ 0.05) during germination (**Figure 5**), corresponding with observations made by Pelembe *et al.* (2004) who noted that malting loss was significantly affected (p < 0.001) by germination time. Similar losses were reported for finger millet malting (Nout and Davies, 1982). However, relatively larger losses have been reported for sorghum malting, with high watering regimes (Morrall *et al.*, 1986). The larger malting losses reported for sorghum may be related to higher malt metabolic activity as a result of the generally longer steeping times used (Morrall *et al.*, 1986) up to 18 h, compared to 3 h in this work.

It should be noted however, that the malting loss data reported here do not take into account additional losses that would occur if the external roots and shoots were removed, as is done with barley malt, and other works involving other grains including millets. Irrespective of the variety, overall, germination over time, led to a significant (p ≤ 0.05) increase in GE from 68.67% at 24 h to 88.83% at 72 h, with no significant (p ≤ 0.05) change between 60 and 72 h, and a general increase in malting loss of 15.19% at 72 h.
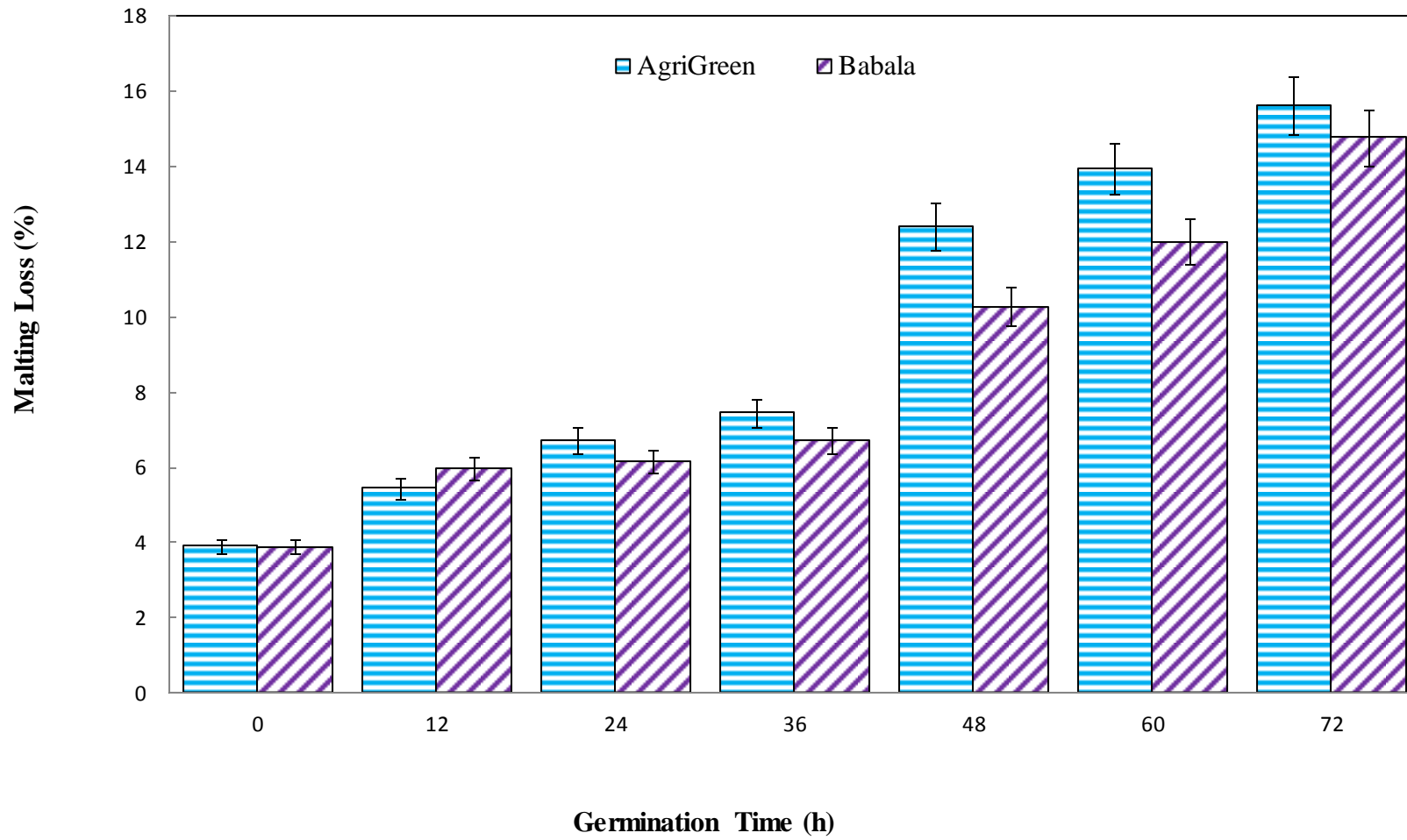
Figure 5: **Malting loss of two varieties of pearl millet over time**
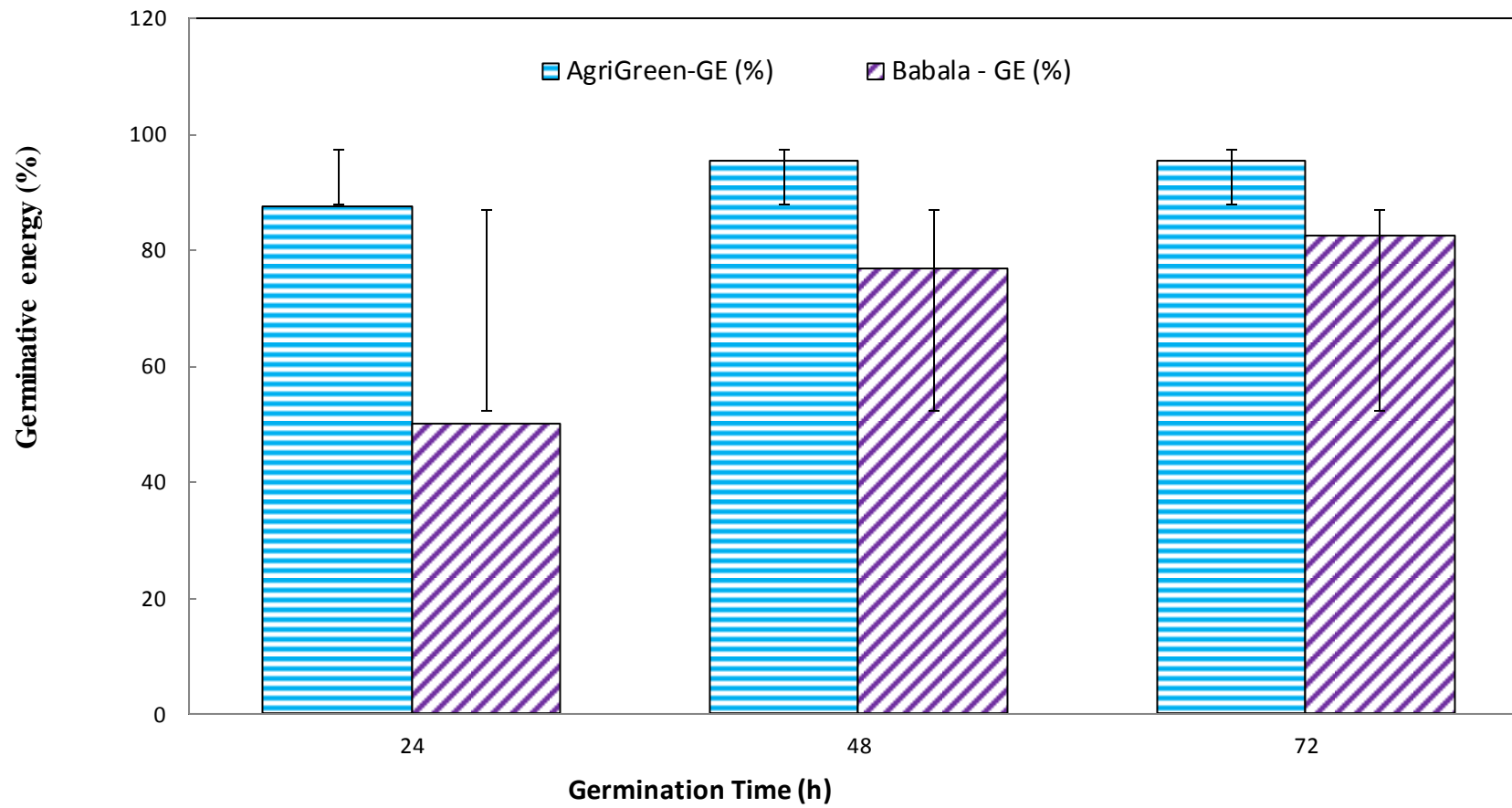
Figure 6: **Germinative energy of two varieties of pearl millet**

Germinative energy is the percentage of grains, which can be expected to germinate if the batch is malted normally at the time of the test. **Figure 6** shows the germinative energy for both AgG and Ba, with AgG exhibiting a significantly ($p \leq 0.05$) higher germinative energy than Babala.

Germination of both varieties would have started at the steeping, but the rate at which the shoots and roots appear from the grains, which is an indication of germinative energy for both, probably differed for several reasons. These include but are not limited to: the rate of water uptake by the grains during steeping; differing optimal germination conditions for the varieties – it is possible the conditions were close to optimal for AgG; or the level of amylase activity which would be indicative of the rate of conversion of starch to essential nutrients for the grains during germination.

Germination of pearl millet has several documented nutritional benefits for the different types of consumers of the malted cereal grain. Some of the benefits in question are related to the amylase activity of the grains during germination. In this experiment, the amylase activity peaked at 36 h of germination. It is however necessary to note that as variety seemingly has an effect on these benefits, specific germination regimens have to be developed for the different varieties of pearl millet prevalent in the different consumption regions of the world, in order to take maximum advantage of benefits that can be achieved from germination.

Overall, irrespective of variety, germination time had a significant ($p \leq 0.05$) effect on the α-amylase activity, malting loss, moisture, protein, fat, ash and carbohydrate content of both millet varieties used in this experiment.

Malting or controlled germination enhances the overall nutritional quality of cereals (Chavan and Kadam, 1989b; Price, 1988; Wu and Wall, 1980) and malted cereals are suitable for malt based speciality foods and value-added products such as milk-based beverages, low

dietary bulk weaning and supplementary foods, amylase rich foods and health foods (Malleshi *et al.*, 1989).

High GE means a high yield in a short period. With the higher GE, AgG, would produce a beverage with a higher nutrient density than Ba in a shorter period. This would mean shorter processing time than for the Ba beverages, cutting on cost and increasing efficiency of the manufacturing process. The malting loss observed in both varieties of pearl millet is because of water loss during the kilning of the green malt after germination. This would have no negative impact on the nutrient content of the beverage powders, but will have a high positive impact on the keeping quality/shelf life of the beverage powders from a microbiological perspective especially.

Figure 7 and **Figure 8** detail the biplot of component loadings for biochemical properties with objects labelled by germination time and pearl millet varieties respectively. The inherent relationship could be described by two components accounting for 73.71 % variation. The variance accounted for by dimension 1 is 46.3 % and dimension 2 is 27.4 %. Dimension 1 is positively correlated to AgG and Ba at 0, 12 and 24 h and is high in fat and moisture content. Dimension 2 is positively correlated to Ba, at 60 h of germination and is high in energy with a high α-amylase activity.

The information observed here are a summary and a reinforcement of earlier discussions on the effect of germination time on the biochemical properties of the two varieties of pearl during the malting process.
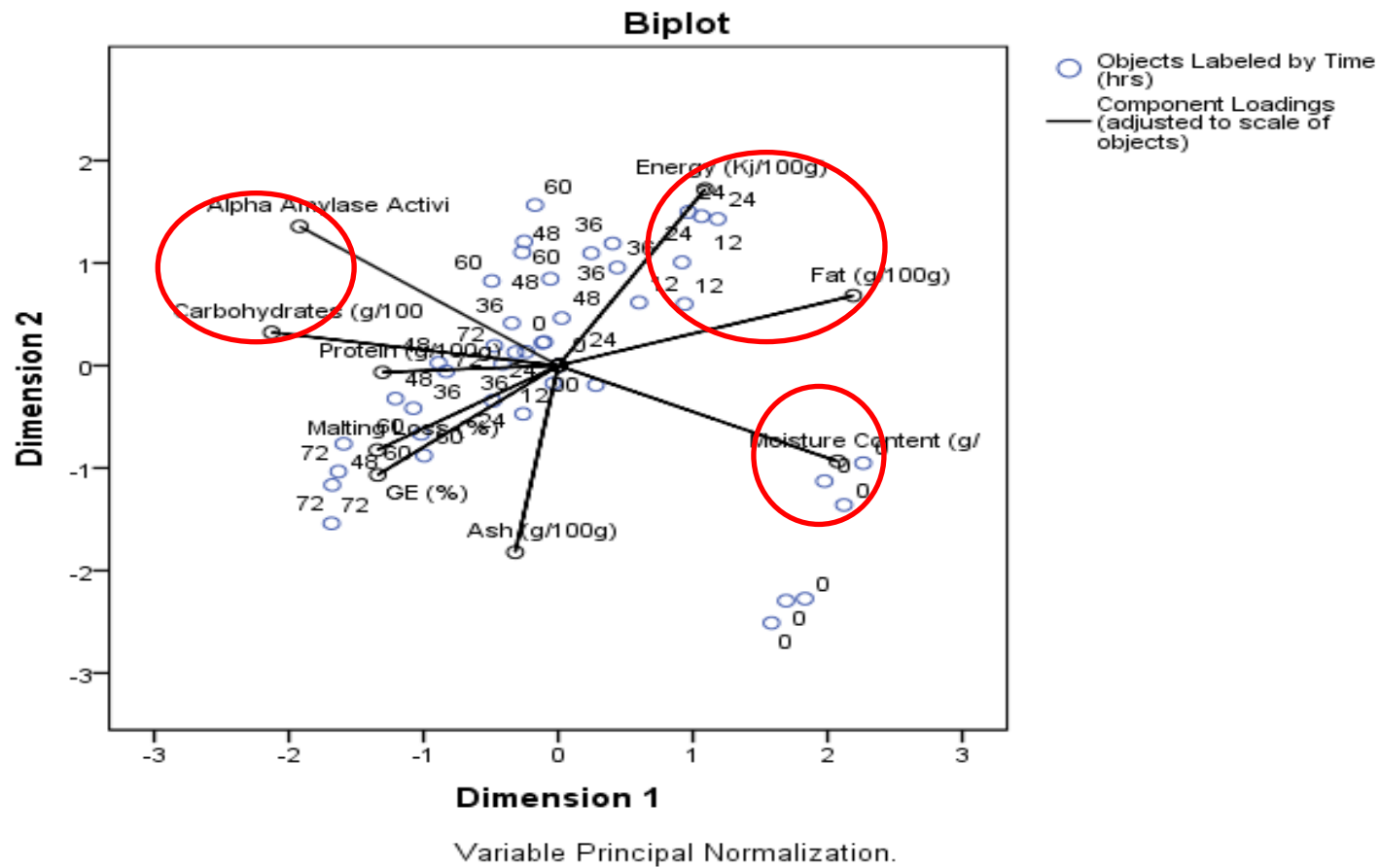
Figure 7: Biplot component loadings (biochemical properties) and objects labelled by time. Circles indicate positive correlation between enclosed components.
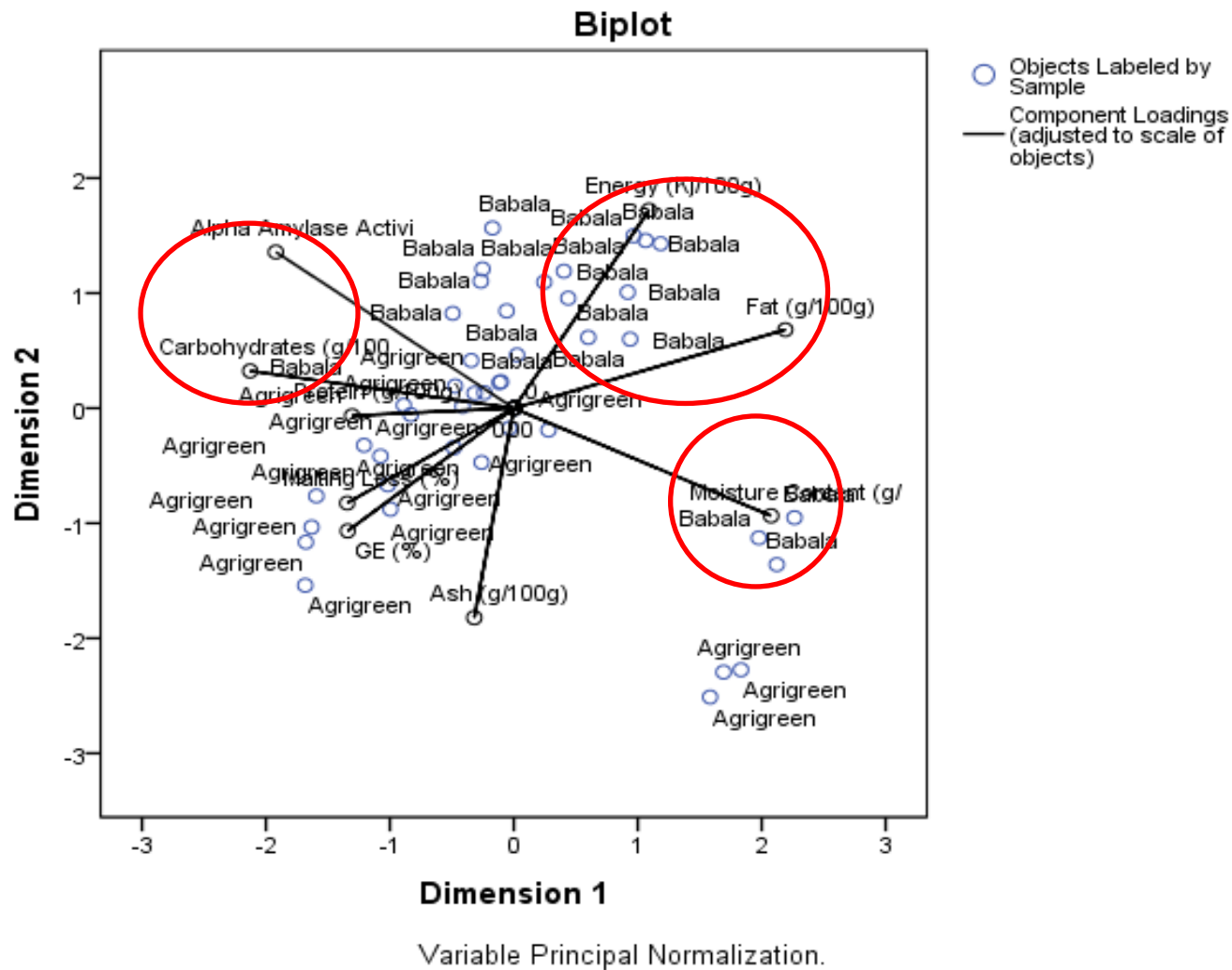
Figure 8: Biplot component loadings (biochemical properties) and objects labelled by pearl millet varieties. Circles indicate positive correlation between enclosed components.

**3.5	Conclusions and Recommendations**

AgG had a higher germinative energy compared to Ba and would therefore be more suitable for sprouting. Malting of AgG and Ba, led to significant ($p \leq 0.05$) malting losses; significant ($p \leq 0.05$) increases in their protein content; but significant decreases in moisture, fat and ash content. Germination increased α-amylase activity peaking at 36 h of germination. Hence, if germination is carried out mainly for the benefit of α-amylase enzyme, then germination must not proceed beyond 36 h, as the activity decreases after this.

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CHAPTER 4: PHYSICAL AND FUNCTIONAL PROPERTIES OF PEARL MILLET PRODUCTS AS AFFECTED BY MALTING, EXTRUSION AND A COMBINATION OF BOTH PROCESSING METHODS

4.1   Abstract

Pearl millet (*Pennisetum glaucum*) flour (PMF) and a pearl millet based instant beverage powder (PMIBP) were prepared by malting, extrusion and a combination of malting and extrusion cooking from two different varieties of pearl millet (Agrigreen (AgG) and Babala (Ba)). For the malted pearl millet, the pearl millets were germinated at $30^{o}C$ and 98% RH for 36 h, kilned at $50^{o}C$ for 48 h then cooled to room temperature, ground and stored in a chiller at $5^{o}C$ until used. Raw, malted and mixture (50 raw:50 malted) pearl millet flour, were extruded using a co-rotating twin-screw extruder under different parameters (screw speed = 400 rpm, feed rate = 14.2 kg/h, H_2O dose rate = 8.7 to 12.5 l/h, heater 1 = $100^{o}C$, heater 2 = 110 to $140^{o}C$, barrel temperature = 83 to $85.5^{o}C$,torque = 20 to 34 N and die size = 5 mm) to obtain the pearl millet instant beverage powder. The effects of the processing methods on some physical and functional properties of the two varieties of pearl millet were evaluated. The different processes significantly ($p \leq 0.05$) affected the colour (L, a & b) of AgG and Ba. The colour difference (ΔE), an indication of the effect of the different treatments on the pearl millet, was highest between RPM and ERPMMPM for AgG (11.30) and between RPM and EMPM for Ba (11.30). The peak viscosity was lowest for MPM of AgG (42.33 RVU) and MPM of Ba (29.75 RVU). The peak times of AgG were 3.66 to 5.38 min and Ba 3.72 to 5.55 mi). Malting and extrusion significantly ($p \leq 0.05$) reduced the peak viscosity of the starches from the raw pearl millet of both varieties of pearl millet as measured by rapid visco analyser (RVA). This can be considered as advantageous with respect to producing an instant beverage powder with a high nutrient content.

4.2   Introduction

Extrusion cooking, particularly in the snack food industry, is a complex process that differs from conventional processing by using high shear rates and high temperatures ($>150^{o}$C) for very short periods (seconds) (Athar *et al.*, 2006).   Wide ranges of thermo-mechanical and thermo-chemical processes are involved during the extrusion cooking process, including shear, Maillard reactions, protein denaturation and hydrolysis.   These processes result in the physical, chemical and nutritional modification of food constituents (Linko *et al.*, 1981).

With advantages that include energy efficiency, lack of process effluents and versatility with respect to ingredient selection and the shapes and textures of products that can be produced, extrusion cooking is a versatile, efficient method of converting raw materials into finished food products (Filli and Nkama, 2007).   Extruded product quality can vary considerably depending on the extruder type, screw configuration, feed moisture and temperature in the barrel session, screw speed and feed rate (Qing-Bo *et al.*, 2005).   Extrusion cooking can make traditional products to be more acceptable in the fast changing society (Filli and Nkama, 2007).   The extrusion cooking process will be employed in this study to produce pearl millet instant beverage powder (PMIBP).

Malting is the germination of cereal grains in moist air under controlled conditions, followed by drying at 50°C for a predetermined length of time.   The primary objective of the germination step is to promote the development of hydrolytic enzymes that are not present in the un-germinated grain, whilst the drying is to arrest the enzymatic activity as well as for flavour development.

The objective of this experiment was to assess the effects of malting, extrusion and a combination of both processes on the physical and functional properties of the pearl millet flours and PMIBP produced.

4.3   Materials and Methods

4.3.1   Source of pearl millet grains, chemicals and equipment:

Two different varieties of pearl millet (*Pennisetum glaucum*) Babala and hybrid Babala (Agrigreen) were obtained from Agricol Pty. Ltd. Cape Town, South Africa.   All chemical reagents obtained from Sigma-Aldrich South Africa.   All equipment used were located in the Department of Food Technology, Cape Peninsula University of Technology, Bellville South Africa and CSIR, Pretoria South Africa

4.3.2   Cleaning and milling of the pearl millet

Cleaning of the pearl millet grains was carried out as described in section 2.1.2.2, page 69. Milling to produce a flour before and after extrusion cooking, was carried out using a hammer mill (CSIR, Sprechert & Schuh, Germany), the product that passed through a 2 mm mesh was collected.   Milling prior to analyses was carried out using a bench-top hammer mill (CPUT, Perten Instruments, Huddinge Sweden).

4.3.3   Malting procedure used for the pearl millet varieties

The malting of the grains was carried out according to the method described in section 3.3.3 page 71 that was used to determine the ideal germination time as related to α-amylase activity (36 h), with some modifications to allow for malting of larger quantities of grain.   Cleaned grains (10000 g) from each variety, were steeped in 3 L 3% NaOH at room temperature (23 – 26°C) for 3 h in 10 L container.   After steeping, the grains were drained (cheesecloth) and washed thoroughly under running tap water, then allowed to drain off excess water.   Three tier plastic vegetable racks (300mm × 400mm × 100m) (Plastics Warehouse, Cape Town) were lined with cheesecloth and the grains were then spread in them.   The plastic vegetable

racks were then put in to a proofing oven (CPUT, MacAdams, Cape Town) at 98% RH and 30°C for 36 h.

Every 12 h during the germination period, the millet was rinsed under running tap water, then drained properly and returned to the proofing oven / germination chamber. After 36 h, the grains were spread out evenly on a stainless steel tray, which was then placed in a forced air cabinet drier (CPUT, Geiger Klotzbucher, Cape Town) at 50°C for 48 h. After drying, the malted grains were vacuum-packed and stored in the cold room at 5°C until analysed.

4.3.4   Extrusion and combination processing of the pearl millet

For the extrusion, flours of the raw pearl millet (RPM), malted pearl millet (MPM) and a mixture (50:50) of RPM and MPM from the two varieties were placed into the hopper of the counter rotating extrusion cooker (CSIR, Werner & Thleiderer, Germany). The processing parameters of the extruder during the extrusion process differed for each of the different samples and were as shown in Table 6. Screw speed (400rpm), feed rate (14.2 kg/h), heater 1 temperature (100°C) were kept constant for all flour samples that were extruded, whilst H_2O dose rate (8.7 and 12.5 l/h) and heater 2   temperature (110 to 140°C) were adjusted to accommodate the level of starch present in each flour sample (the malted PMF have less starch). After extrusion, the extrudates were collected and dried in a forced air cabinet drier (CSIR, Geiger Klotzbucher, Pretoria) at 50°C for 24 hours. After drying, the extrudates were then milled to pass through a 2 mm screen. Products derived from the extrusion process included: extruded pearl millet (ExPM), extruded malted pearl millet (EMPM) and extruded pearl millet mix (ERPMMPM) which is a 50:50 mixture of RPM and MPM.

Table 6:   **Processing parameters of extruder for the different samples**

	BABALA			AGRIGREEN		
	Raw	Malted	Mix	Raw	Malted	Mix
Screw speed (rpm)	400	400	400	400	400	400
Feed Rate (kg/h)	14.2	14.2	14.2	14.2	14.2	14.2
H_2O dose rate (l/hr)	12.5	8.7	8.7	12.5	8.7	8.7
Heater 1 (^{o}C)	100	100	100	100	100	100
Heater 2 (^{o}C)	140	120	130	140	110	130
Barrel Temp (^{o}C)	83	85.5	84.6	84.3	84.3	85
Product Temp (^{o}C)	91	93	91	94	90	91
Torque (N)	20	28	34	20	20	20
Die Size (mm)	5	5	5	5	5	5

Expansion ratio (ER) for the extruded samples (ExPM, EMPM and ERPMMPM) was determined, and all samples (RPM, ExPM, EMPM and ERPMMPM) were analysed for water absorption index (WAI), water solubility index (WSI), colour, colour difference and pasting properties.

4.3.5 Water absorption index, water solubility index, and expansion ratio of the pearl millet flour and pearl millet instant beverage powder

The water absorption index (WAI) of the RPM, ExPM, MPM, EMPM and ERPMMPM were determined by the method of Anderson *et al.* (1969). A sample (2.5 g) was suspended in 30 ml distilled water at 30°C in a previously weighed 50 ml centrifuge tube. This mixture was stirred intermittently over a 30 min period and then centrifuged (Thermo Electron, France) at 3000 g for 10 min. After pouring the supernatant into a tarred evaporating dish, the gel was weighed and the WAI defined as the grams of gel per gram of solids was calculated as follows:

$$WAI \ (\% \ d.b.) = \frac{Weight \ of \ Sediment}{Weight \ of \ dry \ solids} \times 100$$

Flour WSI, defined, as the water-soluble fraction in the sample extract, was determined from the amount of dried solids recovered by evaporating the supernatant from the flour water absorption test (Anderson et al. 1969). The WSI was calculated as follows:

$$WSI \ (\% \ d.b.) = \frac{Weight \ of \ solids \ dissolved \ in \ supernatant}{Weight \ of \ dry \ solids} \times 100$$

The expansion ratio for the extruded PMIBP was determined according to the method of Bhattacharya *et al.* (1986). This was determined by measuring the diameter (mm) of the extrudates using vernier callipers and then dividing these readings by the diameter of the extruder nozzle. Ten readings were determined for each sample and their mean recorded. The ER was calculated as:

$$Expansion\ Ratio\ (ER) = \frac{Extrudate\ diameter}{Die\ diameter}$$

4.3.6 Colour of the pearl millet instant beverage powder

The colour of the RPM, ExPM, MPM, EMPM and ERPMMPM were analysed using a Konica Minolta Spectrophotometer (model CM 5, Konica Minolta Sensing Inc., Japan). An adequate amount of each individual sample to cover the base of the petri-dish (diameter 30 mm) was put, to allow for reflectance measurement. Measurements for each individual sample were performed in triplicate by rotating the dish to three different positions (one reading = average of three readings per rotated position). The colour was recorded in terms of Hunter L, a and b.. L (lightness of the product from 0 for black to 100 for perfect white), a (chromaticity coordinate +a = red and −a = green), b (chromaticity coordinate +b = yellow and −b = blue) (Konica-Minolta, 2003-2010).

The colour difference, a measure of the difference in colour between the different samples and control (RPM of both varieties) was calculated from the data collected using the following equation:

$$Colour\ difference\ (\Delta E) = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Where:

      L = lightness

      a = redness/greenness

      b = yellowness/ blueness

4.3.7 <u>Pasting properties of the pearl millet flours (PMF) and pearl millet instant beverage powder (PMIBP)</u>

The pasting properties of the RPM, ExPM, MPM, EMPM and ERPMMPM were assessed according to the method of Coulibaly *et al.* (2012), using a Rapid Visco Analyser (US, RVA4500, Perten Instruments Australia) with some modifications. Approximately 4 g of extrudates flour was added to 25 ml of distilled water. The heating and cooling cycles were programmed in the following manner. The sample was held at $50^{o}C$ for 1 min, heated to $95^{o}C$ in 3.42 min, held at $95^{o}C$ for 2.5 min, cooled to $50^{o}C$ in 3.80 min and maintained at this temperature for 2 min. The total time for analysis was 13 min., during this time, the following parameters were measured and recorded by the Thermocline software linked to the RVA: peak viscosity (PV), holding strength (HS), breakdown viscosity (BV), final viscosity (FV), setback viscosity (SV) and peak time (PT).

4.3.8 <u>Data Analyses</u>

All data were collected in triplicate. The data were subjected to a multivariate analysis to establish whether there were mean differences between treatments. The Duncan multiple range test was used to separate means where differences existed. Optimal Scaling Principal Component Analysis (CATPCA) was used to determine the relationships between proximate,

physical and functional characteristics and the pearl millet. All data analyses were carried out using IBM SPSS Statistics version 21, 2012.

4.4 Results and Discussion

4.4.1 Effect of malting, extrusion and a combination thereof on water absorption index, water solubility index, expansion ratio and colour of the pearl millet flour and pearl millet instant beverage powder

The effect of extrusion, malting and a combination of both processes on the water absorption index (WAI), water solubility index (WSI) and expansion ratio (ER) of RPM, ExPM, MPM, EMPM and ERPMMPM from AgG is summarised in

Table 7. The WAI, WSI and ER were significantly (p ≤ 0.05) increased by extrusion and a combination of extrusion and malting. Malting, however, significantly (p ≤ 0.05) increased WSI (12.16%) but had no effect on WAI (319.57%). The ER of all the extrusion treatments differed significantly (p ≤ 0.05) from each other. Summarised in Table 8 are the effect of extrusion, malting and a combination of both processes on the WAI, WSI and ER of RPM, ExPM, MPM, EMPM and ERPMMPM made from Babala. Similar to trends of the effects of malting, extrusion and a combination of both processes on AgG were observed for Ba, with WAI and WSI being significantly (p ≤ 0.05) increased by both extrusion and a combination of malting and extrusion. However, malting, as was the case with AgG had no effect on WAI and WSI of Ba. A significant difference was observed amongst all processing methods for ER.

For both AgG and Ba (Table 7 and 8), WAI was significantly (p ≤ 0.05) affected by extrusion, whilst WSI was significantly (p ≤ 0.05) affected by malting. Malting can also be seen to have a significant (p ≤ 0.05) effect on ER of the PMIBP. The significant (p ≤ 0.05) increase in WAI and WSI is in agreement with results reported by Gamalth and

Ganesharanee (2009) and Deshpande and Poshadri (2011) of increased WAI in extruded samples in their studies.

Table 7: Water absorption index, Water solubility index and expansion ratio of pearl millet (Agrigreen) as affected by malting, extrusion and a combination thereof[1,2]

Pearl millet treatment	WAI (% d.b.)	WSI (% d.b.)	ER
RPM[2]	284.54 ± 4.88[a1]	5.14 ± 0.66[a]	----
ExPM	650.92 ± 90.24[b]	8.29 ± 0.82[a]	9.01 ± 0.39[a]
MPM	319.57 ± 43.30[a]	12.16 ± 4.58[b]	----
EMPM	414.67 ± 103.93[c]	12.94 ± 3.95[b]	5.71 ± 0.35[b]
ERPMMPM	534.30 ± 82.43[c]	13.33 ± 0.37[b]	6.59 ± 0.38[c]
OTE	440.80 ± 154.30	10.37 ± 4.02	7.11 ± 1.52

[1] Values are mean ± standard deviation. Different superscripts in columns differ significantly (p ≤ 0.05)
[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect, WAI = Water Absorption Index, WSI = Water Solubility Index, ER = Expansion Ratio.

Table 8: Water absorption index, Water solubility index and expansion ratio of pearl millet (Babala) as affected by malting, extrusion and a combination thereof[1,2]

Pearl millet treatment	WAI (% d.b.)	WSI (% d.b.)	ER
RPM[2]	259.16 ± 8.73^{a1}	6.78 ± 0.09^{a}	
ExPM	510.10 ± 2.73^{b}	11.67 ± 0.49^{b}	9.83 ± 0.33^{a}
MPM	288.76 ± 10.34^{a}	13.32 ± 0.32^{c}	
EMPM	395.85 ± 24.73^{c}	16.16 ± 1.66^{d}	6.97 ± 1.67^{b}
ERPMMPM	526.44 ± 65.21^{b}	10.16 ± 2.89^{b}	8.88 ± 0.63^{a}
OTE	396.06 ± 116.77	11.62 ± 3.49	8.56 ± 1.56

[1] Values are mean $\pm$ standard deviation. Different superscripts in columns differ significantly (p ≤ 0.05)
[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect, WAI = Water Absorption Index, WSI = Water Solubility Index, ER = Expansion Ratio.

The differences in observations to the results of their work and those obtained in this particular work, could be attributed to several factors which include the type of extruder, feed moisture, feed rate, barrel temperature, screw speed, screw profile and die size, which are all important in developing the characteristics of the extruded product (Dobraszczyk *et al.*, 2006). The changes in the functional and physical properties observed in this study are in agreement with (Dobraszczyk *et al.* 2006) who observed that during extrusion processing, food materials are generally subjected to a combination of high temperature, high pressure and high shear, which can lead to a variety of reactions with corresponding changes in the functional properties of the extruded material. The increase in water absorption in this work is one of the many changes in physical and functional properties that occurs because of extrusion, and this is in agreement with (Gomez *et al.* 1988), who also observed a general increase in water absorption of sorghum extrudates. Observations of high WAI for extruded pearl millet made in this work are further corroborated by El-Dash *et al.,* (1984), who concluded that gelatinized starch readily absorbs water to form paste that had higher final viscosity at room temperature than native starch.

The reductions in ER observed during extrusion can be explained according to Guy, (1994) who concluded that typically, protein acts as 'filler' in cereal extrudates and is dispersed in the continuous phase of the extrusion melt, modifying the flow behaviour and characteristics of the cooled extrudates. Proteinaceous materials hydrate in the mixing stage of the process and become soft viscoelastic doughs during the formation of the extrusion melt. The shearing forces generated in the extruder cause breakage of the protein into small particles of roughly cylindrical and globular shapes. At protein levels of 5−15%, the extensibility of the starch polymer foam during its expansion at the die exit is reduced, reducing the degree of expansion. This conclusion is also in agreement with Dobraszczyk *et al.* (2006), who concluded that extrudates show a reduction in expansion with increasing

protein content. At higher levels of protein, severely torn regions in the cell walls of the extrudate are noted, indicating a loss of elasticity in the extrusion melt (Dobraszczyk *et al.*, 2006). The low ER observed in EMPM (which has a lower fat content than un-malted pearl millets for both varieties – reported in next research section) is in agreement with Filli and Nkama (2007) who reported low puff ratios in extruded high fat content cereal grain-legume mixture. This is also in agreement with observations made by Chinnaswamy and Hanna (1988) who noted that the expanded volume of cereal flour decreases with increasing amounts of protein and lipid but increases with starch content. The lower expansion ratio in the mixtures of soybean and groundnut in their work may be attributed to lower protein content of blends of cowpea compared to those of soybean and groundnut except for 100% millet, which recorded the highest expansion ratio. The fat content in the malted grain in this experiment was found to be lower than that in the un-malted grain. Going by the observations made by Filli and Nkama (2007) and Chinnaswamy and Hanna (1988), the ER for the malted grain should be higher than the un-malted grain. But this was not the case in this study, because the malting process does not only breakdown and reduce fat content, but also the starch present in the grain is converted to dextrins simultaneously. Dextrins do not behave like starch under cooking conditions; they do not hold as much water as starch molecules, but dissolve easier in water and under less stringent conditions than starch molecules. Hence, the differing effects of malting and extrusion on WAI, WSI and ER (Tables 7 and 8). High temperature extrusion enables starch to become more fully cooked and thus better able to expand.

4.4.2   Effect of malting, extrusion and a combination thereof on the colour of the instant beverage powder

Tables 9 and 10 summarises the effect of malting, extrusion and a combination of these two processes on the colour of RPM, ExPM, MPM, EMPM and ERPMMPM from both AgG and

Ba. For the AgG samples (Table 9), there was a significant (p ≤ 0.05) difference in the L –
(lightness) values of the product from 0 for black to 100 for perfect white, ±a –
(redness/greenness) and ±b – (yellowness/blueness) values for the different sample types,
with the extruded malt (EMPM) samples exhibiting the largest difference. The Ba samples
(Table 10) exhibited similar trends to the AgG samples as far as the effect of different
processing methods on colour of the finished product is concerned. The L values ranged from
71.60 ± 0.06 for the extruded pearl millet mix (ERPMMPM) to 86.58 ± 0.01 for the raw grain
samples. All processing methods caused a decrease in L (lightness) values, with the extruded
(ExPM, EMPM and ERPMMPM) products been the most affected. The $\pm$ a (redness) values
were from -0.51 ± 0.01 for the raw grains to 3.03 ± 0.00 for the ERPMMPM samples, an
indication of processing methods having a significant effect on this values is also evident in
the trend. The $\pm$ b (yellowness) values for all the samples ranged from 15.23 to 22.53 for
AgG RPM and AgG EMPM respectively and 16.41 to 33.34 for Ba MPM to Ba ExPM
respectively. Malting caused a significant (p ≤ 0.05) decrease in yellowness whilst extrusion
and a combination of both processes increased it significantly (p ≤ 0.05).

According to Ilo and Berghofer (1999), colour is an important characteristic of
extruded foods, which can give information about the extent of browning reactions such as
caramelisation, Maillard reaction, degree of cooking and pigment degradation during the
extrusion process. This is in agreement with (Guy 2001), who concluded that colour
differences between extruded and non-extruded products of the same origin, could be due to
the shear forces generated during extrusion which accelerated the chemical reactions between
amino acids and reducing sugars (Maillards reaction) that take place during extrusion and to
the different cooking temperature, rolling speed, flow rate, water dosing rate and feeding
speed conditions during extrusion (Semasaka *et al.*, 2010).

Table 9: **Colour of pearl millet (Agrigreen) as affected by malting, extrusion and a combination of both processing methods**

Pearl millet treatment	Colour			Colour Difference (ΔE)
	L	a	b	
RPM[2]	81.38 ± 0.01^{a1}	0.95 ± 0.01^{a}	15.23 ± 0.02^{a}	
ExPM	75.77 ± 0.01^{b}	1.15 ± 0.00^{b}	20.95 ± 0.02^{b}	13.48 ± 0.00^{a}
MPM	86.22 ± 0.06^{c}	1.26 ± 0.01^{c}	15.27 ± 0.01^{a}	7.71 ± 0.04^{b}
EMPM	72.89 ± 0.08^{d}	2.50 ± 0.01^{d}	22.53 ± 0.02^{c}	15.36 ± 0.04^{c}
ERPMMPM	73.92 ± 0.01^{e}	2.32 ± 0.00^{e}	22.34 ± 0.04^{d}	15.45 ± 0.06^{d}
OTE	78.04 ± 5.21	1.64 ± 0.66	19.26 ± 3.44	10.40 ± 6.12

[1] Values are mean ± standard deviation. Different superscripts in columns differ significantly ($p \leq 0.05$)
[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

Table 10: **Colour of pearl millet (Babala) as affected by malting, extrusion and a combination of both processing method**

Pearl millet treatment	Colour			
	L	a	b	Colour Difference (ΔE)
RPM[2]	86.58 ± 0.01^{a1}	-0.51 ± 0.01^a	23.94 ± 0.01^a	
ExPM	77.13 ± 0.02^b	1.56 ± 0.01^b	33.34 ± 0.02^b	8.02 ± 0.00^a
MPM	86.70 ± 0.01^c	1.15 ± 0.01^c	16.41 ± 0.04^c	4.84 ± 0.06^b
EMPM	71.76 ± 0.03^d	2.09 ± 0.01^d	27.03 ± 0.02^d	11.30 ± 0.06^c
ERPMMPM	71.60 ± 0.06^e	3.03 ± 0.00^e	25.27 ± 0.10^e	10.39 ± 0.01^d
OTE	78.75 ± 6.98	1.46 ± 1.21	25.20 ± 5.64	6.91 ± 4.25

[1] Values are mean $\pm$ standard deviation. Different superscripts in columns differ significantly ($p \leq 0.05$)

[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

The colour difference calculated for the RPM, ExPM, MPM, EMPM and ERPMMPM from both AgG and Ba gives an indication of the effect of the process used on the change in colour from the RPM flour. All the processes (malting, extrusion and a combination of both) had a significant ($p \leq 0.05$) effect on colour (Tables 9 and 10). The effect of extrusion on the colour change in RPM, during processing to ExPM, MPM, EMPM and ERPMMPM from both varieties of pearl millet was significantly higher than the effect of malting. This difference could be attributed to the high temperatures used during extrusion in conjunction with the presence of sugars (malted samples) and amino acids leading to Maillard browning of the final products. The lack of high temperatures during malting would mean less browning during this process.

The colour difference is also a measure of the acceptability of the RPM, ExPM, MPM, EMPM and ERPMMPM by the consumer. If $\Delta E \geq 1$, it is generally regarded that the consumer will perceive a colour difference between the product and control. The colour difference was greater than 1 for all samples, an indication of the perception of a colour difference by the consumer in all the pearl millet samples.

4.4.3 Effect of malting, extrusion and a combination thereof on the pasting properties of the pearl millet (AgriGreen and Babala) flour

The pasting properties for AgG and Ba are summarised in Tables 11 and 12, respectively. For AgG (Table 11), peak viscosity ranged from 42.33 ± 2.08 for malted pearl millet (MPM) to 719.33 ± 11.59 RVU for raw pearl millet (RPM). The holding strength ranged from 22.00 ± 0.00 for MPM to 657.67 ± 11.59 RVU for (RPM). The breakdown viscosity ranged from 19.33 ± 1.15 for extruded pearl millet malt (EMPM) to 61.67 ± 2.89 RVU for (RPM). The final viscosity ranged from 36.33 ± 1.15 for (MPM) to 1826.00 ± 45.18 RVU for (RPM).

Table 11: Pasting properties of pearl millet (Agrigreen) as affected by malting, extrusion and a combination of both processing methods

Pearl millet Treatment	Peak Viscosity (RVU)	Trough / Holding strength (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Setback (RVU)	Peak Time (min)
RPM[2]	719.33 ± 11.59^{a1}	657.67 ± 11.59^{a}	61.67 ± 2.89^{a}	1826.00 ± 45.18^{a}	1168.33 ± 33.61^{a}	5.38 ± 0.04^{a}
ExPM	97.67 ± 6.66^{b}	63.00 ± 1.00^{b}	34.67 ± 5.69^{b}	93.00 ± 5.00^{b}	30.00 ± 5.57^{b}	3.66 ± 0.46^{b}
MPM	42.33 ± 2.08^{c}	22.00 ± 0.00^{c}	20.33 ± 2.08^{c}	36.33 ± 1.15^{c}	14.33 ± 1.15^{b}	3.67 ± 0.00^{b}
EMPM	73.33 ± 1.53^{d}	54.00 ± 1.00^{d}	19.33 ± 1.15^{c}	88.00 ± 2.00^{b}	34.00 ± 2.65^{b}	3.96 ± 0.08^{b}
ERPMMPM	67.67 ± 4.04^{d}	48.00 ± 1.00^{d}	19.67 ± 3.51^{c}	86.00 ± 8.19^{b}	38.00 ± 7.55^{b}	4.05 ± 0.17^{b}
OTE	200.06	168.93	31.13	425.87	256.93	4.14

[1] Values are mean $\pm$ standard deviation. Different superscripts in columns differ significantly ($p \leq 0.05$)

[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

Table 12: **Pasting properties of pearl millet (Babala) as affected by malting, extrusion and a combination of both processing methods[1,2]**

Pearl millet Treatment	Peak Viscosity (RVU)	Trough / Holding strength (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Setback (RVU)	Peak Time (min)
RPM[2]	932.67 ± 23.59^{a1}	843.67 ± 11.37^{a}	89.00 ± 12.53^{a}	2009.33 ± 49.80^{a}	1165.67 ± 46.82^{a}	5.55 ± 0.04^{a}
ExPM	78.33 ± 3.06^{b}	62.67 ± 1.52^{b}	15.67 ± 1.52^{b}	109.33 ± 2.52^{b}	46.67 ± 2.08^{b}	4.47 ± 0.18^{b}
MPM	29.75 ± 7.54^{c}	12.50 ± 9.81^{c}	17.25 ± 2.36^{b}	34.75 ± 22.20^{c}	22.25 ± 12.61^{b}	3.72 ± 0.06^{c}
EMPM	46.33 ± 12.70^{d}	30.33 ± 6.65^{d}	16.00 ± 6.08^{b}	64.67 ± 2.52^{c}	34.33 ± 5.03^{b}	3.73 ± 0.17^{c}
ERPMMPM	61.00 ± 1.41^{b}	39.50 ± 2.12^{d}	21.50 ± 3.53^{b}	73.00 ± 4.24^{b}	33.50 ± 2.12^{b}	4.10 ± 0.04^{d}
OTE	227.53	195.93	31.60	455.67	259.73	4.29

[1] Values are mean ± standard deviation. Different superscripts in columns differ significantly (p ≤ 0.05)

[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

The setback viscosity was between 14.33 ± 1.15 (MPM) and 1168 ±33.61 RVU (RPM) and the peak time ranged from 3.66 ± 0.46 minutes for the extruded pearl millet (ExPM) to 5.38 ± 0.04 minutes for the raw pearl millet (RPM).

In general, the raw PMF of both varieties exhibited the highest (significant ($p \leq 0.05$) peak, trough, breakdown, final and setback viscosities; as well as peak time. The malted PMF and malted PMIBP however, exhibited the lowest values for the same parameters. These trends are similar to those observed by Obatolu and Cole (2000) in their study of complementary foods made from malted and un-malted millet with soybean and cowpea. They observed that blends, which were based on the whole millet grain, had a higher peak viscosity than blends based on the germinated millet flour, and the paste stability of the blend based on the germinated millet flour was higher than blends based on the whole grain. This reduction in viscosity of the MPM, EMPM and ERPMMPM can be attributed to starch degradation by amylases during the germination process. The viscosity of a paste depends on to a large extent on the degree of gelatinization of the starch granules and the rate of molecular breakdown. In addition to the effect of extrusion, the reduction in viscosity may be attributed to the high level of fat in the millet grains, which consequently decreased the shear effect because of lubrication in the metering zone. Increase in moisture on the other hand, will further lubricate the dough leading to less shearing effect (Filli *et al.*, 2010). Low moisture in the feed can possibly increase frictional damage, particularly when the residence time is high due to low screw speed. Viscosity generally depends on solubility and water holding capacity as well as the structure of components in a food system. Viscosity profile can be thought of as a reflection of the granular changes in the starch granule that occur during gelatinization (Thomas and Atwell, 1997).

The decrease in peak and final viscosity of the ExPM, EMPM and ERPMMPM, could be as a result of the dextrinization of starches in the PMF during extrusion, as a result of the

high temperature and pressures. This is in agreement with Jansen *et al.* (1981) and Likimani *et al.* (1991) who postulated that extrusion can induce starch dextrinization resulting in reduction of viscosity in gruels and a concomitant increase in caloric and nutrient density, as well as Arambula *et al.* (1998) who reported decreased apparent viscosity of extruded instant corn flour when temperature was increased. Davidson *et al.* (1984) reported that viscosity over a heating and cooling cycle have been used to characterize the changes in extruded products in numerous studies. This characteristic is affected by both physical modifications of the granule structure as well as changes to the structures of the starch polymers. They further reported that, the characteristics of the paste viscosity curves were significantly altered by extrusion processing with extrudates showing low values. This is in agreement with observations made in this experiment, with RPM for both varieties showing significantly (p ≤ 0.05) higher viscosity values than the samples that were extruded, malted or processed by a combination of both methods. The reduction in viscosity of the extruded samples may be because of dextrinization of the starch molecules in pearl millet, which agrees with observations made by Jansen *et al.* (1981) and Likimani *et al.* (1991), as stated above. Starch dextrinization during extrusion cooking, however, occurred mostly under processing conditions at very high temperature and low moisture, where shear effects were significant (Gomez and Aguilera, 1983) which are similar to the extrusion conditions used during this experiment. This reduction in viscosity could be beneficial for infant feeding (Pelembe *et al.*, 2002b) or for any person requiring a liquid diet with a high nutritional value (athletes or patients).

Figure 9 and 10 details the biplot of component loadings for physical and functional properties respectively with objects labelled by pearl millet and treatment. The inherent relationship could be described by two components accounting for 84.2% variation. The variance accounted for by dimension, 1 is 68.3% and dimension 2 is 15.9%. Dimension 1 is

positively correlated to Ba and AgG RPM high in PV, FV, HV, SV, BV and PT (Figure 9). The correlation of dimension 1 with AgG and Ba RPM with high viscosity values is expected, as the starch in the RPM is in its native form and gelatinises to form a viscous paste. Processing the pearl millet by malting, extrusion or a combination of both processes on the other hand, leads to degradation of the starch molecules to dextrins, which are shorter chain polysaccharides than starch. This leads to the formation of a thin watery paste on gelatinisation, hence the lower viscosities readings and negative correlation to dimension 1. Dimension 2 is positively correlated to MPM of both varieties of pearl millet, high in lightness (L) and ER. Hence malting resulted in lighter products. From **Figure 10**, it is clear that extruded products (ExPM, EMPM and ERPMMPM) were darker in colour compared to the RPM and MPM as they correlated to a high colour difference (Dimension 2, **Figure 10**).

4.5 Conclusions and Recommendations

Malting and extrusion reduced the viscosity of RPM as indicated by the low viscosities of ExPM, MPM, EMPM and ERPMMPM made from both varieties of pearl millet. This is advantageous with respect to an increase in nutrient density of the resulting beverages prepared from them. This would be suitable for use in the combatting of energy malnutrition observed mostly in young children of low income communities.

Processing of flour from RPM, ExPM, MPM, EMPM and ERPMMPM from both varieties of pearl millet by malting, extrusion and a combination of both processes under the parameters used, had both beneficial and/or no effects on the functional and physical properties. Final viscosity of RPM was higher than that of ExPM, MPM, EMPM and ERPMMPM, an indication of damage to the native starch by the processing methods used.
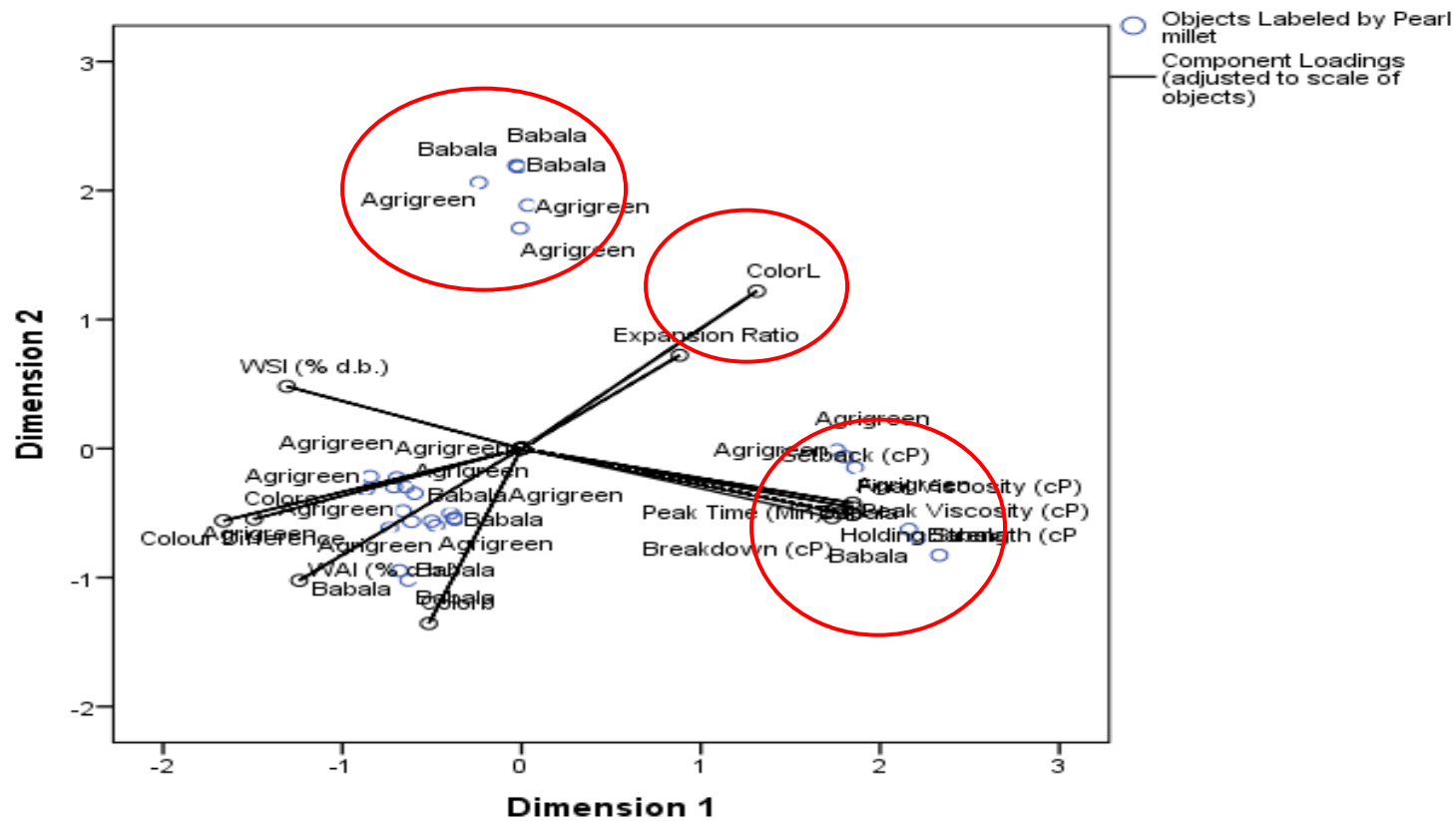
Figure 9: Biplot component loadings (physical and functional properties) and objects labelled by pearl millet variety. Circles indicate positive correlation between enclosed components.
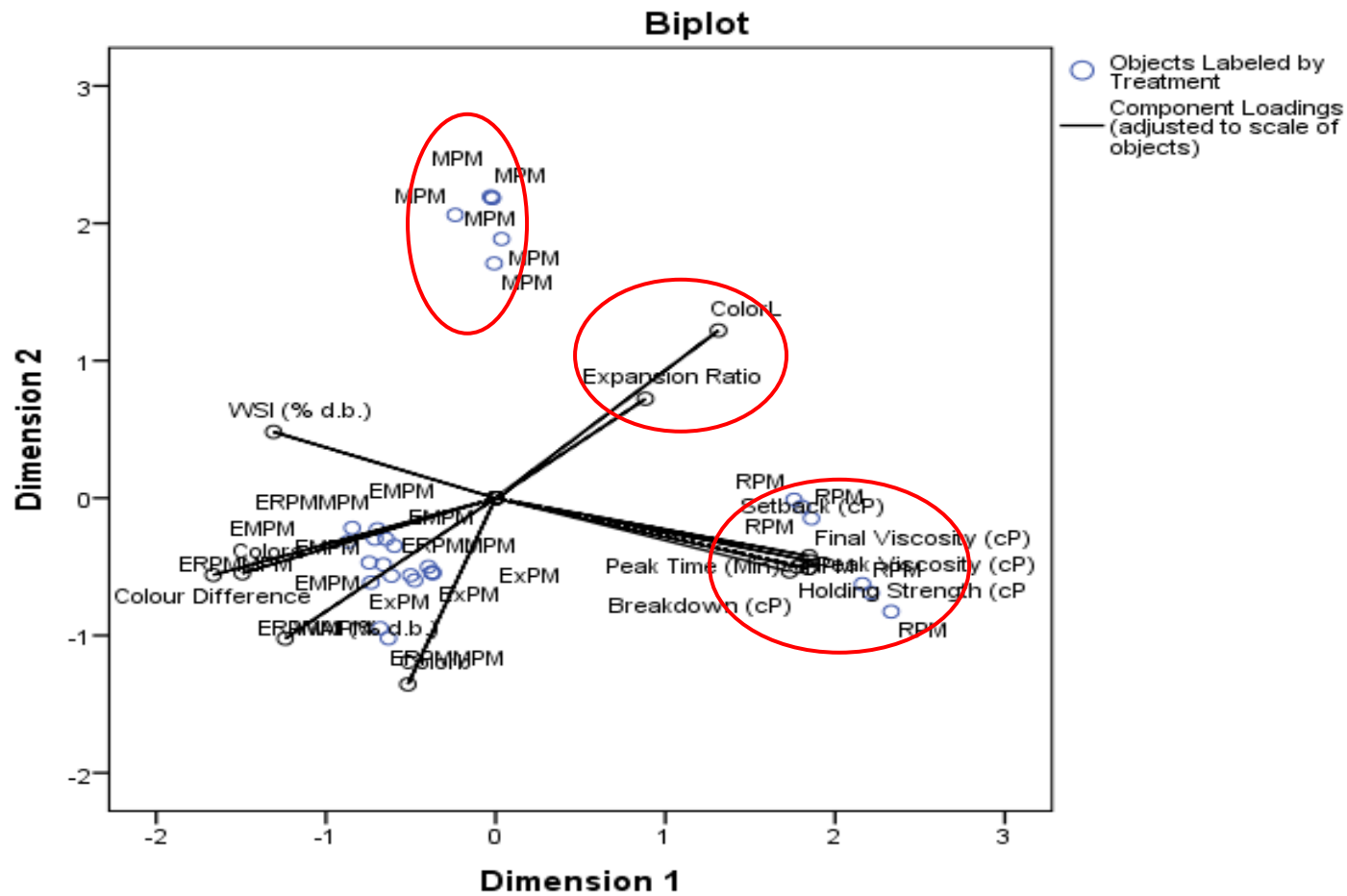
Figure 10: Biplot component loadings (physical and functional properties) and objects labelled by treatment. Circles indicate positive correlation between enclosed components.

Malting lightened the colour of the RPM with high WSI, ER and lower viscosity. Extrusion produced darker products (especially ExPM) with perceivable colour differences from the RPM.

Manipulating processing parameters such as barrel temperature or screw speed amongst others during extrusion processing; or steeping conditions or germination time during malting, would alter the effects of these processes on the properties determined. If the effects are variety dependent (not ascertained in this experiment), development of varieties specifically suited for a particular food product produced using either of these methods alone or in combination will have to be investigated in order to reap maximum benefits from the processes and their advantages.

4.6   References

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CHAPTER 5: NUTRITIONAL, BIOCHEMICAL PROPERTIES, AND SENSORY CHARACTERISTICS OF THE PEARL MILLET BASED INSTANT BEVERAGE POWDER

5.1 Abstract

The effect of the processing methods (malting, extrusion and a combination of both processes), on the nutritional, biochemical, and sensory properties of beverage powders and beverages made from two varieties of pearl millet were evaluated. Combination processing led to a significant ($p \leq 0.05$) decrease in total fat and total dietary fibre (TDF) (3.85 and 22.99 g/100 g, respectively) content of AgriGreen (AgG) extruded malted pearl millet (EMPM); TDF (18.12 g/100 g) content of AgG extruded raw pearl millet-malted pearl millet mix (ERPMMPM). Combination processing, also led to a decrease in the ash, total fat, total dietary fibre, Fe and Zn (1.76, 3.48, 14.26 g/100 g, 7.78 and 4.74 mg/100 g respectively) content of Babala (Ba) EMPM and the ash, total fat, TDF, Fe and Zn (1.88, 4.22, 21.71 g/100 g, 7.24 and 4.14 mg/100 g respectively) content of Ba ERPMMPM. Beverages of 10% total solids (2% sugar for taste) were prepared from the raw and malted pearl millet, by cooking to a rolling boil and offered to an untrained consumer panel consisting of students and staff of the Cape Peninsula University of Technology, under similar sets of conditions in a sensory evaluation room at the Food Technology Department. The panellists rated the beverages for appearance, colour, aroma, flavour, texture and overall acceptability on a 9 point Hedonic scale (1 – like Extremely and 9 – dislike Extremely). In general, Ba RPM was rated 4 - like slightly, and AgG malted pearl millet (MPM) was rated 6 - dislike slightly and all other pearl millet samples from both varieties were rated 5 - neither like nor dislike.

5.2 Introduction

The traditional method of producing instant foods involved producing slurry of the desired final product and proceeding to dry it using a drum drier. This produced a flaked product,

which can be used as is, or ground and sieved to obtain the desired particle size. With the advent of extrusion cooking technology, and diverse production processes associated with the technology food products including instant foods from cereals were developed (Hauck, 1980). This is possible because according to Harper (1981), extrusion cooking gelatinises cereals grain grits and flour, forming expanded products with high water solubility index (WSI), water absorption index (WAI) and water holding capacity (WHC). Moreover, it is also beneficial in the aspects of its high productivity, energy efficiency and reduction in the number of production steps required during processing (Harper, 1981).

More recently though, when the raw material used in the production of instant beverages are in the form of liquids or high viscosity liquids, the method of choice could also be spray drying after cooking (Holsinger *et al.*, 1974; King, 1985). However, if raw materials used are cereals and/or their flours, the process used is either drum drying or extrusion cooking (Anderson *et al.*, 1971). Some of the products are sometimes fortified with nutritive and or sensorial additives (Bookwalter *et al.*, 1971).

The objective of this study, was to evaluate the effect of malting, extrusion and a combination of both methods on the nutritional, biochemical and sensory properties of flours and their beverages, made from two varieties of pearl millet

5.3   Materials and Methods

5.3.1   <u>Source of pearl millet grains, chemicals and equipment; malting, extrusion and combination processing of pearl millet:</u>

Two different varieties of pearl millet (*Pennisetum glaucum*) Babala and hybrid Babala (Agrigreen) were obtained from Agricol Pty. Ltd., Cape Town, South Africa. All chemical reagents were obtained from Sigma-Aldrich South Africa. All equipment used were located in the Department of Food Technology, Cape Peninsula University of Technology, Bellville

South Africa and CSIR, Pretoria South Africa. Cleaning, malting and extrusion of the pearl millet were carried out as per sections 3.3.2 page 71, 4.3.3 page 98 and 4.3.4 page 99 respectively.

5.3.2 Determination of the proximate composition and crude fibre content of beverage powders made from two varieties of pearl millet

The moisture content of the raw ingredients used was determined using the air oven method number 945.38 (AOAC, 2005). The protein content was estimated from the crude nitrogen content of the sample determined using the Kjeldahl method number 979.09 (Nx6.25) by AOAC (2005). Measurement of the total fat content was carried out using a Buchi B815 and B820 extraction and analysis unit, following the method number 996.01 detailed by AOAC (2005). Measurement of the total ash content was carried out using a muffle furnace, following the method number 923.03 detailed by AOAC (2005). The crude fibre content of the sample was determined using the ceramic fibre filter method number 920.86 of AOAC (2005).

5.3.3 Amino acid content of beverage powders made from two varieties of pearl millet

The amino acid content of the millet based instant beverage powder was determined according to the methods of Benson (1965) and Klapper (1982), with slight modifications. Samples (RPM, ExPM, MPM, EMPM and ERPMMPM from both varieties of pearl millet) (3 to 3.9 mg) were individually weighed out on a sensitive Mettler lab balance (AE163 – Mettler Instruments, Zurich), and then transferred into Pyrex bulb-shaped hydrolysis tubes. One ml of 6 M constant boiling HCl containing 0.1% phenol as antioxidant was then added to each hydrolysis tube. This ensured a final sample concentration of approximately 3 to 3.9 mg/ml. The sample tubes were then evacuated using an oil vacuum pump and flame sealed under

nitrogen with a propane flame (Benson, 1965). After 24 hours of hydrolysis in an oven at 110 °C, the tubes were allowed to cool and then cracked open.

The excess acid was evaporated under vacuum with the oil pump. This was done in a vacuum-desiccator containing some dry sodium hydroxide in order to neutralize the acid fumes. Exactly 1 ml of sample citrate buffer pH 2.2, containing norleucine (100 nanomoles internal standard) was added to each dried hydrolysis tube.

Aliquots (10 μl) of the centrifuged samples were then injected into a Waters Amino Acid Analyser (Waters Associates, Medford, MA) and analysed by cation-exchange chromatography using two buffers with an increasing pH gradient using fluorescence (OPA) detection (Klapper, 1982). Buffer A consisted of 0.25 M trisodium citrate, pH 3.05, and buffer B was 0.25 M sodium nitrate, pH 9.5.

5.3.4 Mineral Assay (Ca, Zn and Fe) of beverage powders made from two varieties of pearl millet

Calcium, Iron and Zinc were analysed using the inductively coupled plasma (ICP) spectrometer (Perkin Elmer, Germany). Prior to analysis, samples were digested in a microwave digester (Milestone Microwave Laboratory Systems, Italy). Samples (RPM, ExPM, MPM, EMPM and ERPMMPM from both varieties of pearl millet) (0.5 g) were individually weighed into Teflon vessels to which 3 ml concentrated nitric acid (HNO_3) and 1ml hydrogen peroxide (H_2O_2) were added. Each vessel was closed with its Teflon cover and adapter and tightened with a spring disc. Vessels were positioned on the rotor and secured by placing a circular safety band around them. The rotor was placed onto its base and each vessel tightened with a torque wrench. The microwave oven and fume extractor were switched on and the rotor transferred to the microwave oven. The appropriate program from the instrument user manual was selected and the following parameters entered (Table 13).

Once the operation was complete, the oven was switched off and the rotor taken out of the oven. The vessels were allowed to cool and the contents transferred to a 50 ml volumetric flask and made up to volume using double de-ionised water (Milestone-Microwave-Lab-Systems, 1999).

Working stock solutions of 0, 10, 20, 30, 40 and 50 ppm of the mineral elements Ca, Fe and Zn were prepared from 100 ppm standard solutions by pipetting 10 ml, 20ml, 30ml, 40ml and 50 ml of 100 ppm mineral standards into a 100 ml volumetric flask and making up to volume using double de-ionised water.

The ICP spectrometer was ignited and the ICP 400 software program (Perkin-Elmer, 1996) loaded. The extractor fan, argon gas and the spectrometer were switched on. The peristaltic pump was turned on and de-ionised water was aspirated for 1 min. The torch was ignited and the nebulizer argon flow was set by performing the bullet test. This was achieved by aspirating a 1000 mg/ml solution of Na. The plasma was examined through the viewing window of the torch compartment door and a yellow-orange bullet, extending from the base of the discharge to a point about 2-3 mm above the top of the RF coil, should and was visible in the central channel of the discharge. A satisfactory bullet height was achieved by adjusting the nebulizer argon flow incrementally using the nebulizer adjustment knob. After setting the nebulizer argon flow, the system was allowed to stabilize for about 1h before developing methods and running samples.

Table 13   **Power-time combination for the different steps during the microwave digestion of the pearl millet samples**

	Step 1	Step 2	Step 3	Step 4	Step 5
Power (W)	250	0	250	400	600
Time (min)	1	2	5	5	5

A method was developed by entering the element/mineral parameters into the element mode and the samples, standards and blanks were aspirated and read. The calibrated wavelengths as well as the element parameters were stored in the element mode whilst the element parameters and the element file names were entered and stored in the method mode. The method file name was accessed, the standards, blank, and samples were aspirated and read. A quality control standard of known concentration of each element analysed was determined after every five samples in order to verify the accuracy of the procedure. Upon completion of analyses, de-ionised water was aspirated for 5 min, the plasma was shut down and the software program exited.

The concentrations of minerals were calculated using the concentration from the ICP analyses reports, using the following formula:

$$Mineral\ concentration\ (mg\ 100g^{-1})$$

$$= \frac{Instrument\ concentration\ (ppm) \times Volume\ (ml) \times 100}{Mass\ of\ Sample\ (mg)}$$

Sample solutions were quantified against standard solutions of known concentrations that were analysed concurrently (Perkin-Elmer 1996).

5.3.5 Determination of in vitro protein and starch digestibility of beverage powders made from two varieties of pearl millet

This was carried out according to the method of Saunders *et al.* (1973). A sample (0.2 g) was placed into a 50 ml centrifuge tube; 15 ml of 0.1 N HCl, containing 1.5 mg pepsin, were added and the tube was incubated at 37°C for 3 h. After incubation, the suspension was neutralized by the addition of 3.3 ml of 0.5 M NaOH, then treated with 4 mg of pancreatin in 7.5 ml of 0.2 M phosphate buffer (pH 8.0), containing 0.005 M sodium azide; the mixture was then gently shaken and incubated at 37°C for 24 h. After incubation, the sample was treated with 10 ml of 10% trichloroacetic acid and centrifuged at 5000 $\times$ g for 20 min at room

temperature. Nitrogen in the supernatant was estimated using the micro-Kjeldahl method. Digestibility was calculated using the formula (Ali *et al.*, 2003):

$$Protein\ Digestibility\ (\%) = \frac{Nitrogen\ in\ supernatant \times 100}{Nitrogen\ in\ sample}$$

In vitro carbohydrate digestibility was determined using a method described by Onyango *et al.* (2004b). Sample (RPM, ExPM, MPM, EMPM and ERPMMPM from both varieties of pearl millet) (5 mg) was dissolved in 1ml 0.2 M phosphate buffer (pH 6.9). Porcine pancreatic amylase (20 mg) was dissolved in 50 ml of the same buffer and 0.5 ml added to the sample suspension and incubated at $37^{o}C$ for 2 h. 3,5,-dinitrosalicyclic acid (1ml) was quickly added and the mixture heated for 10 min in a boiling water bath. After cooling, the solution was made up to 25 ml with distilled water and filtered prior to measurement of absorbance in 5 mm cuvette at 510 nm using an Ultrospec 1000 (Pharmacia Biotech, Cambridge, England). A blank for each sample was prepared by incubating the sample first and 3-5- dinitrosalicyclic acid was added before addition of the enzyme solution. A standard curve was prepared using solutions containing known concentrations of maltose monohydrate (0, 10, 20, 30, 40, 60 and 100 µg/ml). Microsoft Excel was used to plot the standard curve and to calculate the concentration of starch digestion products in test solutions. The values were expressed as mg maltose/g starch.

5.3.6   Determination of the total phenolic content (TPC) and antioxidant activity of crude extract of beverage powders made from two varieties of pearl millet

The total amount of phenolic compounds in the pearl millet whole meal flour and product extract was determined using the method described by Silvia *et al.* (1984) with modifications for use with a 96 well plate reader. Firstly, the following solutions were prepared: 1 L of the extraction solvent (1% HCl – methanol solution) in a volumetric flask; in a 15 ml screw cap

tube, 1 ml Folin-Coicalteau phenol reagent (2 N) was added to 9 ml de-ionised water and vortexed to mix well. In a 100 ml media bottle, a 7.5% Na_2CO_3 solution; and in a 50 ml screw cap tube an 800 mg/l Gallic acid standard stock solution, using the extraction solvent.

Then in 6 Eppendorf tubes, 0, 20, 50, 100, 250 and 500 mg/l solutions were prepared from the standard stock solution. 25 µl of each different concentration of the standard and extracts were pipette into the 96 well plate in triplicate, 125 µl of the Folin-Coicalteau phenol reagent was then added and the mixture left to stand for 5 minutes. Then 100 µl of the 7.5% Na_2CO_3 solution was added to each well and the plate left to stand for 2hr at room temperature after which, absorbance in the wells was read using spectrophotometer (Multiskan Spectrum, Thermo Electron Corp., Waltham, MA, USA) at 750 nm. The concentration of phenolic compounds in the extracts was calculated from a calibration curve of the standard, and expressed as gallic acid equivalents (GAE).

The antioxidant activity (by free radical scavenging) of the pearl millet whole meal flour and products were determined using the Trolox (6-Hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid) Equivalent Antioxidant Capacity (TEAC) assay as described by Awika *et al.* (2003) with modifications for a 96 well plate reader. TEAC is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the ABTS + radical cation chromogen in relation to that of Trolox, the water-soluble Vitamin E analogue, which is used as an antioxidant standard.

The reagents for the assay where prepared as follows: ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Diammonium salt: 7 mM (Sigma). Exactly 0.0192 g of ABTS was weighed into a 15 ml screw cap tube, 5 ml distilled water added and mixed until dissolve. This reagent was prepared fresh when required. Potassium-peroxodisulphate: 140 mM (Merck). 0.1892 g) $K_2S_2O_8$ (Sigma) was weighed into a 15 ml screw cap tube, 5 ml distilled water added and solution mixed until dissolved. This reagent was prepared fresh

when required.　　　　　ABTS mix (This must be and was done 24 h before starting the assay): 88 µl of the potassium-peroxodisulphate solution was added to 5 ml of the ABTS solution in a 15 ml screw cap tube and mixed well. This mixture was left in the dark, at room temperature for 24 h before use. The ABTS mix solution was dilute with ethanol to read an absorbance of approximately 2 (±0.1) (Approximately 1 ml ABTS mix and 20 ml EtOH).　　　　　Standard (Trolox also known as 6-Hydrox-2,5,7,8-tetramethylchroman-2-carboxylic acid):1.0 mM (Aldrich): 0.0125 g Trolox was weighed into a 50 mL screw cap tube and 50 ml of ethanol (Saarchem) added, then the solution was mixed until dissolved. This solution was prepared fresh. (When diluted 5x with ethanol this solution should give an absorbance of 0.579±0.015 at 289 nm).

The control (Trolox): 0.0025 g Trolox was dissolved in 50 ml of Ethanol. This solution was prepared fresh and used as the stock control. (When used as is this solution should give an absorbance of 0.579±0.015 at 289 nm). Standard series for the determination were prepared as follows: 6 Eppendorf tubes were marked A-F and to each, the amount of standard stock solution and diluents were added as described in the Table 14. The stock solution was diluted as follows to make a series of standards:

The standards and samples were then added to the clear 96 well plates as follows: 25 µl of Trolox standard (tubes A-F) per well in the designated wells in a clear well plate, 25 µl of the control were added to the Control wells (B7-B12), and 25 µl of sample in triplicate were added to the wells (C1-H12).

The ABTS mix solution was then diluted with ethanol to read an absorbance of approximately 2 (±0.1) (Approximately 1 ml ABTS mix and 20 ml EtOH), 300 µl of this ABTS mix was added to each well using a multichannel pipette.

Table 14: **Preparation of Trolox Standard Series**

Tube	Trolox Standard (μl)	Ethanol (μl)	Trolox conc. (μM)	Well number
A	0	1000	0	A1-A3
B	50	950	50	A4-A6
C	100	900	100	A7-A9
D	150	850	150	A10-A12
E	250	750	250	B1-3
F	500	500	500	B4-6

The plate was left standing for 30 minutes at room temperature to allow colour development before taking an absorbance reading at 734 nm using a Multiskan Spectrum, Thermo Electron Corp., Waltham, MA, USA.

5.3.7   Sensory evaluation of beverages made from two varieties of pearl millet

The consumer panel assessments were conducted at the CPUT food Technology department sensory facilities. The process was carried over a period of 3 days. On day one, panellists were given 10 samples (RPM, ExPM, MPM, EMPM and ERPMMPM beverage) to taste, and on days 2 and 3, panellists got 5 samples to taste. The panel of 78 members over the 3 day period consisted of students and staff of the CPUT food Technology department. The panellists were naive to project objectives.

The beverage powder (80 g) were individually weighed into 3 L stainless steel pots and 200 ml of tap water ($25 \pm 3^{o}C$) and mixed to form a paste. Boiling water (700 ml) was then added slowly while stirring to prevent the formation of lumps. The mixture was brought to a rolling boil. The prepared beverages were then transferred into appropriately labelled 18/8 stainless steel double walled vacuum flasks (Outdoor Warehouse, Cape Town, South Africa).

Freshly prepared RPM, ExPM, MPM, EMPM and ERPMMPM beverages (15-25 ml) were served to the panellists in polystyrene cups to retain temperature and consistency during the evaluation. Beverage samples were coded using random three-digit numbers and served in no particular order. The panellists were provided with water and an empty polystyrene cup to use as a spittoon, and were instructed to rinse their mouths and spit between samples. They were given written instructions and asked to evaluate the products for acceptability based on

its flavour, texture, taste, colour and overall acceptability using 9-point hedonic scale (1 = like extremely to 9 = dislike extremely).

5.3.8 Data Analyses

All data were collected in triplicate. The data were subjected to a multivariate analysis of to establish mean differences between treatments. The Duncan multiple range test was used to separate means where differences existed. Optimal Scaling Principal Component Analysis (CATPCA) was used to determine the relationships between proximate characteristics and the pearl millet varieties as well as between amino acid content and pearl millet varieties. All data analyses were carried out using IBM SPSS Statistics version 21, 2012

5.4 Results and Discussion

5.4.1 Effect of malting and extrusion on the nutritional properties of beverage powders made from two varieties of pearl millet

Malting and extrusion had varying effects on the nutritional properties of RPM, ExPM, MPM, EMPM and ERPMMPM produced from AgG (Table 15). Extrusion led to a significant (p ≤ 0.05) increase in the total fats (3.98 to 4.61 g/100 g); ash (1.75 to 2.03 g/100 g); carbohydrates (81.64 to 83.56 g/100 g); energy (1723.80 to 1789.44 KJ/100 g); Ca (35.05 to 36.23 mg/100 g) and Fe (7.10 to 9.63 mg/100 g). A significant (p ≤ 0.05) decrease in the moisture and TDF (26.59 to 17.11 g/100 g) but had no effect on the protein content of AgG ExPM. Extrusion cooking, like other food processing, may have both beneficial and undesirable effects on the nutritional value of proteins. During extrusion, chemical constituents of the feed material are exposed to high temperature, high shear and/or high pressure that may improve or damage the nutritional quality of proteins in the extruded materials by various mechanisms.

Table 15: Nutritional properties of pearl millet (Agrigreen) processed by malting, extrusion and a combination of both processing methods (d.b.)

	Sample					
	RPM[2]	ExPM	MPM	EMPM	ERPMMPM	OTE
Moisture (g/100 g)	12.56 ± 0.25^{a1}	9.68 ± 0.21^{b}	9.60 ± 0.03^{b}	8.41 ± 0.15^{c}	9.47 ± 0.07^{b}	9.94 ± 1.43
Protein (g/100 g)	12.46 ± 0.22^{a}	12.30 ± 0.28^{a}	12.73 ± 0.64^{a}	12.51 ± 0.16^{a}	12.47 ± 0.09^{a}	12.49 ± 0.32
Ash (100/g)	1.75 ± 0.11^{a}	2.03 ± 0.14^{b}	1.54 ± 0.32^{a}	1.67 ± 0.09^{a}	1.75 ± 0.02^{a}	1.74 ± 0.22
Total Fat (g/100 g)	3.98 ± 0.41^{a}	4.61 ± 0.30^{b}	2.93 ± 0.29^{c}	3.85 ± 0.19^{a}	4.21 ± 0.08^{a}	3.91 ± 0.62
Saturated Fat (g/100 g)	1.11 ± 0.10^{a}	1.27 ± 0.06^{b}	0.83 ± 0.07^{c}	1.07 ± 0.05^{a}	1.16 ± 0.03^{a}	1.09 ± 0.16
MonoUnsat Fat (g/100 g)	1.14 ± 0.13^{a}	1.33 ± 0.10^{b}	0.82 ± 0.09^{c}	1.09 ± 0.07^{a}	1.23 ± 0.02^{a}	1.12 ± 0.19
Polyunsat Fat (g/100 g)	1.72 ± 0.18^{a}	2.01 ± 0.14^{b}	1.28 ± 0.13^{c}	1.69 ± 0.08^{a}	1.82 ± 0.05^{a}	1.70 ± 0.27
TDF (g/100 g)	26.59 ± 3.15^{a}	17.11 ± 0.75^{b}	19.33 ± 1.99^{c}	22.99 ± 2.90^{c}	18.12 ± 2.62^{b}	20.82 ± 4.17
Carb (g/100 g)	81.64 ± 0.34^{a}	83.56 ± 0.90^{b}	85.41 ± 0.64^{c}	85.68 ± 0.35^{c}	84.27 ± 0.06^{b}	84.11 ± 1.57
Energy (KJ/100 g)	1723.80 ± 5.48^{a}	1789.44 ± 3.14^{b}	1763.19 ± 11.53^{c}	1804.34 ± 2.78^{d}	1789.83 ± 3.48^{b}	1774 ± 29.89
Ca (mg/100 g)	35.05 ± 0.25^{a}	36.23 ± 0.04^{b}	38.78 ± 0.45^{c}	40.32 ± 0.25^{d}	36.90 ± 0.14^{e}	37.46 ± 1.96
Fe (mg/100 g)	7.10 ± 0.29^{a}	9.63 ± 0.29^{b}	7.01 ± 0.16^{a}	8.56 ± 0.28^{c}	10.57 ± 0.31^{d}	8.58 ± 1.46
Zn (mg/100 g)	3.43 ± 0.13^{a}	3.30 ± 0.08^{a}	4.18 ± 0.09^{b}	3.19 ± 0.11^{a}	3.16 ± 0.50^{a}	3.45 ± 0.44

[1] Values are mean ± standard deviation. Different superscripts in rows differ significantly ($p \leq 0.05$)

[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

These changes depend on temperature, moisture, pH, shear rate, residence time, their interactions, the nature of the proteins themselves and the presence of materials such as carbohydrates and lipids (Dobraszczyk *et al.*, 2006).

The time-temperature conditions to which foods are exposed during extrusion, are comparable to other high-temperature, short-time (HTST) processes, which is considered preferable in terms of nutrient retention, safety of foods, since growth inhibitors and contaminating microorganisms are more effectively destroyed (Bjork and Asp, 1983). According to Bjork and Asp (1983), extrusion processing, affects the nutritional value of lipids through different mechanisms such as oxidation, *cis-trans* isomerization or hydrogenation. A decrease in fat content of extruded products has been reported by several authors, Fabriani *et al.* (1968) interpreted the decrease in extractable-fat content of extruded products as the result of formation of complexes with other compounds present in the food matrix and/or shear damage caused by the action of the screws and subsequent pressures generated. These could explain the decrease in fat content observed in Babala. The increase, however, in the extractable fat content of AgG, could have been as a result of the exact opposite happening, ie no complexes been formed with other compounds in the food matrix during the processes and/or little or no shear damaged caused by the actions of the screws and subsequent pressures generated.

Malting led to a significant (p $\leq$ 0.05) increase in the carbohydrates, energy, Ca and Zn (81.64 to 85.41 g/100 g, 1723.8 to 1763.2 KJ/100 g, 35.05 to 38.78 mg/100 g and 3.43 to 4.18 mg/100 g respectively). A significant decrease in the TDF and total fat (26.59 to 19.33 and 3.98 to 2.93 g/100 g respectively), and had no effect on the protein, ash and Fe content of AgG MPM. These are in contrast to observations of slightly increased protein content (11%, 7% and 2%, respectively for red sorghum, millet and maize) made by Traore *et al.* (2004), with Shayo et al. (1998) also observing, an increase in protein content of 5% after 48 h of

germination at $30^{o}C$ in 2 varieties of millet from Tanzania. Whilst the increase in protein content in these experiments was attributed to a passive variation due to a decrease in the carbohydrate compounds used for respiration (Opoku *et al.*, 1981), the lack of change in protein content in this particular experiment could be attributed to the shorter germination time (36 h as opposed to 48 h). According to Chavan and Kadam (1989b), a considerable portion of endosperm carbohydrates decrease during germination causing apparent increase in protein and fibre contents of cereals, this could be the reason for no marked changes in the endosperm protein content of sorghum and pearl millet, although the rootlets separated from them contained substantial levels of protein.

The decrease in fat content, are in agreement with observations made by other authors (Elmaki *et al.*, 1999; Opoku *et al.*, 1981; Traore *et al.*, 2004). This decrease could be explained by the fact that lipids are used to produce the necessary energy for the biochemical and physiological modifications that occur in the seed during germination (Elmaki *et al.*, 1999).

Combination processing (malting and extrusion) led to a significant ($p \leq 0.05$) increase in carbohydrates, energy, Ca and Fe (81.64 to 85.68 g/100 g, 1723.8 to 1804.3 KJ/100 g, 35.05 to 40.32 and 7.10 to 8.56 mg/100 g respectively); a significant ($p \leq 0.05$) decrease in TDF (26.59 to 22.99 g/100 g) and no effect on protein and ash content of AgG EMPM.

Combination processing of the raw pearl millet-malted pearl millet mix led to a significant ($p \leq 0.05$) increase in carbohydrates, energy, Ca, and Fe (81.64 to 84.27 g/100 g, 1723.8 to 1789.8 KJ/100 g, 35.05 to 36.90 and 7.10 to 10.57 mg/100 g respectively); a significant ($p \leq 0.05$) decrease in TDF (26.59 to 18.12 g/100 g) and no effect on ash, total fat and Zn content of AgG ERPMMPM.

Table 16 summarises the effect of extrusion, malting and a combination of both processing methods on the nutritional properties of Ba beverage powders.

Table 16   **Nutritional properties of pearl millet (Babala) processed by malting, extrusion and a combination of both processing methods (d.b.)**

	TREATMENT					
	RPM[2]	ExPM	MPM	EMPM	ERPMMPM	OTE
Moisture (g/100 g)	11.91 ± 0.06^{a1}	6.88 ± 0.04^{b}	10.69 ± 0.11^{c}	8.18 ± 0.06^{d}	7.76 ± 0.19^{e}	9.09 ± 1.96
Protein (g/100 g)	12.03 ± 0.18^{a}	12.06 ± 0.08^{a}	12.75 ± 0.04^{b}	12.36 ± 0.26^{c}	12.46 ± 0.11^{c}	12.33 ± 0.31
Ash (100/g)	1.98 ± 0.04^{a}	1.97 ± 0.03^{a}	1.83 ± 0.07^{b}	1.76 ± 0.07^{b}	1.88 ± 0.12^{b}	1.88 ± 0.10
Total Fat (g/100 g)	4.79 ± 0.17^{a}	4.25 ± 0.50^{b}	2.84 ± 0.56^{c}	3.48 ± 0.37^{c}	4.22 ± 0.55^{b}	3.91 ± 0.80
Saturated Fat (g/100 g)	1.24 ± 0.06^{a}	1.16 ± 0.14^{b}	0.78 ± 0.15^{c}	0.96 ± 0.11^{c}	1.15 ± 0.16^{b}	1.06 ± 0.20
MonoUnsat Fat (g/100 g)	1.28 ± 0.04^{a}	1.18 ± 0.20^{b}	0.76 ± 0.20^{c}	0.95 ± 0.15^{c}	1.16 ± 0.22^{b}	1.07 ± 0.24
Polyunsat Fat (g/100 g)	2.27 ± 0.11^{a}	1.92 ± 0.16^{b}	1.29 ± 0.21^{c}	1.57 ± 0.10^{c}	1.92 ± 0.17^{b}	1.79 ± 0.37
TDF (g/100 g)	26.69 ± 4.58^{a}	16.51 ± 0.53^{b}	25.17 ± 7.82^{c}	14.26 ± 2.15^{b}	21.71 ± 5.89^{c}	20.87 ± 6.50
Carb (g/100 g)	81.64 ± 0.09^{a}	86.85 ± 0.48^{b}	84.17 ± 0.62^{c}	86.35 ± 0.63^{d}	85.73 ± 0.41^{d}	84.94 ± 1.99
Energy (KJ/100 g)	1750.11 ± 1.99^{a}	1838.08 ± 12.42^{b}	1735.04 ± 10.60^{a}	1799.15 ± 8.42^{c}	1821.70 ± 13.71^{d}	1788 ± 42.32
Ca (g/100 g)	30.74 ± 0.25^{a}	27.43 ± 0.20^{b}	34.15 ± 0.13^{c}	32.56 ± 0.24^{d}	33.66 ± 0.28^{d}	31.71 ± 2.53
Fe (g/100 g)	9.60 ± 0.43^{a}	9.51 ± 0.09^{a}	7.08 ± 0.45^{b}	7.78 ± 0.13^{c}	7.24 ± 0.33^{b}	8.24 ± 1.17
Zn (g/100 g)	5.36 ± 0.54^{a}	5.51 ± 0.37^{a}	3.97 ± 0.45^{b}	$4.74 \pm 0.05c^{b}$	4.14 ± 0.08^{b}	$4.74\ 0.71$

[1] Values are mean ± standard deviation. Different superscripts in rows differ significantly (p ≤ 0.05)

[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

The extrusion process led to a significant (p ≤ 0.05) increase in carbohydrates and energy (81.64 to 86.85 g/100 g and 1750.11 to 1838.08 KJ/100 g, respectively). A significant (p ≤ 0.05) decrease in total fat, TDF and Ca (4.79 to 4.25, 26.69 to 16.51 g/100 g and 30.74 to 27.43 mg/100 g, respectively) and had no effect on protein, ash Fe and Zn content of Ba ExPM.

Malting led to a significant increase in protein, carbohydrates and Ca (12.03 to 12.75, 81.64 to 84.17 g/100 g and 30.74 to 34.15 mg/100 g, respectively), a significant (p ≤ 0.05) decrease in ash, total fat, Fe, and Zn (1.98 to 1.83, 4.79 to 2.84 g/100 g, 9.60 to 7.08 and 5.36 to 3.97 mg/100 g, respectively) and had no effect on TDF and energy content of Ba MPM. The observations on the effect of malting on proximate composition, mineral (Ca, Fe and Zn) and fibre content of both AgG and Ba were in agreement with observations made by several authors Abdalla *et al.* (1998a); Adeola and Orban (1995); Malleshi and Klopfenstein (1998) to name a few, but differed from observations made by Opoku *et al.* (1981); Suma and Urooj (2011). According to Malleshi and Klopfenstein (1998), during germination, several biochemical, textural and physiological transformations occur in the seeds. The growing root and shoot mainly derive nutrients from the embryo, scutellum and the endosperm and this, results in loss of protein, carbohydrates and minerals from the seed. Consequently, the proportion of some of these nutrients in the malt will be altered. Leaching of water-soluble compounds and metabolism of carbohydrates during germination also contribute for dry matter loss of seeds. This could explain the varying changes in the nutritional properties of the pearl millet after malting.

Malleshi and Klopfenstein (1998), observed that raw sorghum and pearl millet contained 11.8 and 16.1% protein respectively, which did not change appreciably on malting, which is in line with the observations made for protein content of AgG, but differed for that of Ba. They also observed a slight increase in the dietary fibre content of their samples after

malting. This was in contradiction to observations made in this experiment. Also dietary fibre levels reported in their works was markedly lower than that reported in this work.

Combination processing (malting and extrusion) led to a significant ($p \leq 0.05$) increase protein content, carbohydrates energy and Ca (12.03 to 12.36, 81.64 to 86.35 g/100 g, 1750.11 to 1799.2 KJ/100 g and 30.74 to 32.56 mg/100 g respectively) and a significant ($p \leq 0.05$) decrease in the ash, total fat TDF, Fe and Zn (1.98 to 1.76, 4.79 to 3.48, 26.69 to 14.26 g/100 g, 9.60 to 7.78 and 5.36 to 7.74 mg/100 g respectively) content of Ba EMPM.

Combination processing of the raw pearl millet-malted pearl millet mix led to a significant ($p \leq 0.05$) increase in the protein, carbohydrates, energy and Ca (12.03 to 12.46, 81.64 to 85.73 g/100 g, 1750.11 to 1821.70 KJ/100 g and 30.74 to 33.66 mg/100 g) and a significant ($p \leq 0.05$) decrease in the ash, total fat, TDF, Fe and Zn (1.98 to 1.88, 4.79 to 4.22, 26.69 to 21.71 g/100 g, 9.60 to 7.24 and 5.36 to 4.14 mg/100 g respectively) content of Ba ERPMMPM. These variations in values observed, can be attributed to several factors such as differences in the pearl millet varieties experimented with as well as extrinsic factors including growth region, climate and soil type to name a few. The decrease in moisture content of both AgG and Ba was the result of the kilning of germinated grains.

Figure 11 and 12 details the biplot of component loadings for the biochemical properties with objects labelled by pearl millet and treatment respectively. The inherent relationship could be described by two components accounting for 63.1% variation. The variance accounted for by dimension 1 is 38.3% and dimension 2 is 24.8%. Dimension 1 is positively correlated to AgG and ExPM and is high in iron, fat, saturated fat, mono-and poly-unsaturated fat as well as starch digestibility. Dimension 2 is positively correlated to AgG, Ba, MPM and EMPM and is high in carbohydrate, energy, protein and TEAC.
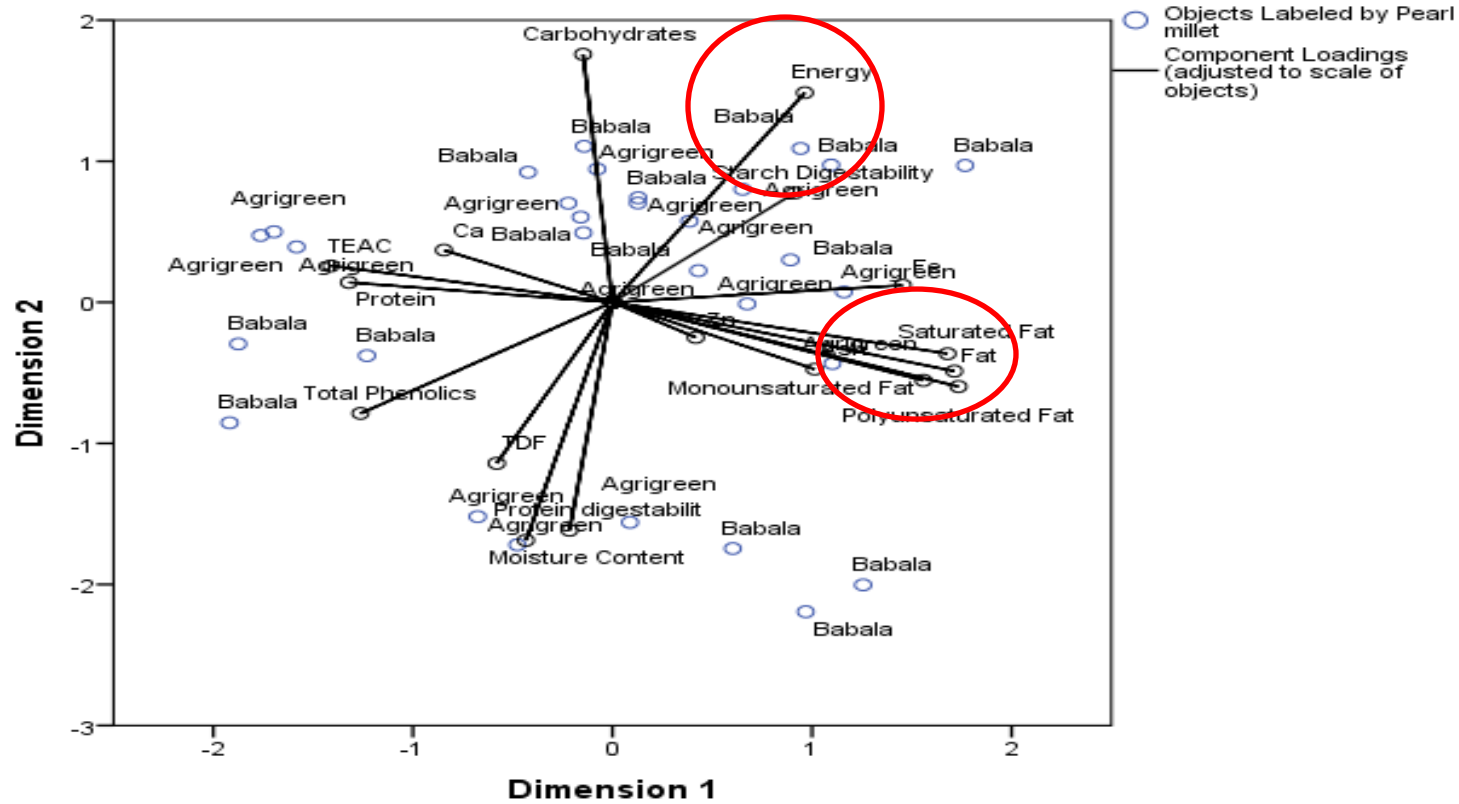
Figure 11: **Biplot component loadings (biochemical properties) and objects labelled by pearl millet varieties. Circles indicate positive correlation between enclosed components.**

Figure 12: Biplot component loadings (biochemical properties) and objects labelled by treatment. Circles indicate positive correlation between enclosed components.

The information observed here is a summary and a reinforcement of earlier discussions on the effect of the processing methods on the two varieties of pearl during the production of the PMIBP.

5.4.2 Effect of malting, extrusion and their combination on the amino acid content of beverage powders made from two varieties of pearl millet

The effects of malting, extrusion and a combination of both methods on the amino acid content of the PMIBP made from the two different varieties of pearl millet AgG and Ba, is shown in Table 17 and 18 respectively. For the AgG variety (Table 17), extrusion led to a significant ($p \leq 0.05$) increase the concentration of the amino acids content in the AgG ExPM. Malting led to a significant ($p \leq 0.05$) increase in all amino acids but glutamic acid, leucine and methionine which remained unchanged in the AgG MPM. Combination of malting and extrusion significantly ($p \leq 0.05$) increased all amino acids but glutamic acid, leucine and arginine in the AgG EMPM. Similar results were obtained for the AgG ERPMMPM with glutamic acid, leucine, lysine and arginine remaining unchanged by the process. None of the treatments led to a decrease in the amino acid content of AgG. Similar results were obtained for Ba (Table 18) with a different set of amino acids remaining unchanged by the different processes applied to product the PMF and PMIBP.

Germination of cereals is known to increase their lysine and tryptophan contents. The subject has been reviewed exhaustively by (Lorenz, 1980 and Chavan and Kadam, 1989b). However, Malleshi and Klopfenstein (1998) only observed similar trends in finger millet as its lysine content increased on malting but no appreciable changes in the lysine content observed during sorghum and pearl millet germination.

Table 17   Amino Acid Content (mg/100 g) of pearl millet (Agrigreen) as affected by malting, extrusion and a combination of both processing methods

AA[2]	Treatment					OTE
	RPM	ExPM	MPM	EMPM	ERPMMPM	
Asp	0.43 ± 0.03^{a1}	0.72 ± 0.03^{b}	0.68 ± 0.04^{b}	0.64 ± 0.03^{b}	0.57 ± 0.06^{c}	0.61 ± 0.10
Thr	0.22 ± 0.00^{a}	0.33 ± 0.02^{b}	0.29 ± 0.00^{c}	0.26 ± 0.00^{d}	0.25 ± 0.03^{d}	0.27 ± 0.04
Ser	0.27 ± 0.00^{a}	0.43 ± 0.03^{b}	0.36 ± 0.02^{c}	0.35 ± 0.04^{c}	0.33 ± 0.02^{c}	0.35 ± 0.05
Glu	1.12 ± 0.05^{a}	1.61 ± 0.16^{b}	1.23 ± 0.04^{a}	1.30 ± 0.14^{a}	1.29 ± 0.14^{a}	1.31 ± 0.19
Gly	0.19 ± 0.00^{a}	0.29 ± 0.02^{b}	0.25 ± 0.01^{c}	0.23 ± 0.01^{c}	0.23 ± 0.02^{c}	0.24 ± 0.04
Ala	0.40 ± 0.01^{a}	0.59 ± 0.05^{b}	0.51 ± 0.01^{c}	0.51 ± 0.04^{c}	0.48 ± 0.03^{c}	0.50 ± 0.07
Val	0.28 ± 0.03^{a}	0.46 ± 0.03^{b}	0.39 ± 0.03^{c}	0.39 ± 0.01^{c}	0.38 ± 0.01^{c}	0.38 ± 0.06
Met	0.06 ± 0.00^{a}	0.11 ± 0.01^{b}	0.05 ± 0.03^{a}	0.10 ± 0.04^{c}	0.09 ± 0.01^{c}	0.08 ± 0.01
Ile	0.23 ± 0.00^{a}	0.34 ± 0.02^{b}	0.27 ± 0.00^{c}	0.29 ± 0.03^{c}	0.27 ± 0.02^{c}	0.28 ± 0.04
Leu	0.53 ± 0.01^{a}	0.79 ± 0.08^{b}	0.59 ± 0.01^{a}	0.63 ± 0.06^{a}	0.63 ± 0.06^{a}	0.64 ± 0.09
Tyr	0.21 ± 0.01^{a}	0.32 ± 0.04^{b}	0.27 ± 0.01^{c}	0.28 ± 0.01^{c}	0.25 ± 0.02^{c}	0.26 ± 0.04
Phe	0.28 ± 0.01^{a}	0.42 ± 0.04^{b}	0.35 ± 0.02^{c}	0.35 ± 0.04^{c}	0.32 ± 0.02^{c}	0.34 ± 0.05
His	0.17 ± 0.00^{a}	0.28 ± 0.04^{b}	0.26 ± 0.01^{b}	0.26 ± 0.04^{b}	0.23 ± 0.02^{b}	0.25 ± 0.05
Lys	0.19 ± 0.00^{a}	0.32 ± 0.02^{b}	0.32 ± 0.01^{b}	0.31 ± 0.09^{b}	0.24 ± 0.03^{a}	0.27 ± 0.07
Arg	0.23 ± 0.01^{a}	0.48 ± 0.06^{b}	0.44 ± 0.13^{b}	0.37 ± 0.08^{a}	0.38 ± 0.02^{a}	0.39 ± 0.09

[1]Values are mean ± standard deviation. Different superscripts in rows differ significantly ($p \leq 0.05$)

[2]AA + Amino Acid; RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

Table 18   **Amino Acid Content (mg/100 g) of pearl millet (Babala) as affected by malting, extrusion and a combination of both processing methods**

| AA[2]	Samples					
	RPM	ExPM	MPM	EMPM	ERPMMPM	OTE
Asp	0.46 ± 0.05^{a1}	0.61 ± 0.04^{b}	0.79 ± 0.00^{c}	0.68 ± 0.03^{c}	0.69 ± 0.02^{c}	0.64 ± 0.11
Thr	0.23 ± 0.04^{a}	0.27 ± 0.01^{b}	0.27 ± 0.00^{b}	0.29 ± 0.00^{b}	0.28 ± 0.04^{b}	0.27 ± 0.03
Ser	0.33 ± 0.04^{a}	0.36 ± 0.01^{a}	0.35 ± 0.00^{a}	0.38 ± 0.01^{a}	0.35 ± 0.00^{a}	0.36 ± 0.03
Glu	1.23 ± 0.21^{a}	1.45 ± 0.09^{b}	1.11 ± 0.00^{a}	1.42 ± 0.08^{b}	1.43 ± 0.16^{b}	1.34 ± 0.19
Gly	0.20 ± 0.03^{a}	0.25 ± 0.02^{b}	0.28 ± 0.00^{b}	0.24 ± 0.00^{b}	0.24 ± 0.00^{b}	0.24 ± 0.03
Ala	0.47 ± 0.07^{a}	0.54 ± 0.03^{b}	0.44 ± 0.00^{a}	0.54 ± 0.03^{b}	0.53 ± 0.03^{c}	0.50 ± 0.06
Val	0.36 ± 0.07^{a}	0.40 ± 0.03^{a}	0.38 ± 0.00^{a}	0.42 ± 0.03^{a}	0.40 ± 0.02^{a}	0.39 ± 0.05
Met	0.09 ± 0.02^{a}	0.08 ± 0.02^{b}	0.05 ± 0.00^{a}	0.12 ± 0.01^{b}	0.12 ± 0.05^{c}	0.09 ± 0.03
Ile	0.27 ± 0.04^{a}	0.29 ± 0.01^{b}	0.26 ± 0.00^{a}	0.33 ± 0.02^{b}	0.33 ± 0.02^{b}	0.30 ± 0.03
Leu	0.62 ± 0.11^{a}	0.71 ± 0.03^{b}	0.55 ± 0.00^{a}	0.72 ± 0.03^{b}	0.69 ± 0.04^{b}	0.66 ± 0.09
Tyr	0.24 ± 0.02^{a}	0.28 ± 0.02^{b}	0.25 ± 0.00^{a}	0.32 ± 0.01^{b}	0.28 ± 0.01^{b}	0.28 ± 0.04
Phe	0.33 ± 0.04^{a}	0.39 ± 0.02^{a}	0.33 ± 0.00^{a}	0.39 ± 0.01^{a}	0.38 ± 0.07^{a}	0.36 ± 0.04
His	0.29 ± 0.06^{a}	0.24 ± 0.00^{a}	0.29 ± 0.00^{a}	0.26 ± 0.01^{a}	0.36 ± 0.08^{b}	0.29 ± 0.05
Lys	0.22 ± 0.02^{a}	0.29 ± 0.09^{a}	0.32 ± 0.00^{a}	0.30 ± 0.09^{a}	0.28 ± 0.03^{a}	0.28 ± 0.06
Arg	0.35 ± 0.06^{a}	0.35 ± 0.04^{a}	0.36 ± 0.00^{a}	0.83 ± 0.37^{b}	0.39 ± 0.17^{a}	0.45 ± 0.25

[1]Values are mean ± standard deviation. Different superscripts in rows differ significantly (p ≤ 0.05)

[2]AA + Amino Acid; RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

Elmalik *et al.* (1986) reported an increase in most of the amino acid contents of sorghum cultivars of varying endosperm texture on germination and the increase being higher in corneous cultivars than the floury cultivars. According to Malleshi and Klopfenstein (1998), malting of sorghum and millets marginally enhances some of their essential amino acids but substantially improves their riboflavin, niacin and ascorbic acid contents.

The various malting processes used in the manufacture of the PMIBP led to either a significant ($p \leq 0.05$) increase or no change in the amino acid content. This is promising as it means that there would not be a need for replacement fortification of the finished product with the lost amino acids. Of the nine essential amino acids required by humans, seven were identified in the ERPMMPM of both varieties of pearl millet; valine and tryptophan were not identified in the various samples. The levels of those quantified were relatively lower than % Nutrient Reference Values (NRV), hence the need to composite millet especially with legumes in order to increase levels of amino acid in the resultant complementary food and hence, its protein quality vis protein digestibility corrected amino acid score (PDCAAS). The NRVs are based on Recommended Dietary Allowances (RDAs) which will meet the needs of nearly all (97 to 98%) healthy individuals to prevent nutrient deficiencies. RDA values are not necessarily enough to maintain optimum nutritional status and prevent chronic disease. These values are therefore considered the minimum amounts necessary to achieve and maintain optimum nutritional status, which will assist in the reduction of disease, specifically degenerative diseases of lifestyle (South-African-Department-of-Health, 2010).

Figure 13 and 14 details the biplot of component loadings for amino acid content with objects labelled by pearl millet and treatment respectively. The inherent relationship could be described by two components accounting for 75.7% variation.
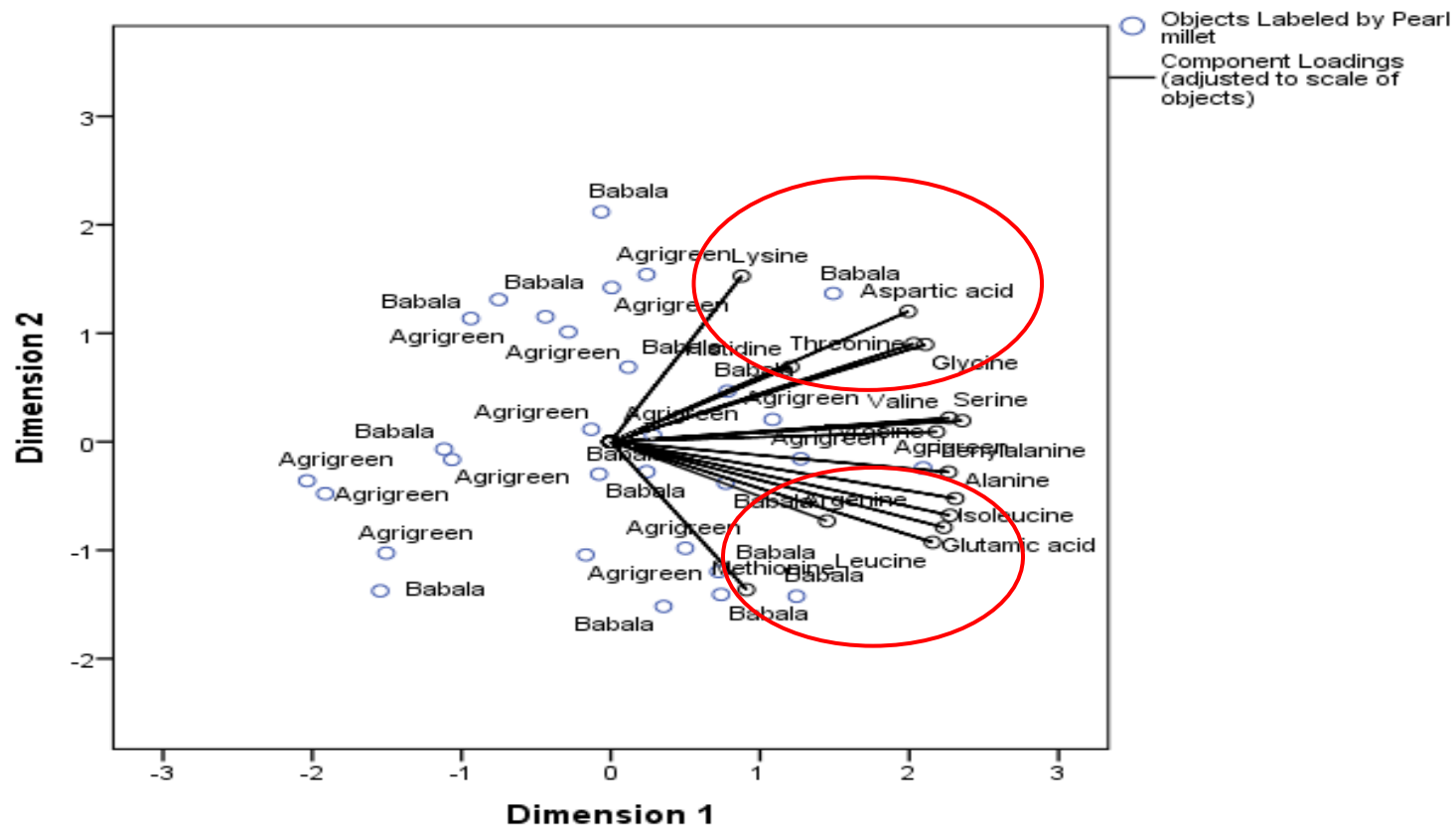
Figure 13: Biplot component loadings (amino acids) and objects labelled by pearl millet variety. Circles indicate positive correlation between enclosed components.
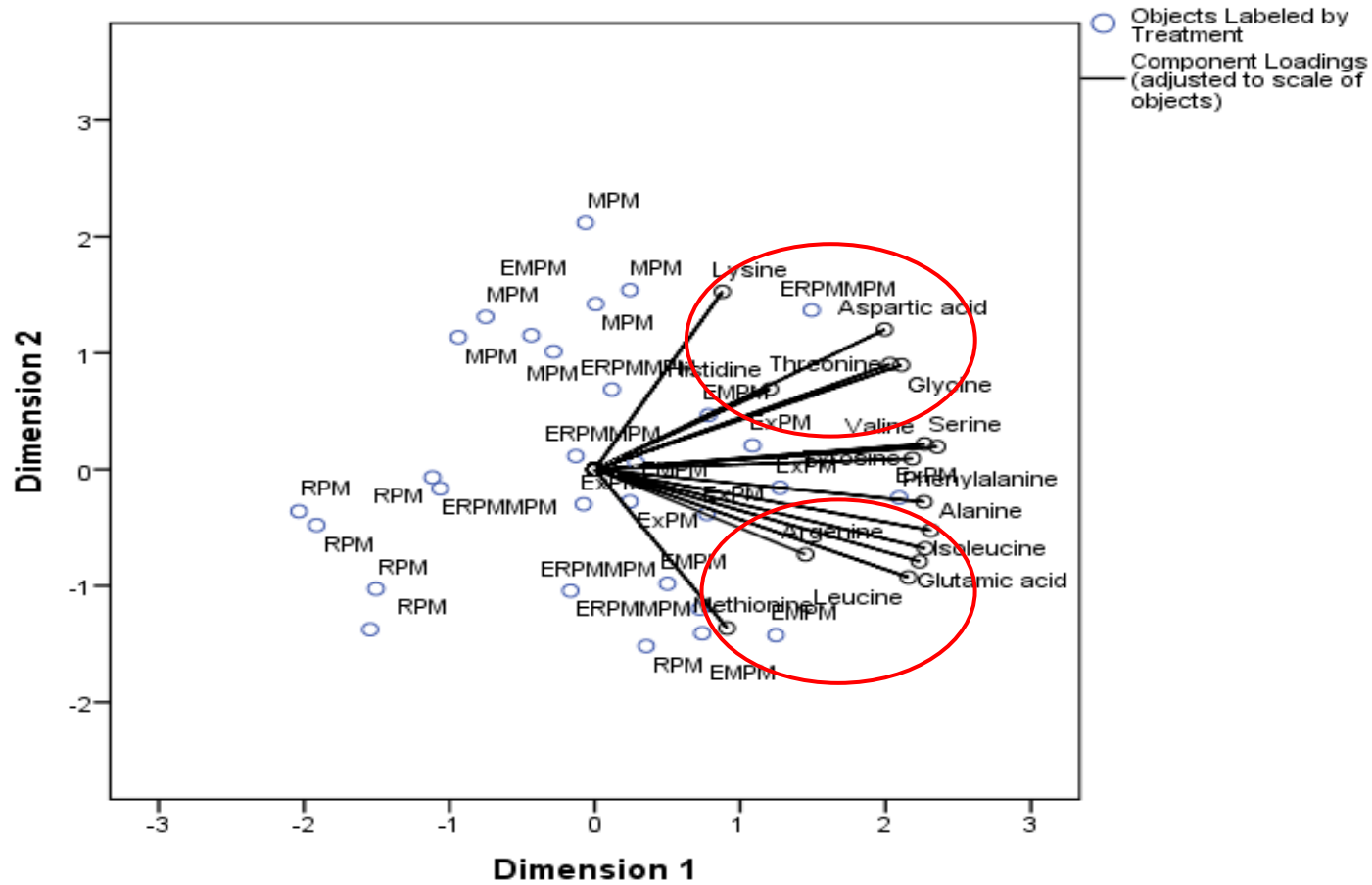
Figure 14: Biplot component loadings (amino acids) and objects labelled by treatment. Circles indicate positive correlation between enclosed components.

The variance accounted for by dimension, 1 is 64.0% and dimension 2 is 11.7%. Dimension 1 is positively correlated to Ba and EMPM high in Ala, Ile, Glu, Leu, and Met. Dimension 2 is positively correlated to Ba, MPM and ERPMMPM high in Lys, Asp, Thr, Gly, Val and Ser. The plots are indicating a lack of or very low amounts of amino acid in the RPM of both varieties, but with processing, there is an increase in amino acid content. This could be happening for several reasons, including the degradation of antinutritional properties, the presence of which could prevent quantification of amino acids in the RPM. According to El Hady and Habiba (2003), soaking reduced phytic content (known antinutrients) in all tested legumes in their experiment. Their data were in agreement with the findings of Alonso *et al.* (1998) and these reductions may be ascribed to the activation of the endogenous phytase during the long soaking treatment and possible enzyme action continued during the germination and drying steps of malting. They also observed a further decrease in the phytic content on extruding their samples at high (180°C).

5.4.3 Effect of malting, extrusion and their combination on the in vitro protein and starch digestibility of beverage powders made from two varieties of pearl millet

Table 19 summarises the *in vitro* protein and starch digestibility for RPM, ExPM, MPM, EMPM and ERPMMPM from both AgG and Ba. Malting significantly (p $\leq$ 0.05) decreased the *in vitro* protein (69.4%) and starch (33.24 mg maltose /100 g starch) digestibility of AgG, whilst extrusion had no effect on protein (73.18%) digestibility but significantly (p $\leq$ 0.05) increased the starch (66.90 mg maltose/100 g starch) digestibility of AgG.

Table 19   **Digestibility of beverage powder produced from two (2) varieties of pearl millet by malting, extrusion and a combination of both processes**

	Agrigreen		Babala	
	Protein digestibility (%)	Starch digestibility (mg maltose / 100 g Starch)	Protein digestibility (%)	Starch digestibility (mg maltose / 100 g Starch)
RPM[2]	87.20 ± 6.96^{a}	40.01 ± 2.42^{a}	96.61 ± 1.18^{a}	35.44 ± 11.24^{a}
ExPM	73.18 ± 10.90^{a1}	66.90 ± 18.37^{b}	67.89 ± 5.10^{b}	74.47 ± 9.55^{b}
MPM	69.94 ± 5.16^{b}	$33.24 \pm 6.13a$	79.70 ± 4.66^{c}	41.93 ± 2.54^{a}
EMPM	70.04 ± 7.68^{b}	65.70 ± 11.45^{b}	69.45 ± 1.38^{b}	41.56 ± 18.03^{a}
ERPMMPM	61.81 ± 9.02^{b}	65.85 ± 12.76^{b}	78.29 ± 0.96^{c}	37.95 ± 6.85^{a}
OTE	72.43 ± 11.02	54.34 ± 18.04	78.29 ± 11.27	46.58 ± 18.05

[1] Values are mean ± standard deviation. Different superscripts in columns differ significantly ($p \leq 0.05$)

[2] RPM = Raw pearl millet; RE = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

Combination processing led to a significant (p ≤ 0.05) decrease in protein digestibility of products from both AgG and Ba, a significant (p ≤ 0.05) increase in the starch digestibility of EMPM from AgG and no change in the starch digestibility of EMPM from Ba. Extrusion of the RPM/MPM mix led to a significant decrease in protein digestibility of both AgG and Ba ERPMMPM, but no change in the starch digestibility of both.

Similar trends appear in the effects of both malting and extrusion on the digestibility of Babala, where malting (79.70%) and extrusion (67.89%) significantly (p ≤ 0.05) decreased protein digestibility. Starch digestibility was significantly (p ≤ 0.05) increased by extrusion (74.47 mg maltose/100 g starch), but unaffected by malting (41.93 mg maltose/100 g starch).

The high protein digestibility of both raw AgG and Ba observed (Table 10) is in contrast to findings by (Sharma and Sehgal, 1992; Walker and Kochar, 1983), attributing their observations to the fact that protein fraction are resistant to denaturation in native state and are not broken down by the digestive enzymes, and the presence of considerable amounts of antinutritional factors respectively. The decrease in digestibility caused by both malting and extrusion of AgG and Ba was also in contrast to findings by (Gahlawat and Sehgal, 1993; Sharma and Sehgal, 1992) who attributed increases in protein digestibility observed, to leaching out of antinutrients like polyphenols and phytic acid during soaking and malting respectively.

Heat treatment of foods may enhance in vitro protein digestibility of food products by altering and breakdown of high molecular weight protein (Boonvisut and Whitaker, 1976) or by destroying the heat labile protease inhibitors. The increase in protein digestibility on malting could also be attributed to the degradation of storage protein (Bhise et al., 1988) which may be more easily available to pepsin attack.

The proteins present in the feed material may undergo structural unfolding and/or aggregation when subjected to heat or shear during extrusion. Intact protein structures

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represent a significant barrier to digestive enzymes; and the combination of heat and shear is a very efficient way of disrupting such structures. In general, denaturation of protein to random configurations improves nutritional quality by making the molecules more accessible to proteases and, thus, more digestible. This is especially important in legume-based foods that contain active enzyme inhibitors in the raw state (Dobraszczyk *et al.*, 2006).

Disulphide bonds are involved in stabilizing the native tertiary configurations of most proteins. Their disruption aids in protein unfolding and thus digestibility. Shearing can contribute to the breaking of these bonds (Dobraszczyk *et al.*, 2006).

Partial hydrolysis of proteins during extrusion increases their digestibility by producing more open configurations and increasing the number of exopeptidase-susceptible sites. Conversely, production of an extensively isopeptide cross-linked network could interfere with protease action, reducing the digestibility (Phillips, 1989).

The inherent property of sprouting seeds, to increase the hydrolytic activity of enzyme may cause the mobilisation of protein leading to the formation of polypeptides, dipeptides and free amino acids. Further during malting, the polyphenols and phytic acids are catabolized as well as their left over amount were removed as malting loss. This may be responsible for increasing the protein digestibility during malting (Pawar and Pawar, 1997).

Considering that most investigators observed an increase in protein digestibility with processing, it is unclear what may have caused the opposite in these experiments, but there are some possible explanations that would need further investigation; the difference in types and varieties of raw materials (grains used) could be a factor in the difference in observations. Also, the processing (exact parameters) conditions for the experiments. It is also possible, that the malting and extrusion parameters, though, leading to a decrease in antinutritional compounds and a breakdown of some protein into polypeptides, dipeptides and amino acids, complexes between proteins and other compounds present (fats, carbohydrates, minerals and

antinutritional factors) were also formed that prevented or lowered protein digestibility. The decrease in in vitro protein digestibility for both AgG and Ba could also be as a direct result of an increase in total phenolic content after malting (**Table 19**).

The *in vitro* starch digestibility for both AgG and Ba (**Table 19**) was improved significantly (p ≤ 0.05) by extrusion cooking, whilst malting only increased starch digestibility for Babala, with no effect on AgG, this is in agreement with (Archana and Kawatra, 2001), who also observed an increase in in vitro starch digestibility of pearl millet. The increase was attributed to malting loss which may represents the removal of antinutrients present in sprouts. According to Holm *et al.* (1987), other factors that have been shown to affect the digestion of starch of food, included; degree of gelatinisation, granule particle size, amylose/amylopectin ratio, starch-protein interaction, amylase/lipid complexes, percentages of resistant or retrograded starch and presence of other non-starch carbohydrates. In seeds, the factors like amylase inhibitors, phytic acid and polyphenols have been reported to inhibit α-amylase (Deshpande and Cheryan, 1984; Thompson and Yoon, 1984), hence decrease in vitro starch digestibility. The levels of these compounds in pearl millet decreases during malting, due to leaching and enzymatic breakdown, this in turn results in increased starch digestibility of malted pearl millet (Archana and Kawatra, 2001). During malting, amylase and phosphorylase might have become active and catalyse amylolysis (Pawar and Pawar, 1997).

5.4.4   Effect of malting, extrusion and their combination on the total phenolic content and antioxidant activity of beverage powders made from two varieties of pearl millet

The effects of extrusion and malting on the total phenolic content and antioxidant activity of AgG and Ba are summarised in **Table 20**.

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Extrusion significantly (p ≤ 0.05) reduced total phenolic content of both AgG (1.78 μg/g)) and Babala (0.93 μg/g), whilst malting significantly (p ≤ 0.05) increased total phenolic content of both AgG (3.68 μg/g) and Ba (4.55 μg/g). Extrusion had no effect on the antioxidant activity (TEAC) of AgG (1.73 μmoleTE/g)) and Ba (1.74 μmoleTE/g), whilst malting significantly increased antioxidant activity (TEAC) of both AgG (6.41 μmoleTE/g) and Babala (7.70 μmoleTE/g). Reduction of TPC by extrusion could be due to direct damage of the phenolic compounds by either the high processing temperatures, high pressures or shear generated by the screws, or a combination of any two or all parameters. The reduction of TPC could be considered advantageous, as some of these compounds are also known to have antinutritional properties.

Contrary to observations of the effect of malting on total phenolics (increase), Archana and Kawatra (1998), reported polyphenol content in untreated (raw) pearl millet grains of 764.45 mg/100 g, and observed a significant (p ≤ 0.05) destruction of polyphenols by malting, the level of destruction was dependent on germination time. It is speculated that leaching of polyphenols during steeping may account for some of this loss. Loss of polyphenols during malting may be attributed to the presence of polyphenol oxidase (Rao and Deosthale, 1982) and to the hydrolysis of tannin-protein and tannin-enzyme complexes which results in the removal of tannins or polyphenols (Farhangi and Valadon, 1981).

Table 20: **TPC and antioxidant activity of beverage powder from two (2) varieties of pearl millet processed by malting, extrusion and a combination of both processing methods**

	Agrigreen		Babala	
	Total Phenolics (ug/g)	TEAC (umoleTE/g)	Total Phenolics (ug/g)	TEAC (umoleTE/g)
RPM[2]	2.67 ± 0.05^{a1}	1.80 ± 0.18^{a}	3.04 ± 0.20^{a}	1.73 ± 0.07^{a}
ExPM	1.78 ± 0.06^{b}	1.73 ± 0.18^{a}	0.93 ± 0.05^{b}	1.74 ± 0.18^{a}
MPM	3.68 ± 0.04^{c}	6.41 ± 0.30^{b}	4.55 ± 0.12^{c}	7.70 ± 0.10^{b}
EMPM	1.34 ± 0.01^{d}	2.14 ± 0.16^{c}	1.30 ± 0.06^{d}	1.78 ± 0.25^{a}
ERPMMPM	1.59 ± 0.08^{e}	2.59 ± 0.09^{c}	1.62 ± 0.01^{e}	3.34 ± 0.04^{c}
OTE	2.34 ± 0.97	3.25 ± 2.06	2.28 ± 1.41	2.94 ± 2.15

[1] Values are mean ± standard deviation. Different superscripts in columns differ significantly (p ≤ 0.05)

[2] RPM = Raw pearl millet; ExPM = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix, OTE = Overall treatment effect.

Contrary to the present study, germination has been reported to reduce the polyphenol content in pearl millet (Osuntogun *et al.*, 1989; Pawar and Pawar, 1997; Sharma and Sehgal, 1992). The increase in total phenolics could be attributed to a possible increase in lignin (Opoku *et al.*, 1981)

5.4.5 <u>Sensory acceptability of the pearl millet based instant beverage prepared from beverage powders made from two varieties of pearl millet</u>

Figure 15 and 16 summarises the sensory acceptability of RPM, ExPM, MPM, EMPM and ERPMMPM beverages made from two varieties of pearl millet (AgG and Ba respectively). The average overall acceptance rating for RPM, ExPM, MPM, EMPM and ERPMMPM from Ba and AgG ranged from 4.71± 0.22 (like slightly) (AgG-RPM) to 6.15 ± 0.23 (Dislike slightly) (AgG-RPM). In general, the different sensory attributes rated by the panelists ranged from "Like Slightly = 4" to "Dislike Slightly = 6". The majority of the panelists "Neither like Nor Disliked" the different beverages. Significant differences (p ≤ 0.05) exists in all the panellists acceptability scores for the sensory attributes for the different products rated. The different backgrounds and possible prior exposure to similar products would affect the ratings of the different products (RPM, ExPM, MPM, EMPM and ERPMMPM from pearl millet) by the panelists.

An improvement in the attributes of the beverages is required in order to improve / increase its overall acceptability. This can be achieved with significantly increased protein content and quality over the un-supplemented pearl millet by the addition of any of the following: soybean, morama bean, or bambara groundnut. These would also act as functional ingredients supplying taste, texture, colour and other properties to variety of foods (Ali *et al.*, 2009).
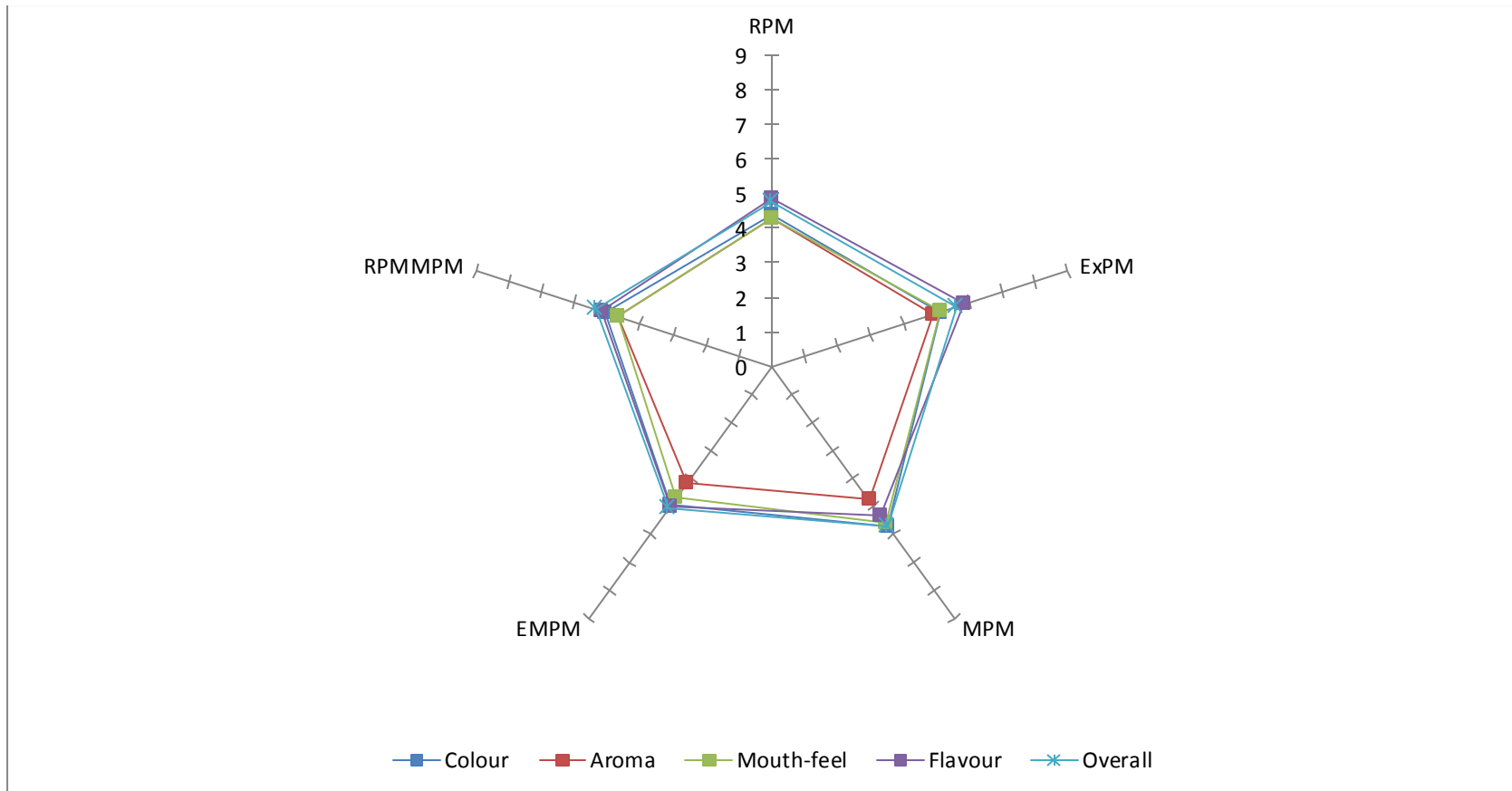
Figure 15: Spider sensory plot for Babala (RPM = Raw pearl millet; ExPM = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix).
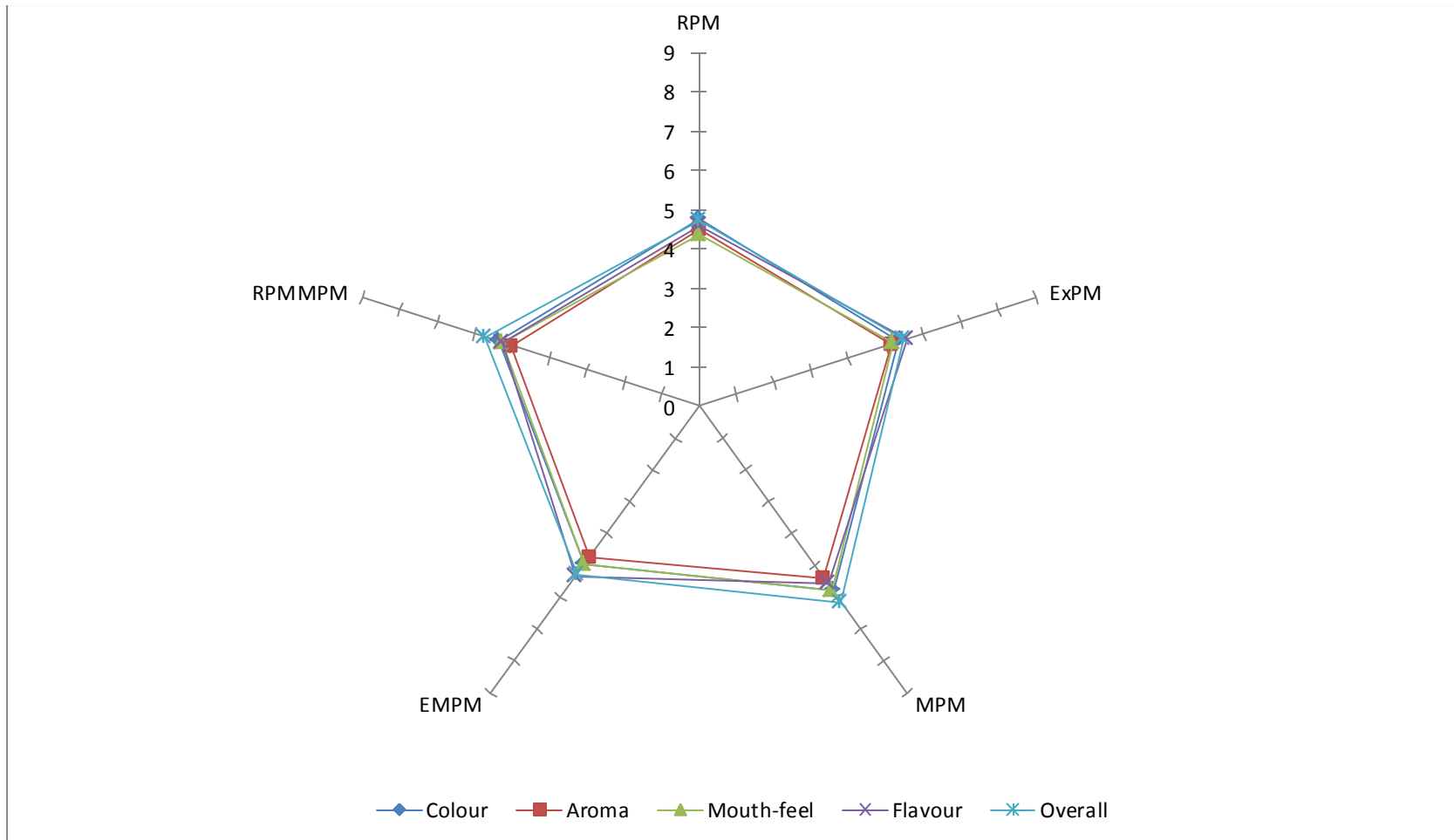
Figure 16: Spider sensory plot for AgriGreen (RPM = Raw pearl millet; ExPM = Extruded pearl millet; MPM = Malted pearl millet; EMPM = Extruded malted pearl millet, ERPMMPM = Extruded raw pearl millet-malted pearl millet mix).

The percentage inclusion of the suggested legumes will have to be determined so as not to adversely affect the flavour, and colour of the final product.

The colour differences calculated from data cin Tables 9 and 10 (section 2.2.3.2 pages 105 and 106), gives an indication of both the perception of a colour difference between ExPM, MPM, EMPM, ERPMMPM and RPM, and the effect of processing methods used for the preparation of the beverage powders.

The perception (visual) of a colour difference between samples, could also be an influencing factor in rating of the other attributes of the beverages, and hence the overall acceptability of the beverage.

5.5   Conclusions

Combination processing of the pearl millet led to a decrease in the TDF content, an increase in carbohydrates, Ca, energy and Fe content and no change in the other nutrients measured. Twelve of the 15 amino acids measured increased significantly following combination processing of the RPM. Protein and starch digestibility also increased following combination processing of both varieties of pearl millet. Whilst total phenolic content was decreased in both AgG and Ba following combination processing of the RPM, antioxidant activity (TEAC) increased significantly in AgG but remained unchanged in Ba. Beverages produced from both varieties of millet though not unacceptable, were not acceptable to the panellists. Improving the colour or rather decreasing the colour difference (ΔE) as well as improving the flavour of the beverages, could inevitably lead to better or increased overall acceptance of the beverages. These could be achieved by increasing the kilning temperature during malting, to affect the development of a more intense flavour profile as well a roasted / toasted colour in the grains. Addition of suitable adjuncts could further boost the nutritional value of the

products, but more importantly, increasing the overall acceptability of the beverages from pearl millet (AgG and Ba).

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CHAPTER 6:   CONCLUSIONS AND RECOMMENDATIONS

The primary aim of this research project was to develop an instant pearl millet based beverage powder, using a combination of malting and extrusion cooking, which is nutrient dense and acceptable to the consumers.   This aim was achieved in parts by undertaking the following: determining the ideal germination time for the two varieties of pearl millet used, at a fixed temperature and humidity; determining the ideal extrusion parameters for the raw, malted and mix (raw and malted) of each hybrid individually; evaluating the effect of malting, extrusion and a combination of both processes on the nutritional, biochemical, physical, functional and sensory properties of PMPMF and PMPMIBP produced from both varieties of pearl millet.

AgG had a higher germinative energy hence, growth yield compared to Ba and would therefore be more suitable for sprouting.   Malting of AgG and Ba, led to significant ($p \leq 0.05$) malting losses in both varieties of pearl millet; significant ($p \leq 0.05$) increases in their protein content; but significant decreases in moisture, fat and ash content.   Malting increased α-amylase activity peaking at 36 h of germination.   Hence, if malting is carried out mainly for the benefit of α-amylase enzyme, then germination must not proceed beyond 36 h, as the activity decreases after this.

PMPMIBP was produced by extruding raw malt and a mix of raw and malted PMPMF from both varieties of pearl millet.   The extrusion process, which involves high temperatures and pressures, cooks and partially dries the product.   The extrudate, which is then ground to give the final product (PMPMIBP) has several advantages.   Less preparation time, which means savings on energy all that is need to prepare the beverage from the PMPMIBP is freshly boiled water.   Long shelf life as it is a dried product.   Increased nutrient density of the beverage from the PMPMIBP, as more solids can be added without appreciable increase in viscosity.

Malting and extrusion reduced the viscosity of RPM as indicated by the low viscosities of ExPM, MPM, EMPM and ERPMMPM made from both varieties of pearl millet. This is advantageous with respect to an increase in nutrient density of the resulting beverages prepared from them. This would be suitable for use in the combatting of energy malnutrition observed mostly in young children.

Production of the flours of RPM, ExPM, MPM, EMPM and ERPMMPM from both varieties of pearl millet by malting, extrusion and a combination of both processes under the parameters used, had both beneficial and/or no effects on the functional and physical properties. The effects of these processes seemed to be variety dependent. Final viscosity of RPM was higher than that of ExPM, MPM, EMPM and ERPMMPM, an indication of damage to the native starch by the processing methods used. Malting lightened the colour of the RPM with high WSI, ER and lower viscosity. Extrusion produced darker products (especially ExPM) with perceivable colour differences from the RPM.

Manipulating operating parameters such as barrel temperature or screw speed amongst others during extrusion processing; or steeping conditions or germination time during malting, would alter the effects of these processes on the properties determined. If the effects are variety dependent, development of varieties specifically suited for a particular food product produced using either of these methods alone or in combination will have to be investigated in order to reap maximum benefits from the processes and their advantages.

Combination processing of the pearl millet led to a decrease in the TDF content, an increase in carbohydrates, Ca, energy and Fe content and no change in the other nutrients measured. Twelve of the 15 amino acids measured increased significantly following combination processing of the RPM. The protein and starch digestibility also increased following combination processing of both varieties of pearl millet. Whilst the total phenolics content decreased in both AgG and Ba following combination processing of the RPM,

consequently, antioxidant activity (TEAC), increased significantly in AgG but remained unchanged in Ba. The beverages from PMPMIBP of both varieties were neither liked nor disliked by the consumer panellists that rated the product, but with more development work aimed at improvement of overall acceptability of the products, panellists (trained and untrained) could come to appreciate the product. Several processing parameters (kilning temperature, kilning time,etc), and characteristics of the beverage powder (colour, viscosity etc.) will need to be adjusted and tested in an effort to improve all the sensory attributes rated as well as the overall acceptability of the beverages, without adversely affecting any of the nutritional, physical and functional properties. Improving the colour or rather decreasing the colour difference (ΔE) as well as improving the flavour of the beverages, could inevitably lead to better or increased overall acceptance of the beverages. These could be achieved by increasing the kilning temperature during malting, to affect the development of a more intense flavour profile as well a roasted / toasted colour in the grains. Addition of suitable adjuncts could further boost the nutritional value of the products, but more importantly, increasing the overall acceptability of the beverages from pearl millet (AgG and Ba).

Pearl millet instant beverage powder can be produced from AgriGreen and Babala varieties of pearl millet by a combination of malting and extrusion. Malting and extrusion decreased the viscosity of the beverage produced significantly ($p \leq 0.05$). This is an indication of the possibility of increasing the energy / nutrient density of the beverage made, by increasing the solid content. This would be suitable for use in the combatting of energy malnutrition observed mostly in young children.

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