UNIVERSITEIT VAN PRETORIA
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YUNIBESITHI YA PRETORIA

Pearl millet milling: Comparison between traditional Namibian fermentation- semi-wet milling and dry milling

by

Stephen Carmelo Barrion

Submitted in partial fulfillment of the requirements

for the degree

MSc (Agric) Food Science and Technology

in the

Department of Food Science

Faculty of Natural and Agricultural Sciences

University of Pretoria

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Dedication

I dedicate this work to my father, Mr Irineo Mendoza Barrion who passed away on 26 June 2003, while this work was being undertaken, my mother, Mrs. Estefania Quitasol Barrion, my grandmother, Lola Luz, my sisters, Carrie and Irene and brother-in-law, Wilfred Schmidt, for their encouragement, support and love throughout my life and during my tenure at the University of Pretoria. They encouraged me every step of the way and supported me through all of the best and worst of graduate school. Thank you for always being there. I would not be who I am today without you. I love you always.

Declaration

I declare that the dissertation herewith submitted for the degree MSc (Agric) Food Science and Technology at the University of Pretoria, has not previously been submitted by me for a degree at any other University or institution of higher education.

Stephen Carmelo Barrion

January 2008

Abstract

Pearl millet milling: Comparison between traditional Namibian fermentation- semi-wet milling and dry milling

By

Stephen Carmelo Barrion

Supervisor: **Professor J.R.N. Taylor**

Co-supervisor: **Professor L.W. Rooney**

Pearl millet is a staple food in Namibia. It is milled into flour by traditional and industrial dry milling processes. This research was conducted to help determine how to improve the nutritional value and acceptability of pearl millet. The traditional milling process involves a lactic acid fermentation step which lowers the pH of kernels. The effects of the traditional Namibian and industrial "dry milling" processes on the physical and nutritional composition of pearl millet grain were compared. Additionally, the effect of steeping three different Namibian pearl millet varieties (Kangara, Kantana and Okashana 2) in lactic acid and water on the colour and the phenolic content of the flour were determined.

Regarding comparing the milling processes, variety Kangara was conditioned and decorticated traditionally with a pestle and mortar and industrially with an abrasive decorticator. The traditional decorticated grain was steeped and sun dried for 24 h before hammer milling, whereas the industrially decorticated grain was roller milled. Tristimulus colorimetry and proximate analyses were conducted on the samples. Concerning acid steeping, kernels were steeped in a pH 3.5 solution and in water as a control. Colour, total polyphenol and c-glycosyl flavone contents were determined. The determination of c-glycosyl flavone content was particularly important because these compounds are considered goitrogenic.

The traditionally milled flour was lighter in colour than industrial milled flour. However, it was significantly lower in protein, ash and c-glycosyl flavone contents in comparison to industrial milled flour. This was due to the removal of more pericarp and germ in the traditional process. The industrial dry milling process therefore produces flour with a

higher nutrient content in terms of protein, fat and minerals. However, the traditional Namibian milling process makes the colour of the pearl millet flour lighter, which is probably the reason that it is more acceptable to consumers.

Kernels steeped in a lactic acid solution were lighter in colour than those steeped in water. Irrespective of the steeping media, the total polyphenol content was significantly lower in steeped kernels compared to those unsteeped. A similar trend was observed for the c-glycosyl flavone content. This indicates that some of these compounds may have leached out during steeping. For all varieties, kernels steeped in lactic acid had a significantly higher total polyphenol content than those in water, probably due to the dissociation of metal-polyphenol complexes in the acidic medium whereby these polyphenols became free and available for measurement. Thus, steeping in a lactic acid solution can lead to better colour improvement of kernels compared to steeping in water. Thus, lactic acid steeping can improve the sensory quality of pearl millet products.

An industrial process can thus be designed to include tempering the grain with food grade lactic acid to produce sour taste and leach out the colour pigments, particularly the c-glycosyl flavones hence lightening the colour of the industrial milled flour. This produces a product with high nutritional content, lighter in colour and has the sour taste that consumers find appealing.

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CHAPTER 1

1. INTRODUCTION

1.1. Statement of the problem

According to the Food and Agriculture Organization (FAO), pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the sixth most grown cereal in the world (FAO, 2005). Out of the 28 million tons of millet produced globally (1992-1994 average), 15 million tons are pearl millet. It is considered one of the most important crops in semi-arid areas of Africa and India (Hadimani, Muralikrishna, Tharanthan and Malleshi, 2001) because it contributes to food security in these regions (ICRISAT and FAO, 1996). It is grown on about 26 million hectares, principally for grain and feed (Elyas, El Tinay, Yousif and Elsheikh, 2002). Eighty percent of millet produced globally is used for food and the remainder is divided between feed (7%) and other users such as seed, beer and waste (ICRISAT and FAO, 1996). In Africa and Asia, pearl millet is grown for human consumption (Abdalla, El Tinay, Mohamed and Abdalla, 1998a). In developed countries one million tons is used as animal feed (ICRISAT and FAO, 1996).

Data from the FAO (2005) show that in Namibia from 2000 to 2005 the total amount of pearl millet produced was two to five times greater than the total production of all other cereals. According to ICRISAT and FAO (1996), within the hottest and driest regions of the world, such as Namibia, pearl millet will remain a major source of energy and a crucial part of food security.

This highlights the importance of pearl millet for human consumption in Namibia where food security has become dependent on urban agriculture. Urban agriculture in this context refers to the informal transferring of food from rural areas to migrants living in urban areas. Urban migrants receive significant amounts of Namibia's pearl millet, locally known as *"Mahangu"*, from relatives living in the rural areas to meet their food needs.

In Namibia, pearl millet is milled into flour, which is fermented, and made primarily into a thick porridge *"oshifima"*, thin porridge *"etete"*, breads "*oshikwiila*", cakes and cookies *"uukuki"*. The pearl millet flour can also be used in combination with sorghum flour to

make alcoholic beverages such as *"oshikundu"* or *"omalavu"* (Mallet and Du Plessis, 2001).

Flour used for food preparation is produced by milling, either through traditional or mechanical processes. According to Taylor and Dewar (2001) the objective of milling is to achieve an anatomical separation of the pericarp, germ and endosperm, as well as to reduce the endosperm into fine particles. Decortication involves removing, the outer layers of the grain. For example, the pericarp and testa of the pearl millet kernel, in which anti-nutrients are concentrated are removed, which may increase the availability of nutrients (Chowdury and Punia, 1997). However, decortication is also associated with the loss of nutrients such as vitamins and minerals which are found in the pericarp layers of cereals including pearl millet (Serna-Saldivar, Clegg and Rooney, 1994).

In Namibia, decortication of pearl millet is traditionally achieved in a manual process which involves pounding with a pestle and mortar. More recently, the introduction of small scale decortication and milling machinery has made the process easier, which has contributed to increased pearl millet flour production. The traditional Namibian milling process for pearl millet involves soaking decorticated grain in water and allowing a lactic acid fermentation process to take place. The fermented grain is then removed from the water and dried in the sun. Once the grain is semi-dry it is milled into fermented pearl millet flour. The milled grain is then completely dried.

In 1999, Namib Mills (Pty) Ltd, the largest industrial milling company in Namibia, started milling pearl millet on an industrial scale. This industrial-scale process is a dry milling process, which differs significantly from the traditional milling process. Both milling processes improve the palatability of pearl millet grain, making it suitable for human consumption. Flour produced by the two milling processes, however, may differ in nutritional composition and functional properties. In addition, the pearl millet fermented flour is lighter in colour than industrial milled flour, making it more appealing to consumers. The reason for the lightening effect of pearl millet grain milling is not well understood but it has been attributed to c-glycosyl flavones that change colour with pH (Reichert, 1979).

This research project therefore investigated the effects of these two milling processes on the nutritional and functional properties of pearl millet flour.

1.2 LITERATURE REVIEW

In this review, our knowledge of the kernel structure, chemical and nutritional composition of the pearl millet grain will be discussed. Literature describing traditional and industrial milling processes for pearl millet and related grains will also be reviewed. In addition, what is known about the effects of these processes on the physical, chemical and nutritional value of pearl millet and related grain flour will be examined.

1.2.1 Pearl millet kernel structure

The overall structure of the pearl millet kernel (Fig. 1) is similar to that of sorghum (Serna-Saldivar and Rooney, 1995), except that the pearl millet kernel is smaller in size and has a relatively smaller endosperm and proportionally larger germ than sorghum (Abdelrahman, Hoseney and Varriano-Marston, 1984). The pearl millet kernel (Fig. 1) comprises three main components: the pericarp, the germ (embryo) and the endosperm, with a distribution of 7.2 to 10.6%, 15.5 to 21% and 71 to 76%, respectively (Abdelrahman et al., 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. Differences exist among different varieties of pearl millet.

The surface of the pearl millet kernel is covered with a thin, waxy cutin layer which plays a role in reducing weathering effects on the kernel (Taylor, 2004). The pericarp consists of large blocky cells that contain concentric layers of pigmented tissues but no starch granules (McDonough and Rooney, 1989). The pearl millet 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 can be 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. The presence of a thick pericarp can mask the presence of pigment 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 sorghums (McDonough and Rooney, 1989).

Beneath the seed coat lies a single layer of aleurone cells that forms the first layer of the endosperm cells (Zeleznak and Varriano-Marston, 1982). These cells which have thick cell walls contain protein bodies and oval lipid bodies in their cytoplasm. Aleurone cells may also contain pigments which contribute to the overall colour of the kernel. Pearl millet starchy endosperm may be 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, Rooney and Earp, 1986).

The pearl millet starchy endosperm can be subdivided into three distinctive areas: 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, Hoseney and Casady, 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 more loosely packed resulting in less 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).

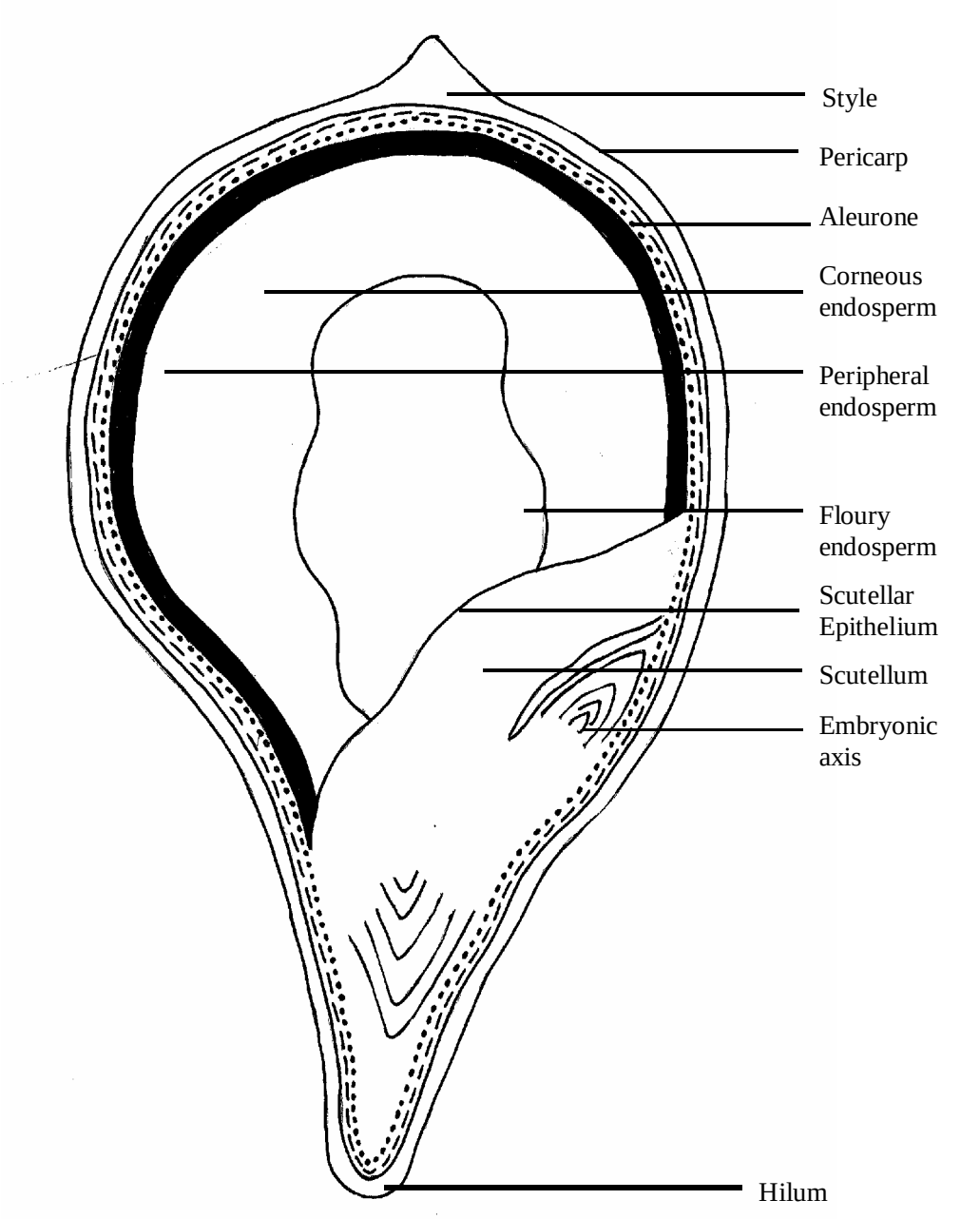

Fig. 1. Diagram of a section through a pearl millet kernel.

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, in addition, characterized by the presence of a discontinuous protein matrix.

The last major structural component of the pearl millet kernel is the germ (embryo). As stated, 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 (McDonough and Rooney, 1989) 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). The embryonic axis on the other hand, has the function of incorporating the radicle, the hypocotyl to which the cotyledon is attached and the shoot apex containing the plumule (Bewley and Black, 1994).

1.2.2 Nutritional composition of pearl millet

1.2.2.1 Carbohydrates

The major component of pearl millet grain, as in all cereals, is starch (Table I). The starch content may vary from about 50 to 75% (Hadimani and Malleshi, 1995; Hoover, Swamidas, Kok and Vasanathan, 1996; Oshodi, Ogungbenle and Oladimeji, 1999; Hadimani et al., 2001), which is similar to that of sorghum.

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% (reviewed by Taylor, 2004). Hoover et al. (1996) and Hadimani et al. (2001) showed that the starch amylose content may be as high 28.8 to 38% in some varieties. This shows that there is 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).

Aside from starch, the carbohydrates in pearl millet grain include free sugars and non-starch polysaccharides (Hadimani et al., 2001). Similar amounts of total soluble sugars are found in sorghum and pearl millet, 2.3 and 2.6%, respectively (Serna-Saldivar and Rooney, 1995). The 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 a 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). Nandini and Salimath (2001) found 3% soluble fibre and 5% insoluble fibre in defatted whole grain flour from one pearl millet variety. Glucose, arabinose and xylose were the major non-starch polysaccharide fractions of pearl millet (Hadimani et al., 2001). This indicates that the pentosans are composed predominantly of arabinose and xylose, whereas the glucose comes mainly from cellulose. Serna-Saldivar and Rooney (1995) stated that the dietary fibre components such as cellulose, hemicellulose, lignin, pectins and gums in cereals are found in the pericarp and endosperm cell walls.

Table I. Typical Chemical Composition (%) of Whole Pearl Millet Grain
(adapted from Taylor 2004)

Component ***(db) (g per 100 g)***	**Whole grain (%)**
Starch	63.1 – 78.5[1] (71.8)[2]
Protein	8.6 – 19.4 (14.5)
Fat	1.5 – 6.8 (5.1)
Dietary Fibre	8.0 – 9.0 (8.5)
Ash	1.6 – 3.6 (2.0)

1. Range of values
2. Typical values

1.2.2.2 Proteins

Pearl millet has a higher protein content and amino acid score than sorghum (Serna-Saldivar and Rooney, 1995). The crude protein content of most pearl millet cultivars ranges between 10 and 15% (DeFrancisco, Varriano-Marston and Hoseney, 1982a; McDonough et al., 1986; Hoover et al., 1996; Chowdhury and Punia, 1997; Elyas et al., 2002). This is a typical range, even if there is a wide variability in the protein contents of different pearl millet genotypes (Hulse, Liang and Pearson, 1980).

Pearl millet protein can be categorized 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. Taylor (2004) stated that prolamins may comprise 31 to 41% of the total protein in pearl millet. The prolamins in the pearl millet endosperm as in other cereals, serve as storage proteins (Eggum, Bach Knudsen, Munck, Axtell and Mukuru, 1982). Marcellino, Bloch and Gander (2002) reported three major types of prolamins called A-, B- and C- pennisetins with molecular weight values around 27, 22 and 12 kDa. The albumins and globulins account for 25% of the total protein content (Hadimani et al., 2001).

Pearl millet is considered to have one of the best protein qualities in terms of amino acid score (Almeida-Dominguez, Serna-Saldivar, Gomez and Rooney, 1993). For example, 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 it still remains the most limiting essential amino acid. 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, 2004). 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. This decreases from the exterior to the interior of the kernel (McDonough 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 contributes to the corneous endosperm texture, which facilitates good milling properties (DeFrancisco, Shepherd, Hoseney and Varriano-Marston, 1982b). Relatively few protein bodies are found in the floury endosperm. McDonough and Rooney (1989) reported that a considerable amount of the pearl millet protein is present in the germ.

1.2.2.3 Fat

Typically, pearl millet grain has a fat content of approximately 5.1% (Taylor, 2004), which is higher than what is typically found in sorghum of 3.2% (Serna-Saldivar and Rooney, 1995). Maize, however, has a higher fat content (Taylor, 2004). 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, Hoseney and Varriano-Marston, 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). The fat content is reduced by decortication. For example, Hadimani and Malleshi (1993) reported a reduction in pearl millet fat content from 5.2% before decortication to about 4% after decortication. 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, Jacobs and Marquart, 2001).

1.2.2.4 Vitamins

The niacin and carotene content of pearl millet are lower than in sorghum (Serna-Saldivar and Rooney, 1995). In a study conducted by Simwemba, Hoseney, Varriano Marston and Zeleznak (1984) it was concluded that niacin in pearl millet is moderately affected by the location where the crop is grown. A typical value for niacin is 2.7 mg/100 g, compared to 4.8 mg/100 g in sorghum (Serna-Saldivar and Rooney, 1995). Niacin is partially bound to

carbohydrates which is made available by treatment with alkali (Gregory III, 1996; Taylor, 2004). In West Africa, wood ash is used in the preparation of a porridges which makes the niacin more available (Taylor, 2004). This seems not to be the practice in Namibia.

Vitamin B_{12} amounts of 0.07 µg/100 g in pearl millet flour and 0.05 µg/100 g in bread made from pearl millet flour have been reported (Khalil and Sawaya, 1984). Similarly, a reduction of panthothenic acid from 1.40 mg/100 g in pearl millet flour to 0.71 mg/100 g in pearl millet bread was also reported. It is assumed that these vitamins are reduced by the application of heat during baking. Low values of 0.5 mg/100 g β-carotene were found in pearl millet compared to 2.9 mg/100 g found in sorghum (Serna-Saldivar and Rooney, 1995). There is apparently a small difference in the Vitamin E content of pearl millet and sorghum. Pearl millet has been reported to have a Vitamin E content of 1.9 mg/100 g and 1.2 mg/100 g in sorghum (Serna-Saldivar and Rooney, 1995).

Similar values of thiamin and riboflavin have been found in pearl millet and sorghum. Typical values are 0.4 mg/100 g and 0.2 mg/ 100 g, respectively (Serna-Saldivar and Rooney, 1995). Riboflavin, niacin and thiamin are mainly in the germ and pericarp of pearl millet (Simwemba et al., 1984). They are, however, relatively low in the endosperm. Hence, degermination and milling causes significant losses in the B vitamins (Taylor, 2004).

1.2.2.5 Minerals

Minerals such as calcium, phosphorus, zinc, sorghum, 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) found a calcium content of 22 mg/ 100 g (wet basis) in whole pearl millet flour. 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). Decortication and milling significantly reduces the levels of these

micronutrients (Chowdhury and Punia, 1997; Hadimani and Malleshi, 1993; Slavin et al., 2001).

1.2.2.6 Minor components

1.2.2.6.1 Phenolics in pearl millet

The total polyphenol content 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 phenols in pearl millet grain. However, Elyas et al. (2002) reported lower values ranging between 294 and 319 mg/100 g.

Phenolics are characterised by the presence of an aromatic ring with one or more hydroxyl groups (Shahidi and Naczk, 2003). They act as antioxidants (Rice-Evans, 2001; Kaur and Kapoor, 2001; Pretorius, 2003) and may prevent various diseases associated with oxidative stress (Cotelle, 2001) such as cancers, cardiovascular disease and inflammation (Sagin and Sozmen, 2004). Phenolics include phenolic acids, flavonoids and tannins.

1.2.2.6.2 Phenolic acids in pearl millet

Phenolic acids are derivatives of phenylalanine and to a lesser extent tyrosine (Shahidi and Naczk, 2003). Ammonia is formed as a by-product when phenylalanine is catalyzed by the enzyme phenylalanine ammonia lyase (PAL). The resulting phenylalanine derivative is the phenolic acid *trans*-cinnamic acid. Similarly, tyrosine can be converted into 4-hydroxy-cinnamic acid by tyrosine ammonia lyase (TAL). An introduction of a hydroxyl group at the *para* position of the ring of cinnamic acid, results in the formation of *p*-coumaric acid. This compound is further subjected to hydroxylation at positions 3 and 5 by hydroxylases and possibly by methylation via O-methyl transferase with S-adenosylmethionine as a methyl donor. This leads to the formation of caffeic, ferulic and sinapic acids. Pearl millet phenolic acids include ferulic, coumaric, gentisic, cinnamic, caffeic, vanillic, protacatechuic, p-OH benzoic, syringic and sinapic acids (McDonough et al., 1986).

1.2.2.6.3 Ferulic acid

According to Shahidi and Naczk (2003) the content of alkali-labile ferulic acid (Fig. 2) (ALFA) in pearl millet bran may be up to 10 times higher than in rice, maize, oat, rye and wheat bran. They reported amounts of 133 to 241 mg/100 g ALFA. Similarly, Reichert (1979) found 158 mg/100 g of ALFA in pearl millet.

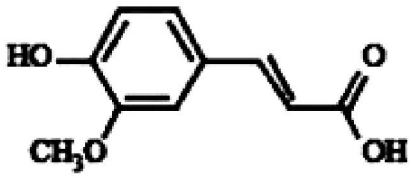

Fig. 2. Structure of ferulic acid (Pretorius, 2003).

1.2.2.6.4 Flavonoids in pearl millet

According to Hollman and Arts (2000) flavonoids are polyphenolic compounds characterized by a diphenylpropane (C_6-C_3-C_6) carbon skeleton (Fig. 3a). They consist of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle (Gee and Johnson, 2001).

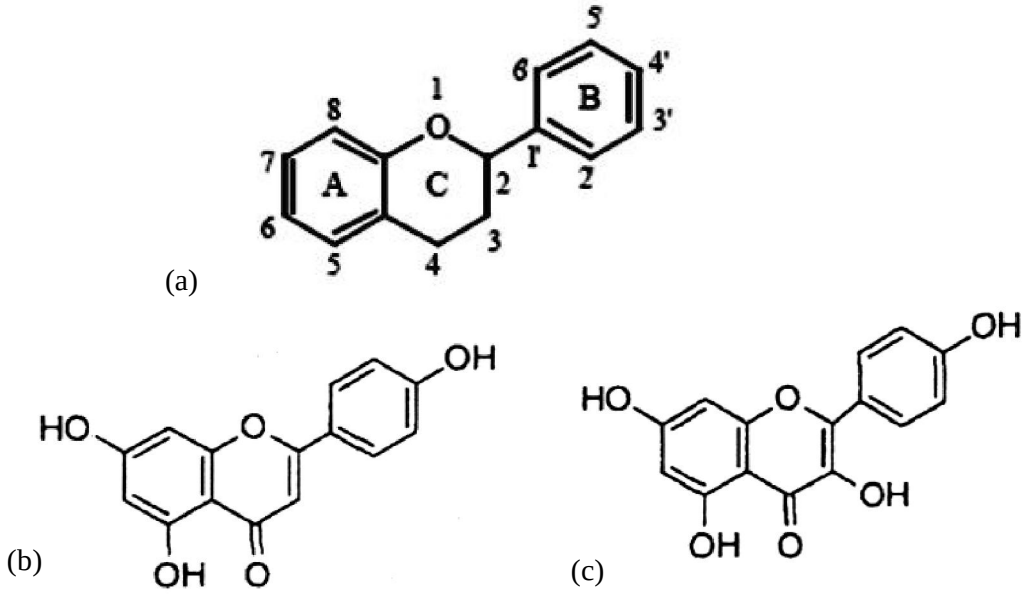

Fig. 3. Flavonoid type phenolics (a) Basic structure of flavonoids (Cotelle, 2001)
b) flavone and (c) flavonol (Peterson and Dwyer, 1998).

The first “A” ring is aromatic and the second “C” ring is an oxygen atom containing heterocyclic ring attached to a third aromatic “B” ring (Peterson and Dwyer, 1998). Flavonoids differ in their structure from each other at the “C” ring. Structural variations within the rings differentiate flavonoids in pearl millet. Those of particular relevance to this review are the flavones and flavonols (Fig. 3b and c), which are found in pearl millet (Reichert, 1979; Akingbala, 1991).

Birzer and Klopfenstein (1988) reported that flavonoids occur as glycosides (with sugar moieties) and aglycones (without sugar moieties). Glycosylation renders the molecule more water soluble and less reactive to free radicals (Cotelle, 2001). The sugar most commonly involved in glycoside formation is glucose but others include galactose, rhamnose and xylose. Flavones and flavonols are structurally similar (Hollman and Arts, 2000). Flavonols, as compared to flavones have an additional hydroxyl group at the 3-position. Flavones such as apigenin and its glycosides are found in the aleurone and pericarp layers of pearl millet grain and as stated, may influence grain colour (Reichert, 1979).

1.2.2.6.5 Potential goitrogens

C-glycosyl flavones are flavonoid-type compounds found in most millets including pearl millet. These include vitexin (Fig. 4), glucosyl vitexin, and glucosyl orientin (Reichert, 1979; Akingbala, 1991). Primarily, they contribute to the pigmentation of pearl millet. However, according to Gaitan, Lindsay, Reichert, Ingbar, Cooksey, Legan, Meydrech, Hill and Kubota (1989) and Elnour, Liedén, Bourdoux, Eltom, Khalid and Hambraeus (1997) these compounds may interfere with thyroid function resulting in goitre.

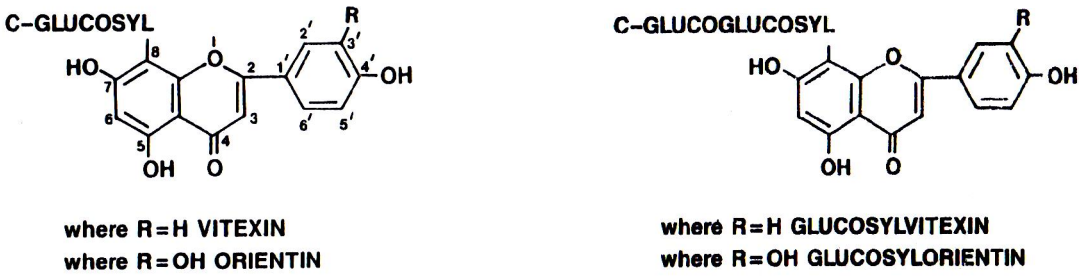

Fig. 4. Pearl millet goitrogens (C-glycosyl flavones) (Gaitan et al. 1989).

Generally, the glycosyl flavone content of pearl millet may be as high as 0.1% (Gaitan et al. 1989). Reichert (1979) found whole pearl millet contained 124 mg/100 g c-glycosyl flavones (measured as glucosylvitexin equivalents). However, others reported 119 mg/100 g to 275 mg/100 g other pearl millet varieties (Klopfenstein, Leopold and Cecil, 1991; Akingbala, 1991). Akingbala (1991) reported a variety of pearl millet with 76.6 mg/100 g (i.e. less than 0.1%) c-glycosyl flavone content.

The c-glycosyl flavones are located in the aleurone and pericarp layers of the pearl millet kernel (Reichert and Youngs, 1979) and form part of the grey pH sensitive pigment. They are methanol soluble. They cause the colour of pearl millet flour to change from grey to yellow-green in basic conditions and grey to creamy white in the presence of an acid. With progressive decortication, the concentration of c-glycosyl flavones decreases (Reichert, 1979). This author found that the c-glycosylflavone concentration decreased from 124 mg/100 g to 90 mg/100 g after 10% of the kernel was removed. Gaitan et al. (1989) similarly reported that the concentration of c-glycosyl flavones was greatest in the bran, while the flour contained the lowest concentration.

According to Birzer and Klopfenstein (1988) flavonoids undergo chemical changes when ingested by animals. As a result of these changes, glycosides are hydrolyzed to aglycones, while B-rings undergo methylation and c-ring fission occurs (Fig. 5). This increases anti-thyroid activity as well as additive anti-thyroid effects.

1.2.2.6.6 Tannins

Tannins are a sub-group of polyphenols (Bravo, 1998). Pearl millet is reported not to contain tannins (McDonough et al., 1986; McDonough and Rooney, 1989; Taylor, 2004). However, Elyas et al. (2002) detected the presence of tannin in pearl millet with levels of 0.12% and 0.24% expressed as catechin equivalent (CE). This may be an artifact as many assay methods used to quantify polyphenol content do not differentiate between condensed and uncondensed phenolic compounds (Taylor and Belton, 2002).

1.2.2.6.7 Phytate

As with other grains, pearl millet contains phytic acid (Badau, Nkama and Jideani, 2005). It is the principal store of phosphorus in seeds (Taylor, 2004). Typically, phytic acid in

pearl millet ranges between 172 to 327 mg/100 g. However, higher levels of 354 to 795 mg/100 g have been reported (Abdalla et al., 1998b). Phytic acid or phytate (myo-inositol) when it is in a salt form, has strong chelating properties (García-Estepa, Guerra-Hernández and García-Villanova, 1999). It binds with multivalent cations like iron, calcium, magnesium and zinc, reducing the absorption of these minerals by humans. Additionally, it binds to protein and starch reducing their bioavailability (Kheterpaul and Chauhan, 1991; El Hag, El Tinay and Yousif, 2002). Phytic acid is concentrated in the aleurone layer and germ (Taylor, 2004).

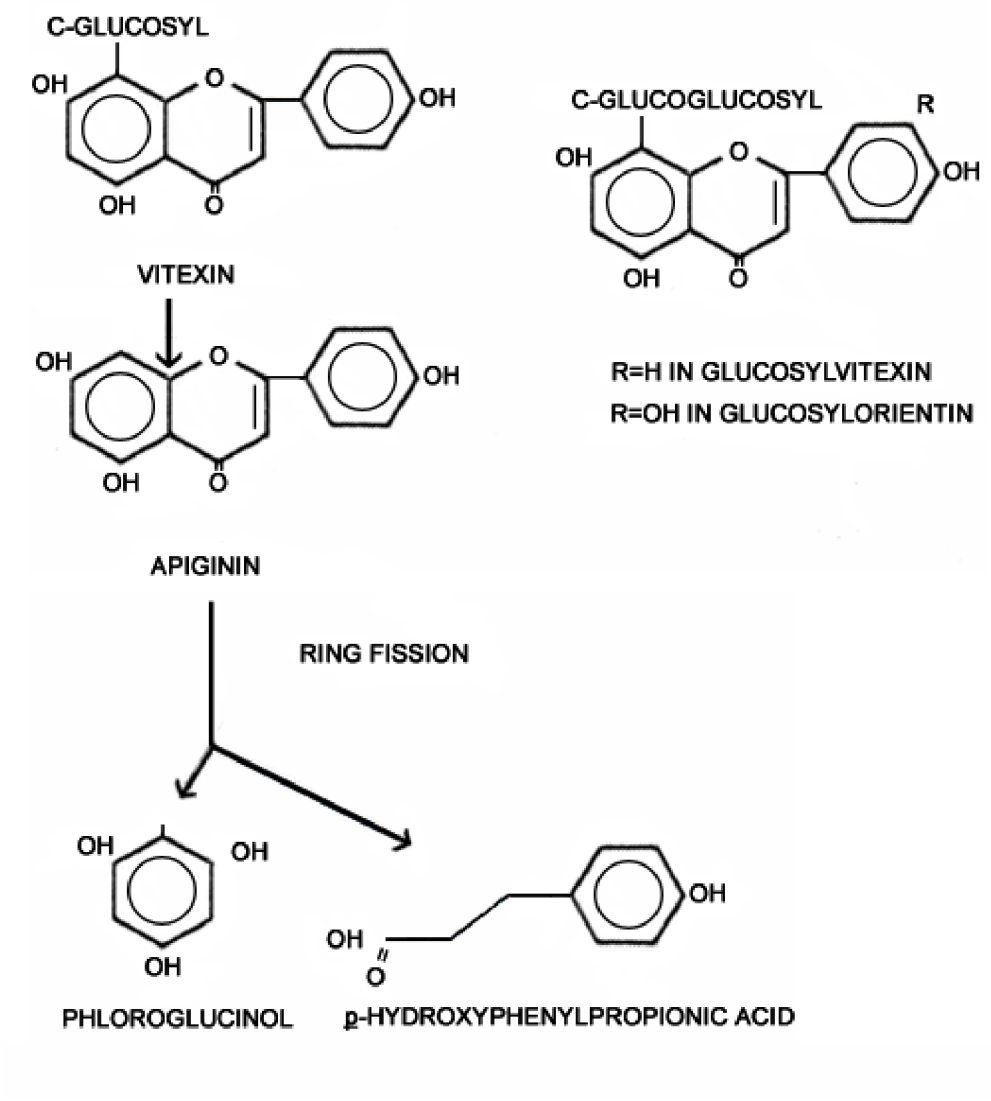

Fig. 5. Pearl millet flavonoids may be metabolically split to form known goitrogens (adapted from Birzer and Klopfenstein, 1988).

A study by El Hag et al. (2002) found that a combination of decortication and fermentation reduced the phytic acid content from 943 mg/100 g to 380 mg/100 g (60% reduction) and 1076 to 580 mg/100 g (48% reduction) in two pearl millet cultivars.

1.2.3 Primary Processing

1.2.3.1 Milling

In Namibia, pearl millet milling is generally a two stage process (Dendy, 1995). However, it is possible that grain may be milled in one process to make whole meal flour. In two stage pearl millet milling, the first stage, which is decortication, separates the pericarp and germ from the starchy endosperm, to improve the palatability and storage quality of flour (Taylor, 2004). In the second stage, the endosperm is reduced into fine particles (Taylor and Dewar, 2001). In Namibia the second stage usually involves a hammer milling process. Thus, hammer mills have been developed to produce pearl millet flour for household and small scale industrial use in Namibia (Dendy, 1995).

The one stage pearl millet milling process, with a hundred percent extraction rate, does not involve any loss of nutrients. However, in the two stage milling process certain nutritional losses occur due to the removal of the pericarp layers and germ, where most of the micro and macro-nutrients are found in high concentrations. Therefore, it may be advantageous to develop milling methods that reduce this loss or develop means to optimize nutrition by recombining the kernel components.

1.2.3.2 Traditional milling

Traditionally, Namibian women decorticate pearl millet grain by hand pounding, so that the end product is more palatable for consumption (personal observation, 2002). In the traditional Namibian milling process, pearl millet grain is decorticated manually using a wooden pestle and mortar (Mallet and Du Plessis, 2001). Thereafter, it is pulverized into flour manually with the mortar and pestle or mechanically using a hammer mill. This traditional process consists of five major unit operations: conditioning, decortication, steeping (lactic acid fermentation), sun drying and pulverising.

1.2.3.2.1 Conditioning

Prior to decortication, the grain is conditioned (tempered) by first moistening the grains with water to facilitate the removal of the pericarp from the grains (Mallet and Du Plessis, 2001). Bassey and Schmidt (1989) reported that the addition of 300 g/kg of water to sorghum and pearl millet grain results in the swelling of the pericarp layer which reduces its adherence to the endosperm. The diffusion of the moisture into the grain is the result of a moisture gradient and the structural composition of the grain (Jain and Bal, 1997). Water enters through small openings around the hilum. This results in an increase in moisture content of the germ and the surrounding tissues, thus changing their texture from a glassy polymer into a rubbery texture, which is more easily separated. Tempering also causes the aleurone layer and pericarp cells to become more malleable allowing them to be removed easily during decortication (Dexter and Wood, 2001). Additionally, tempering causes the plasticising of the endosperm which assists in the milling of wheat grains into flour.

1.2.3.2.2 Decortication

After conditioning with water (approximately 26% of their weight), the grain is hand pounded by use of a mortar and pestle (personal observation). The moisture level used for conditioning grain may at first appear to be high. However, since hand pounding is typically done in the open and under the sun, most of the moisture used for conditioning grain is lost through evaporation. Thereafter, the grain is skillfully winnowed to remove the pericarp. In general, the pestle and mortar are made of wood, with the latter weighing approximately 5 kg (Dendy, 1995). Mortars used for pearl millet decortication can be 43 to 45 cm high with a diameter of about 37 cm, while the pestle is usually about 1 m long and 6 cm in diameter with bulbous ends (Eggum et al., 1982). These figures are in agreement with observations made in this research project (Section 2.1 Fig. 7 c).

According to Scheuring and Rooney (1979), decortication, sometimes referred to as pearling or dehulling, is the removal of pericarp layers from cereal grain. It aids in improving the colour, texture and cooking quality of grain. Theoretically, the most desirable decortication would remove only the outer pericarp layers of the pearl millet grain, which are rich in polyphenols and retain the nutritious germ attached to the endosperm (Bassey and Schmidt, 1989). This process could result in a lighter colour of

flour, as more of the aleurone layer and pericarp layers containing phenolics are removed. However, in practice, the decortication process is repeated until an acceptable pericarp free product is obtained, as it is not an exact process.

1.2.3.2.2 Steeping (lactic acid fermentation)

The fully decorticated grains are steeped and fermented for 24 to 48 hours in water at a temperature of 29°C (personal observation, 2002). Lactic acid fermentation takes place, decreasing the pH of the steeping water. The sharpest decrease in pH occurs within the first two hours of fermentation (Kheterpaul and Chauhan, 1991). As fermentation progresses, the rate of decrease in pH slows. Kheterpaul and Chauhan (1991) reported a change in pH from 6.4 to 4.4, 4.0 and 3.6 at temperatures of 20, 25 and 30°C respectively, after 72 hours of steeping and fermentation.

Changes in the pH of the water influences the colour of the resulting flour (Reichert, 1979). The c-glycosyl flavones, which are in part responsible for pigmentation in pearl millet are particularly affected (Akingbala, 1991). Reichert (1979) reported that acidic conditions cause the metal present in the metal-flavanol complex to dissociate and alters the grey pigment present in the pearl millet into a creamy-white colour. An acidic pH was most effective in the depigmentaton of pearl millet to obtain white flour (Rathi, Kawatra and Sehgal, 2004a; Rathi, Kawatra, Sehgal and Housewright, 2004b). In addition, lowering of the pH contributes to the development of the sour taste desired by consumers (Mallet and Du Plessis, 2001).

1.2.3.2.3 Sun drying

After soaking, water is decanted off and the decorticated, steeped and fermented grain is sun dried (Abdalla et al., 1998a). Sun drying is done on concrete floor or on long tables made of wooden slats or on polythene sheets (Taylor, 2004). This process is repeated after milling the grain into flour to obtain shelf-stability.

1.2.3.2.4 Pulverising

The semi-wet, decorticated, fermented grains are pulverized by hammer milling into flour (Mallet and Du Plessis, 2001). Examples of hammer mills used in Namibia include the Kikuyu machine with 0.3 mm and 0.8 mm screens and the Drotsky mill with a 1.2 mm

screen. Most of the mills, for example the Drotsky M16 model, operate with three-phase electrical motors with 15 kW to 22 kW capacities. A few operate with tractors with petrol or diesel engines.

1.2.3.3 Industrial milling

The industrial dry milling process for pearl millet in Namibia comprises several processes, including the use of complex continuous systems of precision roller mills, sifters and air classifiers. The process generally consists of five unit operations: conditioning (tempering), decortication, roller milling, sifting and purification.

Pearl millet grains are conditioned (tempered) prior to industrial milling (personal observation). The grain is placed in steeping bins and water is poured onto them. The water penetrates the surface of the grain to assist in softening the protein-starch bonds that give the grain its characteristic hardness (De Francisco et al., 1982a). Due to varying diffusion resistances offered by the structural components of the grain, the moisture diffusion and absorption differs in the varying tissues, yielding fractions of different particle sizes when milled (Jain and Bal, 1997).

After conditioning, the pearl millet grain is decorticated using an abrasive dehuller. It is inaccurate to use the term dehuller since pearl millet does not have a hull (Taylor, 2004). A more appropriate term to use is an abrasive decorticator. Abrasive decortication is a process in which the pericarp layers of pearl millet are removed subsequently by friction and abrasion operations (Reichert and Youngs, 1976). The abrasion period depends on the design and the rate of grain flow through the dehuller (Bassey and Schmidt, 1989). Dehullers such as PRL (Prairie Research Laboratory) dehuller can decorticate grain at a rate of 50 to 100 kg/hr (Dendy, 1995). They consist of a metal shaft with evenly spaced out grinding stones or abrasive disks. This is enclosed within a semi-circular sheet-metal barrel, which serves as container for the grain. The disks rotate at a speed of 1500 to 2000 rpm, coming in contact with the grain and abrading off the outer layers. The fine bran particles are removed by means of a fan (Taylor and Dewar, 2001).

1.2.3.3.1 Roller milling

Little literature on the roller milling of pearl millet is available. According to Dendy (1995) roller mills designed for wheat and maize milling can be used on pearl millet grain to

produce good quality flour. This type of machinery is utilized in countries such as Namibia where pearl millet is processed on an industrial scale.

There are three sets of rolls involved in the roller milling process of pearl millet grain (personal observation, 2002). The first set of rolls is known as the break rolls. These are corrugated and run spirally along the axis of the roll (Hoseney, 1994). The slower moving roll tends to hold the pearl millet kernels, while the faster roller strikes the kernels as it passes between them. The gap between the rolls is such that the pearl millet kernel passes between the rolls without being crushed (personal observation, 2002). Instead, the rolls shear the grain open in order to make the pericarp layers come away from the endosperm. Although a small amount of flour is produced during this “break system”, the main objective is not to produce a lot of flour but rather to maximize the separation of the pericarp from the endosperm.
After each set of rolls sifting takes place (Hoseney, 1994). This is done by sieving machines (plansifters) with different screen and cloth meshes. These sieves gyrate continuously and rapidly allowing the stock (pearl millet material being ground) to be separated (personal observation, 2002). The first sieves remove large pieces of pericarp containing endosperm. These are then sent to purifiers and subsequently to reduction rolls.

A purifier is essentially an inclined sieve that is coarser from the front and finer to the back (Hoseney, 1994). Its main function is to separate unwanted material by both weight and size. The sieve oscillates and an air current passes upward through the sieve. Vibration and airflow contribute to stratification of the stock (pearl millet material not ground) and separation of material. The sieve permits heavy particles to fall through while other parts are suspended in the air in layers, as determined by their density. The less dense particles of pericarp are carried away, while the material that passes through the finest sieve cloth of the purifier is flour. The remaining particles not fine enough to pass through the sieves (overs) are directed to a second set of rollers known as the reduction rolls (personal observation, 2002).

The reduction rolls in the pearl millet roller milling process are smooth, not corrugated (personal observation, 2002). The purpose of this reduction system is to reduce the overs to fine flour. After each grinding process, the stock is sifted, the flour removed, and the coarser particles are sent to a third reduction roll where the processes repeated. The

separation by size and grade at each stage of the pearl millet milling process produces first break flour, second break flour and clean bran. The first and second break flours are blended and brought to bulk storage bins where they are filled into sacks and weighed.

Unlike the pearl millet situation, the application of roller milling technology to sorghum has been reviewed comprehensively by Taylor and Dewar (2001). In South Africa, small roller mills with two or three pairs of rollers and a vibrating screen device have been developed. These pairs of rollers consist of coarse fluted “break” rolls, finer break rolls and in some instances smooth “reduction” rolls (Taylor and Belton, 2002). A typical small commercial roller mill (capacity of 500 kg/hour) has coarse break rolls with eight flutes per 25 mm and finer break rolls with 22 flutes per 25 mm (Kebakile, Rooney and Taylor, 2007). It also has vibrating sieves with different aperture sizes that range from 1.00 to 0.710 mm (Tyler standard 16 and 26). These sieves, which separate the milled stocks are arranged in descending order by size to produce flour with varying levels of coarseness and fineness. It is reported that sorghum meal of higher extraction, and slightly lower ash and fat content is produced by roller milling the preconditioned sorghum grain to a moisture content of 16% than by hammer milling decorticated grain (Gomez, 1993).

1.2.4 Conclusions

The nutritional quality of pearl millet, with the exception of its protein quality and high fat content, is comparable to most other cereals. To improve the palatability and storage quality of pearl millet flour, pearl millet is milled through a traditional or industrial process. In the traditional processing of pearl millet, a steeping stage is included, which reduces the pH of the flour. This contributes to lightening colour of the flour and the development of the sour taste desired by consumers. The flour from the industrial milling process, however, is darker as it does not involve the steeping process. In both processes, decortication is performed, which results in losses of certain micronutrients and phenolic compounds. No studies have been done to compare the compositional differences between the flour produced by traditional milling and industrial processes. Given the importance of pearl millet to Namibia, it is important to establish the influence of the two milling processes on the nutritional composition, colour, phenolic content and other functional properties, which may lead to the improvement of both milling processes.

1.3 Objectives

- To determine and compare the effects of the traditional Namibian milling process and industrial “dry milling” processes, on the nutritional composition and anti-nutrients (polyphenols, goitrogens) of pearl millet grain.

- To determine and compare the effects of the traditional Namibian and industrial “dry” milling processes on the physical properties of pearl millet grain.

1.4 Hypotheses

The traditional milling process will produce flour of lower nutrients (protein, fat and ash content) than the flour from the industrial dry milling process because they are lost during the fermentation step, which is part of the traditional milling process and not of the industrial milling process.

It is expected that the traditional milling process, which also has lactic acid steeping process, has the potential for increasing the grain's goitrogenic properties. This is because not all polyphenols and flavonoids are removed during the decortication process of milling. According to Klopfenstein, Leipold and Cecil (1991) enzymatic processes are activated when the grain is wet and these enzymatic processes may convert flavonoid compounds in the grain to goitrogens, phloroglucinol and p-hydroxylphenylpropionic acid.

Traditional milling using a pestle and mortar removes less of the anti-nutrients compared to mechanical decortication. Decortication with a pestle and mortar would only remove anti-nutrients present in the outer layers (pericarp and testa) of the pearl millet kernel, for example, the flavonols found in the pericarp tissue (Akingbala, 1991). However, mechanical decortication removes the flavonols present in the pericarp tissue and those flavanols found in relatively greater amounts in the aleurone, the germ and part of the peripheral endosperm (Akingbala, 1991). Therefore a lower concentration of anti-nutrients is expected in the industrial dry milled samples compared to the traditionally milled samples.

The traditional milled flour is "whiter" in colour because of the "bleaching effect" on the pH sensitive flavonoid pigments (Reichert and Youngs, 1979) during the lactic acid fermentation process.

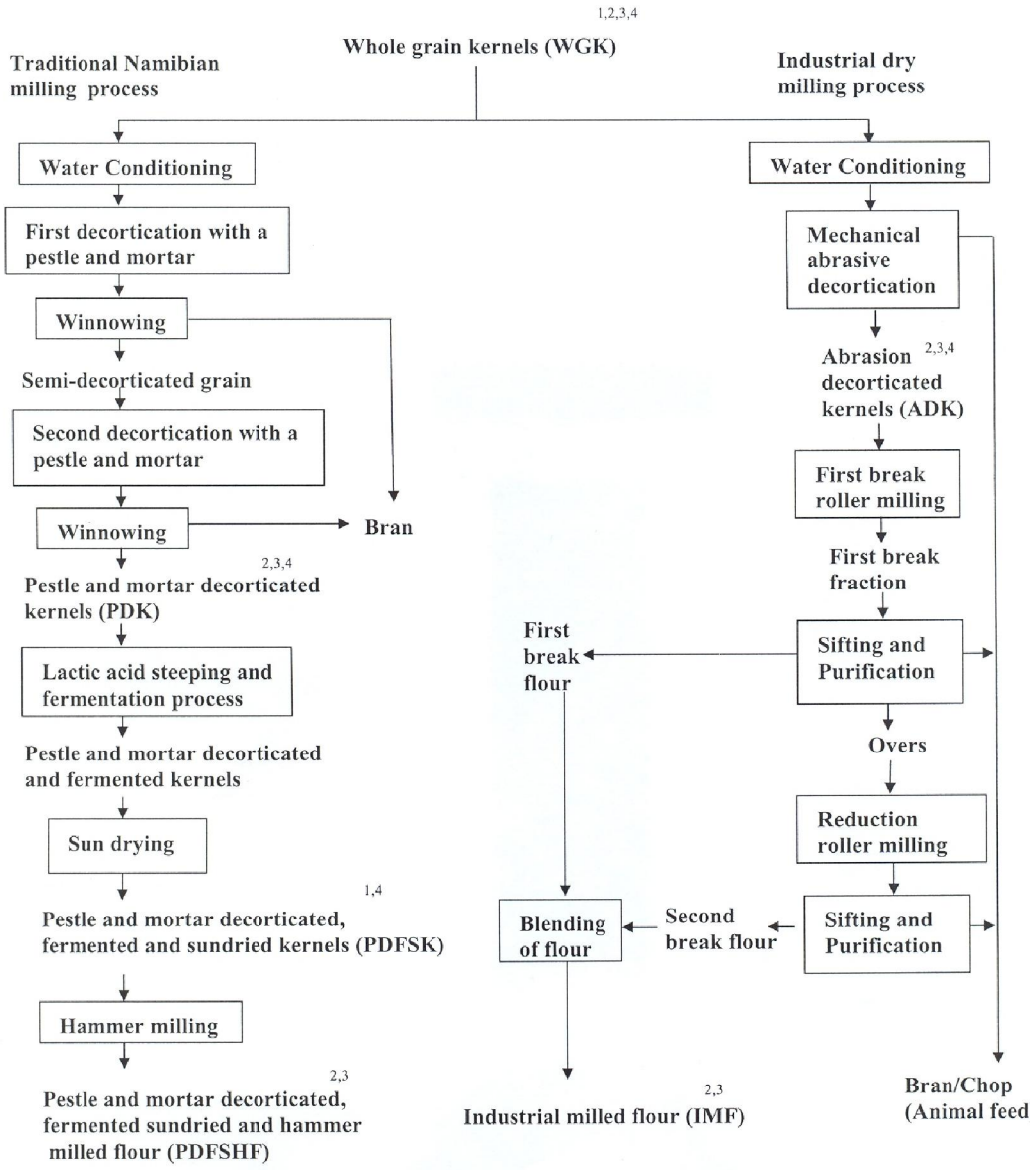

Fig. 6. Flow diagram of the traditional Namibian milling process and industrial dry milling process of pearl millet used in this research (Superscripts refer to analyses carried out on indicated samples as outlined in Phases I and II below).

Research comprised two phases:

Phase I

1. Characterisation of the pearl millet grain: Grain size, Endosperm texture, Pericarp colour, Grain hardness and Thousand kernel weight.

2. Proximate and Physico-chemical analyses: Moisture content, Crude fat, Crude protein, Total starch, Fibre by difference and Colour of resultant milled product e.g. whole grain kernels (WGK), pestle and mortar decorticated kernels (PDK), pestle and mortar decorticated, fermented and sun dried kernels (PDFSK), pestle and mortar decorticated, fermented, sun dried and hammer milled flour (PDFSHF), abrasion decorticated kernels (ADK), industrial milled flour (IMF).

3. Anti-nutrient analyses: Total polyphenols and Goitrogens (c-glycosyl flavones)

4. Staining and Light microscopy of whole grain kernels, abrasion decorticated kernels, pestle and mortar decorticated kernels, pestle and mortar decorticated, fermented and sun dried kernels.

Phase I dealt with the effects of the traditional Namibian (steep then mill) and industrial dry milling processes on the nutritional composition of pearl millet flour and is covered in Chapter 2.1.

Phase II

Phase II dealt with most aspects as in Phase I applied to three different pearl millet varieties. Further, the effects of acid steeping on the colour and phenolic content of pearl millet varieties were determined in Chapter 2.2.

CHAPTER 2

RESEARCH

A flow diagram of the experimental design used in this research is shown in Fig. 6

2.1 Effects of the traditional Namibian (steep then mill) and industrial dry milling processes on the nutritional composition of pearl millet flour

Abstract

Pearl millet is the major staple food in Namibia. It is milled into flour by traditional and industrial “dry milling” processes. The effects of the traditional Namibian and industrial “dry milling” processes on the physical and nutritional composition of pearl millet grain were compared. Pearl millet variety, Kangara, was conditioned and decorticated traditionally with a pestle and mortar and industrially with an abrasive decorticator. The traditional decorticated grains were steeped and sun dried for 24 h before hammer milling, whereas the industrially decorticated grains were roller milled. Four kernel fractions and five flour fractions were collected for structural and physical analysis. Staining and light microscopy revealed that pestle and mortar decortication removed more pericarp than abrasion. Tristimulus colorimetry showed that the pestle and mortar decorticated kernels and flour were lighter in colour because more pericarp was removed. The traditional milling process significantly reduced the flour protein, ash and c-glycosyl flavone contents relative to the industrial milling process. This is presumably due to the removal of more pericarp and germ in the traditional process. Anti-nutrient analysis revealed an overall decrease in c-glycosyl flavones with both decortication methods. It is suggested that because the traditional Namibian milling process makes the colour of the pearl millet flour lighter, the flour is probably more acceptable to consumers. However, because the industrial milling process removes less of the pericarp and germ, it produces a flour with a better nutrient content as compared to the flour produced by the traditional Namibian milling processes.

2.1.1 Introduction

Millets are major sources of energy and protein for approximately 130 million people in Sub-Saharan Africa (Obilana, 2003). Pearl millet (*Pennisetum glaucum* (L.) R. Br.), known as "mahangu" in Namibia, is the most important staple food in that country (Du Plessis, 2001). In Namibia, pearl millet is utilised mostly at household level, where it is consumed in the form of thick and thin porridges, boiled grains, fermented drinks, pancakes and other food products.

Most Namibians prefer a slightly fermented pearl millet meal produced by the traditional semi-wet milling process to industrially dry milled flour. The milling process as generally applied to pearl millet consists of two stages: decortication and kernel size reduction (Dendy, 1995; Taylor and Dewar, 2001). In the traditional milling process, pearl millet grain is first conditioned then decorticated using a wooden pestle and mortar. The cleaned grain is steeped for 1 to 2 days at room temperature, allowing lactic acid fermentation to take place, before it is milled into flour (Mallet and Du Plessis, 2001).

On a small scale level, the traditional milling process is done by hand which is time and energy consuming (Dendy, 1995). Women and children spend long hours to prepare pearl millet grain for daily food. Only 600 to 700 g meal/hour can be made. In addition, traditional methods that produce the preferred flour are not suitable for commercial operations (Mallet and Du Plessis, 2001). This is mainly due to the number of unit operations and processing time involved in the traditional milling process that would make mechanisation difficult. For these reasons new industrial milling technologies have been developed to improve milling efficiency.

At the industrial level, pearl millet is milled into flour on a large scale through dry milling. Mechanical decortication is carried out based on the principle of abrasion. Thereafter, the decorticated grain is passed through a set of roller mills, sieves and purifiers to produce a dry non-fermented flour.

Hadimani and Malleshi, (1993) and Serna-Saldivar, Clegg and Rooney (1994) found decortication affected the physico-chemical properties of pearl millet flour. However, there is no literature comparing the traditional and industrial pearl millet milling processes. The

objective of phase I of this research was therefore to determine and compare the effects of the traditional Namibian and industrial "dry milling" processes on the physical and nutritional composition of pearl millet grain. Specifically, the relative effects of the two milling processes on the nutritional quality of pearl millet were compared.

2.1.2 Materials and Methods

2.1.2.1 Pearl millet grain

Kangara variety (SDMV 92040) was planted under irrigation at a commercial farm in Otavi in the Oshikoto region of Namibia in February 2002 and harvested in July 2002. The grain was used for both traditional Namibian and industrial dry milling processes

2.1.2.2. Traditional milling process

The pearl millet whole grain (WGK) was collected from an industrial mill silo and taken to the Women's Action for Development (WAD) centre at Mahanene Research Station in the Omusati Region, Northern Namibia to be used for the traditional milling process. The traditional Namibian milling process used is outlined in Fig. 6.

Ten kilograms of WGK were cleaned to remove the chaff and other impurities such as dried leaves and stalks by winnowing using shallow and deep woven baskets (Fig. 7a). Before decortication, water was added to condition the grain to give approximately 25% moisture content (Fig. 7b). More water was added to replace that which was lost due to evaporation. Grains were conditioned for approximately 30 minutes. The conditioned grain was then decorticated by hand pounding using a pestle and mortar (Fig. 7c) until most of the pericarp was removed, as discerned visually.

To remove the loose pericarp, the grain was winnowed using small, flat and shallow baskets (Fig. 7d). The decorticating and winnowing processes were repeated to

Fig. 7. The traditional Namibian steep then mill process (a) Cleaning of pearl millet grain, (b) Conditioning of grain with water prior to decortication (c) Decortication with a pestle and mortar.

Fig. 7 continued (d) Winnowing of kernels (e) Steeping and Lactic acid fermentation of pestle and mortar, decorticated kernels (f) Washing and sun drying of kernels (g) Milling of kernels with a hammer mill.

remove the remaining pericarp (assessed visually). Samples of the pestle and mortar decorticated kernels (PDK) were taken and set aside for analysis. All milling fractions were vacuum sealed in polyethylene bags and were stored at approx. 8°C until analyses were conducted.

The decorticated grain was soaked in tap water at a temperature of 28ºC in a ratio of 1:2 (w/w) in plastic containers for 24 hrs (Fig. 7e). During that time lactic acid fermentation took place. After steeping, the grain was sun dried for 24 hours on polyethylene sheets (Fig. 7f) to a moisture content of approximately 10% to produce fermented, sun dried kernels (FSK). A sample of FSK was collected for analysis. The remainder was hammer milled into flour (PDFSHF) using a Kokuyu BTC SNU 508.607, Japan) (Fig. 7g) with a screen opening size of 800 µm.

The samples obtained for analysis were: whole grain kernels (WGK), pestle and mortar decorticated kernels (PDK), pestle and mortar decorticated, fermented, sun dried kernels (PDFSK) and the pestle and mortar decorticated, fermented, sun dried hammer milled flour (PDFSHF) for proximate and microscopic analyses. Directly prior to analysis, the whole grain kernels (WGK) and pestle and mortar decorticated kernels (PDK) were milled into flour with a laboratory hammer mill fitted with 800 µm opening screen.

2.1.2.3 Industrial milling Process

The grain was commercially milled at Namib Mills, Otavi, Namibia. Fig. 6 shows a flow diagram of the industrial dry milling process. The grain was cleaned to remove the impurities such as stones and pieces of metal with the use of sieves and aspirators. It was then conditioned with water in steeping bins for two hours. The conditioned grain was then carried by augers to the mechanical abrasion decorticator. Samples of the abrasion decorticated kernels (ADK) were collected. The remainder was then subjected to roller milling with first break (corrugated) rollers. The first break fraction underwent sifting and purification. After purification, the clean flour called first break flour went to the blending container. The bran was collected in another container. The remaining fractions (overs) left on the screen were further milled with reduction (smooth) rollers to produce a second break fraction. This fraction was then sifted, purified and blended to give the second break flour which was mixed with the first break flour to make the final industrial milled flour

(IMF). Exact details of the equipment and process technology used in the industrial process are not disclosed due to protection of proprietary information.

Samples taken for analysis were: whole grain kernels (WGK), abrasion decorticated kernels (ADK) and the final industrial milled flour (IMF). The samples were milled and stored as described above.

2.1.2.4 Physical analyses

2.1.2.4.1Grain characterisation

Grain hardness (proportion of vitreous to floury endosperm) was determined by cutting twenty kernels longitudinally in half and assessed against standards, according to the method of Taylor (2001). A sound pearl millet kernel, germ side up was secured onto a piece of rubbery gum to keep it in place. Using a scalpel the grain was halved longitudinally in a manner that each half contained an equal portion of germ. The cut kernels were compared against standards. The results were expressed as percentage of hard, medium and soft grains. The grains with a higher proportion of vitreous endosperm are considered harder than those with a higher proportion of floury endosperm.

2.1.2.4.2 Light microscopy

The grain samples were stained as described by Scheuring and Rooney (1979). A May-Grunwald dye solution was prepared comprising 0.5% methylene blue and 0.5% eosin-Y in methanol. Kernels were halved longitudinally using a sharp razor blade. One half of each kernel was fitted on an aluminum stub with the aid of double–side adhesive tape. Each half was viewed using a light microscope (Nikon Optiphot, Tokyo, Japan).

2.1.2.4.3 Milling recovery rate (yield)

The milling recovery rate (the amount of material recovered after each unit operation of the milling process) was determined by comparing the weights of what was produced from 1000 abrasion decorticated kernels (ADK), 1000 pestle and mortar decorticated kernels (PDK) and 1000 pestle and mortar decorticated, fermented and sun dried kernels (PDFSK) with the original weight of 1000 whole grain kernels (WGK). Before weighing, the grains were oven dried at ±103°C for 3 hr and then sorted using a sieve with an opening size of 2

mm. Grains that did not pass through the sieve were counted using a laboratory seed counter (Numigral Seed Counter, Villeneuve La Garenne, France).

2.1.2.4.4 Colour

The colour of all pearl millet grains and flour milling fractions was measured by Tristimulus colorimetry (Hunter Lab Colorquest, Hunter Associate Laboratories, Reston, USA), using the L, a, b scale.

2.1.2.5 Chemical analyses

2.1.2.5.1 Moisture

This was determined using a one stage air oven method, AACC Method 44-01, Moisture–One stage air oven method (AACC International, 2000a). Samples were dried by heating at ±103°C for three hours.

2.1.2.5.2 Ash

Ash was determined by the AACC Method 08-01, Ash- Basic Method (AACC International, 2000b) wherein samples were incinerated at 550ºC in a muffle furnace until a light grey ash with a constant weight was obtained.

2.1.2.5.3 Crude protein

Crude protein was determined using combustion nitrogen analysis (LECO model FP528 Protein/Nitrogen Analyzer, St Joseph, USA). A factor of 6.25 was used to convert nitrogen to protein.

2.1.2.5.4 Crude fat

Crude fat was determined by the Soxhlet method. The fat was extracted with petroleum ether with a boiling range of between 30°C and 60°C.

2.1.2.5.5 Total starch

Total starch was measured as glucose after gelatinisation by pressure-cooking, and hydrolysis by α-amylase and amyloglucosidase (Taylor, 1992). Glucose was determined colorimetrically with potassium ferricyanide reagent, which reacts with reducing sugars leading to a reduction in absorbance at 420 nm.

2.1.2.5.6 Dietary fibre

Dietary fibre was calculated as components other than water, starch, ash, fat and protein by difference.

2.1.2.5.7 Test for the presence of pigmented testa

The bleach test was performed on the grains (Waniska, Hugo and Rooney, 1992). Grains were soaked in a solution of 5% NaOH in 3.5 % (w/v) sodium hypochlorite solution (household bleach) (Taylor, 2001). The presence of a black-pigmented testa layer indicates that grains are rich in tannins. The absence of a black testa layer showed that the pearl millet grains were tannin-free.

2.1.2.5.8 Total polyphenols

This was measured according to the International Organization for Standardization (ISO) Jerumanis ferric ammonium citrate procedure (ISO, 1988). Tannic acid (Merck Cat. no.: 1.59446.0010, HS-No 3201 9090) was used as a standard in order to permit inter laboratory comparison of results and the polyphenols were expressed as % tannic acid equivalents.

2.1.2.5.9 Goitrogens (c-glucosylflavones)

Total c-glycosyl flavones were measured according to the method of Reichert (1979) as modified by Akingbala (1991). Milled pearl millet flour (0.1 g) and 10 ml hexane were added into centrifuge test tubes with stoppers fitted loosely. The meniscus was marked. The test tubes were heated in water bath at 60ºC for 20 minutes. The test tubes were then vortex mixed at 5 minute intervals. Hexane was added to the level of the marked meniscus to compensate for the hexane lost through evaporation. The test tubes were centrifuged at

300 x g for 5 minutes. The supernatant was decanted and the defatting process was repeated. The test tubes were then placed in a water bath at 80ºC for 1 hour to evaporate residual hexane. Methanol (10 ml) was added to the dried oil-free residue. The meniscus was marked and the contents vortex mixed. Tubes were placed in a water bath at 75ºC for 20 minutes and shaken at 5 minute intervals. Methanol was added to the level of the marked meniscus to compensate for the methanol lost through evaporation. The test tubes were centrifuged at 300 x g for 5 minutes. The supernatant was decanted and collected. The extraction process was repeated for the second time and added to the first supernatant collected. Sodium methoxide, 5 ml (2.5% (w/v)) was added to the methanol extract, and the absorbance measured at 387 nm. C-glycosyl flavone content was calculated as mg glucosyl vitexin equivalent per 100 g pearl millet, dry weight basis (Akingbala, 1991).

2.1.2.6 Statistical analysis

One way Analysis of Variance (ANOVA) with the Least Significant Difference (LSD) Test was performed on the data from the different grain and flour analyses. Analysis of each sample was repeated three times.

2.1.3 Results and Discussion

2.1.3.1 Grain characterisation

It has been suggested that the physical attributes of pearl millet grains such as their texture, pericarp thickness and shape are crucial factors that determine their decortication and milling performance (McDonough and Rooney, 1989). Table II shows that the Kangara pearl millet variety had a medium texture (i.e. a relatively high proportion of floury to vitreous endosperm) and was straw/yellow in colour with a thin pericarp. The whole grain had a thousand kernel weight of 9.99 g, which is higher than the 2.3-7.1 g and 8.7 g, respectively, reported by Hadimani and Malleshi (1993) and Barragán Delgado and Serna-Saldivar (2000) for other pearl millet varieties. This indicates that the pearl millet grain used in this work was slightly larger in size than other pearl millet varieties found in other studies.

Table II. Kernel physical characteristics of the Kangara variety used in the traditional and industrial processes

Parameters	**Kangara**
Kernel colour	Straw/Yellow
Pericarp thickness	Thin
Endosperm colour	White
Kernel size	Large
Kernel shape	Globular
Endosperm texture	Medium
Pigmented testa	None
1000 kernel weight (g)[1]	9.99 ±0.35

1. Values are means ± standard deviations of 3 replicate experiments.

The large size of the Kangara variety may be explained by the fact that it was grown under irrigation in addition to genetic variation between varieties.

Pearl millet kernels that are globular in shape and are large in size, such as the sample of Kangara, have good decortication attributes (McDonough and Rooney, 1989). However, kernels with a larger proportion of vitreous endosperm tend to decorticate better than those with a larger proportion of floury endosperm (Beta, Rooney and Taylor, 2000).

2.1.3.2 Light microscopy

When WGK were stained using the May-Grunwald dye, most kernels remained unstained. This may be due to their waxy cuticle that prevented the penetration of the dye. However, a few of the whole kernels were partially stained green (Fig. 8a (i)). The green stain indicates that part of the waxy cuticle may have been removed through abrasion during threshing. It was more noticeable in the hilum and stylar areas than in any other parts of the grain. This is probably explained by the fact that the hilum has a microscopic opening that allows the entry of moisture required for the germination of grain. In this case the dye penetrated the opening. As can be seen when the grain was sectioned, the undecorticated areas remained unstained, confirming that the dye was unable to penetrate the kernel (Fig. 8a (ii)).

In kernels decorticated by pestle and mortar (PDK), (Fig. 8b (i)), the green stain was more concentrated on the uneven surfaces of the pericarp. The green colour was probably due to the presence of pericarp not removed by decortication. However, the waxy cuticle had

been removed and hence the grain was stained. The pink colour was probably due to the presence of protein in the endosperm. In the PDK most of the pericarp and germ was removed, while most of the endosperm remained intact. Fig. 8 b (ii) illustrates dissected PDK with fractures occurring near the germ. These fractures may have resulted from forces applied during hand pounding.

Fig. 8c (i) and (ii) show that in PDFSK the endosperm was fractured, probably due to the impact of the pestle and mortar and the effect of steeping and sun drying. Furthermore, PDFSK had a lighter pink colour than PDK. This may be attributed to the solubilisation of protein during lactic acid fermentation. The dissected grain of PDFSK shows that the pericarp and germ had been partially removed.

In the industrial milling process, the outer layers of the pericarp were slightly abraded away (ADK), while a larger portion of the germ remained intact (Fig. 8d (i)). ADK (Fig. 8d (i)) were observed to stain more uniformly green compared to PDK (Fig. 8b (i)). This indicates that some of the pericarp was removed from ADK compared to PDK. Also more pericarp was removed in PDFSK (Fig. 8c) compared to PDK (Fig. 8b) and ADK (Fig. 8d), which probably also caused the kernels to fracture.

Decortication caused significant reduction in grain kernel weight irrespective of the method of decortication. The lowest milling recovery yield was obtained with the PDFSK, whereby a reduction in milling loss of 11.9% was obtained compared to 10.1 and 7.3% respectively for PDK and ADK (Table III). This is in agreement with the findings discussed in the light microscopy section. As mentioned, the lower milling recovery yield of the PDFSK may be due to the loss of grain matter during steeping (lactic acid fermentation) which may be attributed to leaching and microbial activity. Nevertheless, milling losses recorded in the present study were lower than the previous values of 25% (Barragán Delgado and Serna-Saldivar, 2000) reported for another variety of pearl millet. Differences in losses may be due to different levels of decortication among these studies.

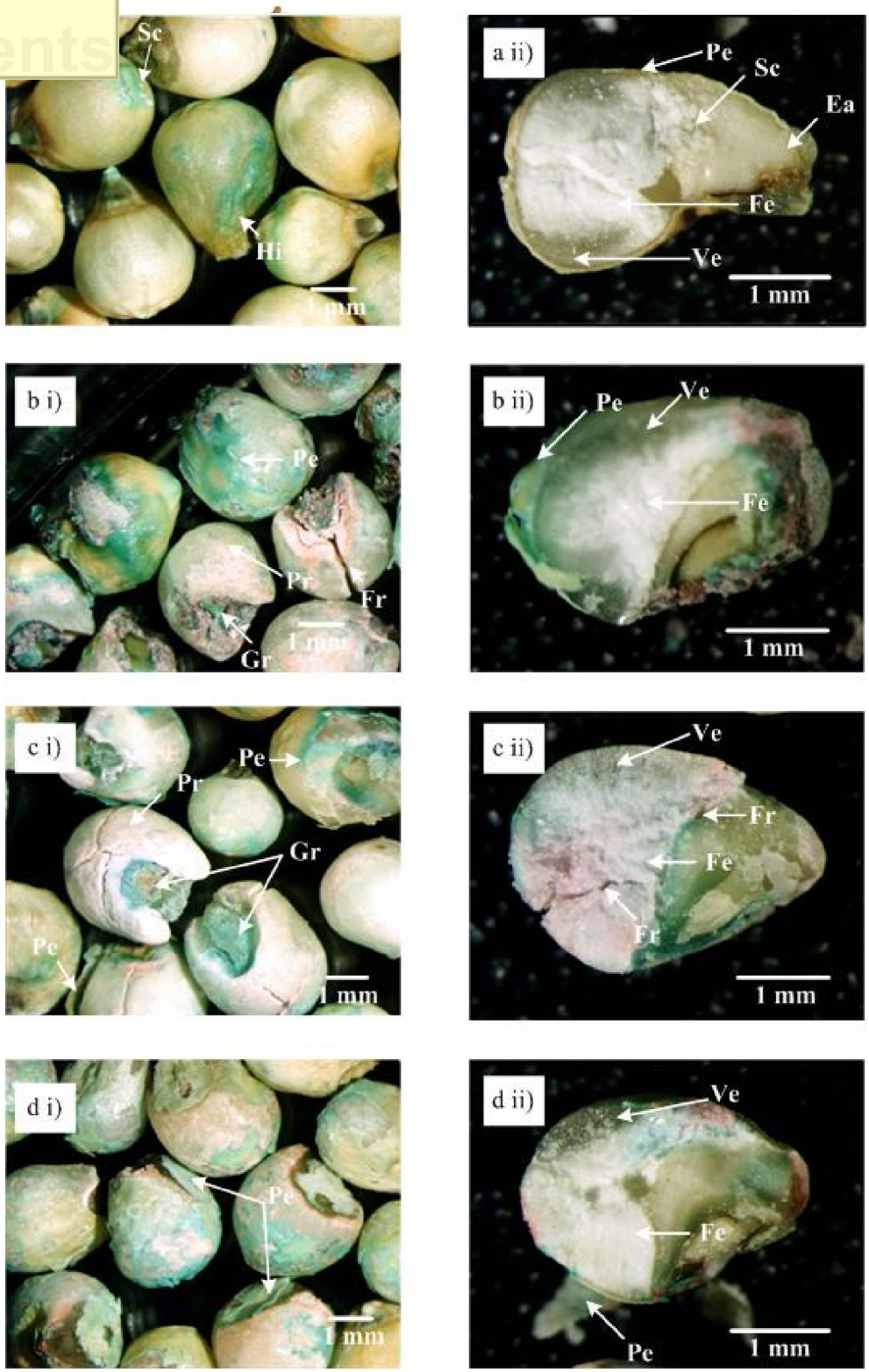

Fig. 8. Light micrographs of stained whole and decorticated pearl millet kernels. (a) whole grain kernels (WGK), (b) pestle and mortar decorticated kernels (PDK), (c) pestle and mortar decorticated, fermented and sun dried kernels (PDFSK), (d) abrasion decorticated kernels (ADK). (i) Pearl millet kernels (ii) cross section of pearl millet kernels. (Ea=embryonic axis, Fe=floury endosperm, Fr= Fractures, Ve=vitreous endosperm, Gr=germ removed, Hi=hilum, Pe=pericarp, Pr=protein, Sc=scutellum).

Table III. Effect of the traditional Namibian and industrial "dry" milling processes on milling recovery rate (yield)

Physical Component	**Whole grain Kernels (WGK)**	**Pestle and mortar decorticated kernels (PDK)**	**Pestle and mortar decorticated, fermented and sun dried kernels (PDFSK)**	**Abrasive decorticated kernels (ADK)**
Milling recovery rate [1] (yield) (%)	100 c ±0.35	89.9 ab ±0.20	88.1 a ±0.02	92.7 b ±0.01

1. Values are means ± standard deviations of 3 replicate experiments. Values with different letters within a row differ significantly ($p<0.05$)

PDK had the lightest colour (L=62.3) (Table IV). This may be because more of the pericarp, which contains coloured pigments (Reichert, 1979), was removed in PDK than in ADK. Reichert (1979) reported that flavonoids are responsible for the colour of the pearl millet grain and flour (Refer to Section 2.2 (Fig. 9). Thus, the removal of the pericarp, which contains flavonoids, by decortication, would result in lighter kernels. PDFSK was darker than PDK. This may be due to non-enzymic browning reactions during sun drying.

The red (a) and yellow (b) colours were more intense for WGK than the other kernels because of the pericarp which contains colour pigments. ADK and PDK had less intense red and yellow colours than PDFSK. This may also be attributed to the non-enzymic browning reactions that may have taken place during sun drying of PDFSK. In addition, enzymic browning increases with a drop in pH may have also contributed to the red and yellow colours in PDFSK.

Table IV. Effect of the traditional Namibian and industrial "dry" milling processes on the colour of pearl millet kernels as measured by Tristimulus Colorimetry

Pearl millet kernels	**Colour**[1,2]		
	L	**±a**	**±b**
Whole grain kernels (WGK)	53.3a ±0.28	1.9c ±0.21	16.7c ±0.48
Pestle and mortar decorticated kernels (PDK)	62.3d ±3.05	1.0a ±0.21	14.4a ±0.22
Pestle and mortar decorticated, fermented and sun dried kernels (PDFSK)	59.5c ±1.15	1.4b ±0.10	15.4b ±0.54
Abrasive decorticated kernels (ADK)	55.7b ±0.56	1.1a ±0.05	14.6a ±0.43

1. "L" measures colour intensity with L = 0 for darkness, and L = 100 for lightness. + a = increase in red colour, - a = increase in green colour, + b = increase in yellow colour, -b = increase in blue colour.

2. Values are means ± standard deviations of 3 replicate experiments. Values with different letters within a column differ significantly ($p<0.05$)

After laboratory milling of PDK, PDFSK and ADK, colour analysis of the flours (Table V) confirmed that decortication had a lightening effect on pearl millet. All the flours from decorticated kernels were lighter than WGF. As stated, this is presumably due to the removal of the pericarp layers, which are rich in flavonoids. IMF was the darkest flour from decorticated grains. This is because the first break flour is blended with the second break flour, which contains more pericarp, as observed in Fig. 6 and also as indicated by the higher milling yield of ADK (Table IV). The a and b values of the flours were lower in comparison to those of the kernels (Table V). In fact, the a values of the flours became negative. This indicates a shift of colour from red in the kernels to green in the flours. This may be due to the distribution of colour pigments after milling.

Table V. Effect of the traditional Namibian and industrial "dry" milling processes on the colour of pearl millet flour as measured by Tristimulus Colorimetry

Pearl millet flour	**Colour[1,2]**		
	L	**±a**	**±b**
Whole grain Flour (WGF)	74.2a ±0.07	-0.31c ±0.02	13.0d ±0.10
Pestle and mortar decorticated flour (PDF)	77.3d ±0.13	-0.70a ±0.04	11.9bc ±0.25
Pestle and mortar, fermented, sun dried and hammer-milled flour (PDFSHF)	76.5c ±0.08	-0.60b ±0.02	10.9a ±0.23
Abrasive decorticated flour (ADF)	76.7c ±0.19	-0.67a ±0.02	12.2c ±0.03
Industrially milled flour (IMF)	74.8b ±0.24	-0.60b ±0.06	11.7b ±0.29

1. "L" measures colour intensity with L = 0 for darkness, and L = 100 for lightness. + a = increase in red colour, - a = increase in green colour, + b = increase in yellow colour, -b = increase in blue colour.

2. Values are means ± standard deviations of 3 replicate experiments. Values with different letters within a column differ significantly ($p<0.05$)

2.1.3.3 Nutrient composition

The effects of the traditional Namibian and industrial "dry" milling processes on the nutrient composition of pearl millet flour are given in Table VI. All flours from the kernels subjected to decortication, except ADF, had significantly lower protein content than WGF. Of note is PDFSHF which had the lowest protein content of all the flours. This may be due to the removal of more protein rich aleurone layer and germ during decortication and fermentation than the rest of the flours. In the case of fermentation, the decrease in protein content may be due to the action of enzymes such as proteases and deaminases from moulds and anaerobic bacteria that degrade protein and convert it to ammonia during the steeping (lactic acid fermentation) process (Abdalla et al, 1998a). ADF had the same protein content as WGF because less aleurone and germ were removed during abrasive decortication. This is indicated by the intense green staining of ADK (Fig. 8d (i). The protein contents agree with the milling recovery rate.

The decrease of 5.6% in protein content of PDF falls within the range of 5-9% protein loss earlier reported for pestle and mortar dehulled sorghum and millet (Reichert and Youngs, 1977). Abdalla et al. (1998a) reported that traditional Sudanese fermented pearl millet flour known as “damirga” contained an average of 7.5 to 11.4% less crude protein compared to whole grain pearl millet flour.

Ash content was significantly lower ($p \leq 0.05$) in decorticated flours. This difference in ash content indicates differences in the extent to which the outermost layers of the decorticated grains are lost during decortication by the various methods. PDFSHF had the lowest ash value of all the flours. This suggests a greater loss of pericarp during pestle and mortar decortication, as indicated by its lowest milling recovery rate (Table III) and by the faint pink staining of PDFSK (Fig. 8c (i). In addition, water-soluble vitamins and minerals may have leached out during the steeping process reducing ash content.

Fat content followed a similar trend as ash content. The decrease in fat content of flours from kernels subjected to decortication is attributed to the removal of the germ, which is rich in fat (Hadimani and Malleshi, 1993). The more extensive fat removal observed in the fermented grain flour may be due to the hydrolytic action of lipases produced by micro-organisms.

The starch content of flours from kernels subjected to decortication were significantly higher ($p \leq 0.05$) than that of WGF. The PDFSHF had the highest starch content. This is probably due to more removal of the pericarp and germ during decortication and fermentation. This in turn led to more loss of the other components such as ash and fat, whereas starch present in the endosperm remained.

Whole grain flour had the highest dietary fibre (12.9%), whereas flour from abrasive decorticated grain had the lowest dietary fibre (10.2%). This is because decortication removes the pericarp where the dietary fibre is concentrated. Hence, the more substantial decrease in dietary fibre with more extensive milling of the grain. The dietary fibre figures are probably a slight overestimate as simple sugars should also be subtracted.

There was no significant difference in total polyphenols in all four flour fractions. This indicates that the levels of total polyphenols were so low in Kangara that they were

essentially not detectable. These values obtained for the four flour fractions (~140 mg/100 g) were much lower than those found by Elyas et al. (2002) who reported that the total polyphenol content for pearl millet was 319 and 294 mg/100 g. This may be due to varietal differences since that author used Composite Population III and Baladi varieties, whereas the Kangara variety was used in this study. No polyphenol values for PDF, PDFSHF and IMF were found to compare with my results. Contrary to the findings of Elyas et al. (2002), who reported tannin levels (0.24%) in pearl millet, insignificant amounts of condensed tannins were found in the flours. The contradictory findings could be due to the possibility that Elyas et al. (2002) used a different method than the Chlorox bleach test and Vanillin HCl method which were used in this study. This is because Elyas et al. (2002) did not describe the method they used.

There were significant differences in the total C-glycosyl flavones in all four flours. The highest amount of c-glycosyl flavones was found in WGF and the lowest in the PDFSHF. The value for WGF was similar to those obtained by Akingbala (1991) who reported c-glucosylvitexin levels of 76.6 mg to 275.7 mg/100 g in flour from whole pearl millet grains. There was a substantial reduction in the c-glycosyl flavone concentration in all the other flour fractions, relative to WGF. PDFSHF had the lowest c-glycosyl flavone content. This may be attributed to loss of c-glycosyl flavone content through decortication and steeping. IMF had lower c-glycosyl flavone content than ADF and PDF. This may be attributed to the removal of the pericarp tissue, which contains the highest concentration of c-glycosyl flavones in the kernel. Reichert (1979) and Akingbala (1991) also reported that with increased levels of decortication, the c-glycosyl flavone content of pearl millet decreased.

Table VI. The effect of the traditional Namibian and industrial "dry" milling processes on the chemical composition (dry basis) for Kangara pearl millet[1]

Chemical components	Whole grain flour (WGF)	Pestle and mortar decorticated flour (PDF)	Pestle and mortar decorticated, fermented and sun dried hammer-milled flour (PDFSHF)	Abrasive decorticated flour (ADF)	Industrially milled flour (IMF)
Protein (N x 6.25) (%)	14.8d ± 0.1	13.9c ± 0.1	13.2a ± 0.1	14.6d ± 0.2	13.4b ± 0.0
Ash (%)	1.64d ± 0.09	1.21bc ± 0.02	0.76a ± 0.11	1.27c ± 0.02	1.12b ± 0.07
Fat (%)	4.86e ± 0.03	3.96b ± 0.01	3.84a ± 0.04	4.67d ± 0.90	4.08c ± 0.06
Starch (%) (Carbohydrates)	59.8a ± 1.2	68.8b ± 0.7	70.8c ± 1.3	69.3bc ± 0.6	69.8bc ± 1.4
Dietary fibre by difference (%)	12.9	12.1	11.4	10.2	11.7
Total Polyphenols (mg/100 g)	150.0a ± 0.0	140.0a ± 0.0	80a ± 0.1	140a ± 0.0	140.0a ± 0.0
Total (C-glycosyl flavones) (mg/100 g)	118.0e ± 1.4	70.6c ± 1.9	60.7a ± 0.3	76.8d ± 1.8	64.8b ± 0.3

1. Values are means ± standard deviations of 3 replicate experiments. Values with different letters within a row differ significantly ($p<0.05$)

2.1.4 Conclusions

The industrial dry milling process yields pearl millet flour with a higher nutrient content in terms of protein, fat and ash than the traditional Namibian (steep then mill) process. This is because the industrial dry milling process removes less of the pericarp and germ. In contrast, the traditional Namibian milling process yields flour with a higher starch content. In terms of the physical effects, traditional decortication removes more outer pericarp layers of pearl millet than abrasive decortication. As a result, the traditional milling process produces a lighter coloured flour that is presumably more appealing to the consumer compared to flour produced by industrial milling. The traditional Namibian milling process reduces the c-glycosyl flavone content more than the industrial dry milling process. This is because more of the pericarp where the c-glycosyl flavones are located is removed during the traditional milling process. The industrial dry milling process retains more nutrients than the traditional Namibian (steep then mill) process but the product may be less preferred by the consumer.

2.1.5 Literature cited

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2.2 Effects of acid steeping on the colour and phenolics of whole and decorticated pearl millet grain

Abstract

In Namibia, pearl millet is traditionally milled through a process of decortication and steeping in water for 24 hours, a lactic acid fermentation. The resulting low pH is believed to lighten the flour colour. The objective of this study was to determine the effect of steeping three different Namibian varieties of pearl millet (Kangara, Kantana and Okashana 2) in lactic acid and water on the colour and the phenolic content of the flour. Whole grain kernels of Kangara, Kantana and Okashana 2 were yellow, brown and grey, respectively. Steeping in a lactic acid solution was done in order to imitate the effect of lactic acid fermentation. This resulted in the kernels becoming lighter in colour. Irrespective of the steeping media, the total polyphenol and c-glycosyl flavone contents were significantly lower in steeped kernels compared to those unsteeped. This indicates that some of these compounds may have leached out during steeping. Surprisingly, for all varieties, kernels steeped in lactic acid had a significantly higher total polyphenolic content than those in water, probably due to the dissociation of metal-polyphenol complexes in the acidic medium whereby these polyphenols became free and available for measurement. Decortication caused a significant decrease in the c-glycosyl flavone content of Kangara. As thought, steeping in a lactic acid solution leads to improvement in colour of kernels compared to steeping in water. Thus, lactic acid steeping is probably used to improve the sensory quality and microbial safety of pearl millet products.

2.2.1 Introduction

Pearl millet (*Pennisetum glaucum* (L.) Br. R), also known as "mahangu" in Namibia, is one of the most important drought-tolerant crops grown in Africa and Asia (ICRISAT and FAO, 1996). However, the grain of many pearl millet varieties is pigmented and this pigmentation has important sensory and nutritional implications. These pigments belong to the flavonoid group of polyphenols (Shahidi and Naczk, 2003). They are present in the pericarp, aleurone and endosperm tissues of pearl millet grain and contribute to the unattractive grey colour and mousy taste of products made from its flour (McDonough and Rooney, 1989). The c-glycosyl flavones are examples of phenolic flavonoid type compounds present in pearl millet (Taylor, 2004) that may give the grain its characteristic pigmentation. In addition to colour effects, these c-glycosyl flavones have been reported to be goitrogenic (Birzer and Klopfenstein, 1988; Gaitan et al., 1989; Elnour et al., 1997).

Rathi et al. (2004a) reported that the removal of c-glycosyl flavones to obtain white flour is one of the key objectives of pearl millet processing. However, some major technical constraints such as its small kernel size and the tight attachment of the pericarp to the endosperm need to be taken into account to meet this objective.

Pigment removal by decortication and by steeping of grain in water or acidic conditions has been investigated. Akingbala (1991) reported that the degree of decortication and steeping in water and in acid reduces the amount of c-glycosyl flavones, thereby increasing the whiteness of the resultant flour. The latter is probably related to the fact that the polyphenolic pigments present in pearl millet are pH sensitive and that treatment with an acid may result in whiter flour (Reichert, 1979). Another study, Rathi et al. (2004a) reported the effect of acid depigmentation on the nutritional and sensory quality of pasta made from pearl millet. These researchers depigmented pearl millet grains by soaking it in 0.2 M HCl for 18 hours. They found that pasta made from flour of depigmented pearl millet grain was lighter than that made from untreated pearl millet grain.

In Namibia, the main pearl millet varieties grown in the Northern Communal Areas (NCA) are Kangara, Kantana and Okashana 2, which are mostly consumed as pearl millet flour at household level (Mr Simon Kamwele Awala, Agricultural Research Officer, Department of Agriculture, Research and Training, Ministry of Agriculture, Water and Forestry in Namibia, personal communication). Kangara is an early maturing variety (50-55 days to

flowering and maturing to 75-85 days) developed by the SADC/ICRISAT/Sorghum and Millet Improvement Programme (SMIP) at Matopos Research Station in Zimbabwe (Monyo, Gupta, Muuka, Ipinge, Chambo, Mpofu, Chintu, Mogorosi and Mutaliano, 2002). The grain is white to creamy white in colour and kernel shape is globular. The grain is 50-72% ≥ 2.6 mm in size with a 1000 kernel weight of 11-13 g and of intermediate hardness (Monyo et al., 2002).

Kantana is an open-pollinated landrace type variety (flowering at 65 days) which originated in Kavango, Namibia (Mr Simon Kamwele Awala, Agricultural Research Officer, Department of Agriculture, Research and Training, Ministry of Agriculture, Water and Forestry in Namibia, personal communication). This variety has been extensively used as a local standard to compare with improved varieties in breeding experiments. The Division of Crop Research of the Department of Agricultural Research and Training (DART) (Namibia) identified that Kantana had a good yield potential and tried unsuccessfully to purify it (Mr Simon Kamwele Awala, Agricultural Research Officer, Department of Agriculture, Research and Training, Ministry of Agriculture, Water and Forestry in Namibia, personal communication). The Namibian National Farmers Seed Growers Cooperative (NNFSGC) further attempted purification of this variety at the Etunda Irrigation Project during the 2001/02 planting season. The grain colour is a mixture of brown and grey in its exposed areas, due to its impure nature. The kernel shape is obovate and kernel size small. This variety has a 1000 kernel weight of 11.7 g and the kernel is soft (Mr Simon Kamwele Awala, Agricultural Research Officer, Department of Agriculture, Research and Training, Ministry of Agriculture, Water and Forestry in Namibia, personal communication).

Okashana 2 was also developed by SMIP at the Matopos Research Station in Zimbabwe (Monyo et al., 2002). The grain colour is light grey, it is globular in shape and kernel size is large (50% to 72 %, ≥ 2.6 mm. The grain has a 1000 kernel weight of 11 to 13 g with intermediate grain hardness.

The main objective of Phase II of this study was to determine the changes in the phenolic content and colour of grain and flour that occurs in decortication, steeping in water and in acidic medium and during the traditional Namibian pearl millet milling process.

2.2.2 Materials and Methods

2.2.2.1 Pearl millet varieties

Three varieties, Kangara (SDMV 92040), Kantana (landrace type variety) and Okashana 2 (SDMV 93032) were used. They were grown in the experimental plots of Etunda Irrigation Project and obtained from the Namibian National Farmers Seed Growers Cooperative (NNFSGC) in Mahanene, Omusati Region, Namibia. These varieties were grown under identical agronomic conditions in January 2002 and harvested in June 2002. These varieties were chosen to represent pearl millet grains with different genotypic characteristics such as variation in colour, predominantly grown and consumed in the NCA of Namibia.

2.2.2.2. Physical analyses

2.2.2.2.1 Grain characterisation

As described in previous chapter.

2.2.2.2.2 Light microscopy

As described in previous chapter.

2.2.2.2.3 Thousand kernel weight

This was determined by counting 1000 kernels of each variety, using a laboratory seed counter (Numigral Seed Counter, Villeneuve La Garenne, France) and weighing them. This was done in triplicate.

2.2.2.2.4 Soaking of grains

Whole grain kernels of Kangara, Kantana, Okashana 2 and abrasion decorticated kernels of Kangara (decorticated using a PRL dehuller in the industrial dry milling process, as described in the previous chapter) were soaked in a solution of distilled water containing 0.4 g/l sodium azide (control), and in a pH 3.5 solution, prepared by titrating 0.1 M lactic acid with 0.1 M NaOH containing 0.4 g/l sodium azide (treatment). Soaking was done in a ratio of 1:8 (grain to solution, w/v) in a 1 litre glass bottle with its cap loosely closed. The contents of the bottles were stirred with a glass rod before and after being incubated for 24 hours at 25ºC. After this, the grains were drained, placed on metal trays covered with paper

towels and oven dried for 24 hours at 40ºC to simulate normal sun drying. Dried grains were milled into fine flour using a hammer mill (Laboratory Mill 3100 Falling Number, Huddinge, Sweden) fitted with an 800 µm opening sieve. The milled products were vacuum-sealed in plastic bags and stored at approx. 8°C until further analysis.

2.2.2.2.5 Colour determination

As described in previous chapter.

2.2.2.3 Chemical analyses

As described in previous chapter.

2.2.2.4 Statistical analysis

As described in previous chapter.

2.2.3 Results and Discussion

2.2.3.1 Physical characteristics of kernels of the Namibian pearl millet varieties

Whole kernels of Kangara were globular in shape and straw/yellow in colour (Fig 9a (i)) compared to the obovate, greyish brown Kantana kernels (Fig 9b (i)). Like Kangara, Okashana 2 kernels (Fig 9c (i)) were also globular in shape but had an intense grey colour. The intense grey colour in Okashana 2 may indicate a higher c-glycosyl flavone content (Reichert, 1979) in comparison to the other varieties. The Kangara variety was straw/yellow colour as opposed, to the white/creamy white colour described by Monyo et al. (2002).

Kangara had a soft endosperm texture compared to Kantana and Okashana 2 which had medium endosperm textures. Kangara had a narrow and incomplete vitreous endosperm. The vitreous endosperm region of Kantana and Okashana 2 were continuous but comprised less than 50% of the endosperm. This indicates that both Kantana and Okashana 2 had medium endosperm textures. The medium endosperm texture of Okashana 2 observed in this study is consistent with the findings of Monyo et al. (2002).

Fig. 9. Light micrographs of the whole kernel appearance of pearl millet cultivars.
(a) Kangara (b) Kantana (c) Okashana 2 (i) whole grain kernel (WGK) (ii) cross section of WGK.

Table VII. Kernel physical characteristics of Namibian pearl millet varieties

Parameters	Kangara	Kantana	Okashana 2
Kernel colour	Straw/Yellow	Brown	Grey
Pericarp thickness	Thin	Thin	Thin
Kernel size	Large	Small	Large
Kernel shape	Globular	Obovate	Globular
Endosperm colour	White	White	White
Endosperm texture	Soft	Medium	Medium
Pigmented testa	None	None	None
Thousand kernel weight (g)[1]	10.0b ± 0.4	9.2a ±0.0	10.5b ±0.2

[1]Values are means ± standard deviations of 3 replicate experiments. Values with different letters within a row differ significantly ($p \leq 0.05$)

Among the varieties, Kantana had the smallest kernel size and a lowest thousand kernel weight (Table VII). The thousand kernel weight of the pearl millet varieties used in this study are within the range of 6.8 g to 14.3 g reported by McDonough et al. (1986). All kernel physical characteristics of the Kangara variety in Table VII. were similar to those reported in Section 2.1, (Table II), except the endosperm texture. The endosperm texture of the Kangara used in the study discussed in Section 2.1 was medium, whereas that of Kangara used in this phase of the study was soft. This may be explained by the fact that these two samples were grown at different locations with different environmental conditions. The kernel weights obtained in this study were relatively lower than those reported by Monyo et al. (2002) and (Mr Simon Kamwele Awala, Agricultural Research Officer, Department of Agriculture, Research and Training, Ministry of Agriculture, Water and Forestry in Namibia, personal communication). This is probably because the kernels were cultivated under different environmental conditions.

The colour of the whole grain kernels (WGK) and milled whole grain flour (WGF) are shown in Table VIII. Kangara WGK was the lightest in colour, while Okashana 2 was the darkest. Okashana 2 had significantly lower a and b values compared to the other varieties. This indicates that it had a more greyish colour than the other varieties. This is in agreement with their visual appearance (Fig. 9).

Table VIII. Colour of whole grain and milled whole grain (flour) of Namibian pearl millet varieties as measured by Tristimulus colorimetry

Parameter	Kangara	Kantana	Okashana 2
Whole grain kernels (WGK)			
L[1]	53.3c[2]	46.9b	43.7a
	±0.3	±0.6	±0.9
a	1.9b	3.1c	0.4a
	±0.2	±0.1	±0.2
b	16.7b	16.2b	11.6a
	±0.5	±0.1	±0.6
Whole grain flour (WGF)			
L	74.2c	72.0b	68.0a
	±0.1	±0.1	±0.2
a	-0.3b	-0.0c	-0.9a
	±0.0	±0.0	±0.0
b	13.0c	12.6b	9.4a
	±0.1	±0.1	±0.1

1. "L" measures colour intensity with L = 0 for darkness, and L = 100 for lightness. + a = increase in red colour, - a = increase in green colour, + b = increase in yellow colour, -b = increase in blue colour.

2. Values are means and standard deviations for 3 replicates; means with different letters within a row differ significantly ($p<0.05$)

All the colour value parameters of the kernels changed after milling into WGF. The L values increased irrespective of grain variety. This is probably because the colour pigments are mainly present in the pericarp. When milled these are evenly spread throughout the flour. Hence, the flour became lighter. Kangara WGF was the lightest in colour and Okashana 2 WGF the darkest. This trend is the same as for WGK.

The chemical composition of the pearl millet varieties is given in Table IX. Protein content was significantly lower in Kantana, whereas the starch content was significantly higher than in the other varieties. This indicates that Kantana may have a lower germ to endosperm ratio than the other varieties, as can be observed (Fig. 9). The range of protein observed is similar to the 10.8% and 14.9% reported by Elyas et al. (2002). The starch content of the Namibian pearl millet varieties is also within the range of 50 to 75% reported by Hadimani and Malleshi (1995) Hoover et al. (1996) Oshodi et al. (1999) Hadimani et al. (2001).

Table IX. Chemical composition of Namibian pearl millet varieties

Chemical Components	Kangara	Kantana	Okashana 2
Protein (g/100 g)	14.8c[1] ± 0.1	11.0a ±0.0	13.8b ±0.1
Starch (g/100 g)	59.8a ± 1.2	69.4c ±0.3	64.2b ±0.2
Ash (g/100 g)	1.6b ± 0.1	1.5b ±0.0	1.3a ±1.3
Fat (g/100 g)	4.9a ± 0.0	5.4b ±0.8	5.5b ±0.1
Dietary fibre by difference (g/100 g)	18.9	12.7	15.2
Total polyphenols (mg/100 g)	151.7a ±2.0	311.1b ±3.0	330.1b ±8.0
C-glycosyl flavones (mg/100 g)	118.0a ±1.4	140.3a ±12.3	175.3b ±17.0
Presence of pigmented testa	Absent	Absent	Absent
Catechin equivalents (mg/100 g)	0.01a ± 0.01	0.03ab ± 0.01	0.05b ± 0.00

1. Values are means ± standard deviations of 3 replicate experiments. Values with different letters within a row differ significantly ($p<0.05$)

Ash was significantly lower in Okashana 2 (1.3 g/100 g) than the other varieties. This suggests that Okashana 2 may have a thinner pericarp than the other varieties. This, however, was not evident visually (Fig. 9). With the exception of Okashana 2, the other varieties were within the range of 1.6 to 3.6 g/100 g reported by Taylor (2004).

Kangara had a significantly lower fat content than the other varieties. This suggests that Kangara had a smaller germ in comparison to the other varieties, as was observed (Fig. 9). The crude fat values obtained in this study fall within the range of 4.6% to 5.6% reported by Hoover et al. (1996). Kantana had the lowest dietary fibre content while Kangara had the highest. This may indicate that Kantana had a thin pericarp compared to Kangara. However, there was no obvious difference in pericarp thickness between the varieties when observed visually using reflecting light microscopy (Fig. 9). The dietary fibre content was 12.7% to 18.9% in the different pearl millet varieties. This total dietary fibre content found is higher than the 8.5% reported by Taylor (2004). This difference may have been due to errors in the chemical analyses since these were determined indirectly by difference. Additionally, the difference may also be due to genotypic and environmental factors.

Kantana and Okashana 2 had significantly higher total polyphenol content than Kangara. This is presumably related to their darker colour in comparison to Kangara (Table VIII). The total polyphenol content for these pearl millet varieties 151.7 mg/100 mg and 330.1 mg/100 mg fell within the range reported by McDonough and Rooney (1989). C-glycosyl flavone content was significantly higher in Okashana 2 than in the other varieties. This may explain why Okashana 2 was more grey than the other varieties. The c-glycosyl flavone content in pearl millet is associated with its characteristic grey colour (Reichert, 1979). The values obtained for the c-glycosyl flavones in these varieties fell within the range of 76.7 mg/100 g and 275 mg/100 g values reported by Reichert (1979) and Akingbala (1991). Based on the Chlorox Bleach test and the Vanillin-HCl assay these Namibian pearl millet varieties were found to be tannin free (Table IX). These results agree with those of McDonough et al. (1986) and McDonough and Rooney (1989) that pearl millet does not contain tannins.

2.2.3.2 Effect of steeping on the total polyphenol and c-glycosyl flavone contents

Table X shows that the pearl millet total polyphenol content was significantly higher, except Kantana WGK, when steeped in lactic acid solution (pH 3.5) compared to the distilled water control. Okashana 2 WGK had the highest polyphenol content of all samples steeped at pH 3.5. This suggests that after being subjected to acidic conditions, more polyphenols in the steeped kernels became soluble in the methanol extractant. This means that steeping at pH 3.5 probably causes the dissociation of possible metal-polyphenol complexes (Reichert, 1979). Consequently, the polyphenols became free and available for measurement. It is important, however, to note that irrespective of the steeping medium and variety, the total polyphenol contents were at least two times lower in the steeped kernels compared to the unsteeped (Table IX). This indicates that some phenolic compounds leached out during steeping. Decortication had no significant effect on the total polyphenols.

The c-glycosyl flavone contents of the samples did not change significantly for the kernels steeped in a lactic acid solution compared to those steeped in the distilled water control, except for Okashana 2 which increased (Table X). This was probably due to the leaching of the c-glycosyl flavones into the endosperm due to prolonged steeping (24 hours) of this experiment.

Table X. Effect of steeping on the levels of total polyphenols and c-glycosyl flavones of Namibian pearl millet varieties

	Kangara Whole Grain Kernels (WGK)		**Kangara Abrasive Decorticated Kernels (ADK)**		**Kantana Whole Grain Kernels (WGK)**		**Okashana 2 Whole Grain Kernels (WGK)**	
	Distilled water control	**Lactic acid solution**	**Distilled water control**	**Lactic acid solution**	**Distilled water control**	**Lactic acid solution**	**Distilled water control**	**Lactic acid solution**
Total polyphenols (mg/100 g)	32.8ab[1] ±1.0	39.2c ±2.8	0.0a ±1.0	45.8bc ±2.5	61.7bc ±2.0	85.9c ±3.0	50.6bc ±3.1	154.8d ±4.0
Total C-glycosyl flavones (mg /100 g)	83.8b ±8.5	78.6b ±0.0	56.5a ±9.3	55.5a ±0.3	74.3ab ±2.2	76.4ab ±6.8	74.5ab ±9.0	113.4c ±25.2

1. Values are means and standard deviations for 3 replicates; means with different letters within a row differ significantly ($p<0.05$)

Similar results were obtained in a study by Akingbala (1991), where pearl millet grain was steeped in 0.2 M HCl for four hours and sixteen hours. It was found that steeping pearl millet grain for four hours removed more c-glycosyl flavones than steeping at sixteen hours.

The increase in c-glycosyl flavone content in Okashana 2 after steeping in lactic acid solution suggests that in this variety, the c-glycosyl flavones were more pH sensitive and steeping at pH 3.5 probably caused a greater extractability of these compounds. However, it is more probable that the increase in c-glycosyl flavone content in Okashana 2 was a measurement artifact. This is indicated by the fact that it had the highest coefficient of variance (22%).

As in the case of the total polyphenol content, the total c-glycosyl flavone contents were lower in the steeped kernels compared to the unsteeped (Table IX) irrespective of the steeping medium and variety, indicating that the c-glycosyl flavones leached out into the steeping medium. Decortication also caused a significant decrease in the c-glycosyl flavone content. This indicates that decortication removes the pericarp layers where the c-glycosyl flavones are concentrated. Akingbala (1991) decorticated pearl millet (80% yield) and found that the c-glycosyl flavones decreased significantly and that the pearl millet flour became lighter.

The effects of steeping on the colour of the pearl millet grain are shown in Table XI. Generally, steeping at pH 3.5 caused significant increases in the L values, indicating that the kernels became lighter in colour. Kangara (WGK) and Okashana 2 (WGK) had the highest increases in lightness and all others showed a much smaller but similar increase. The lighter colour may be an effect of pH, which alters the degree of ionization of the phenolic compounds (Singleton, 1972). Consequently, the phenolics' ability to chelate with copper, iron, aluminium and other metal ions to form polyphenol metal complexes responsible for pigmentation, is decreased. These findings are in agreement with those of Akingbala (1991) who also found that steeping of pearl millet at 0.2 M acidic medium may increase the lightness of the flour.

In comparison to Kangara WGK, Kangara ADK gave a significantly lighter flour after decortication. This is due to the removal of the pericarp where the pigments are concentrated and the reaction rate between acid and the c-glycosyl flavone during steeping to form colourless c-glycosyl flavonol-derived compounds (Reichert, 1979). The a and b values showed statistically significant changes for some samples. However, these changes were too small to have a meaningful contribution to the overall colour of the kernels. In comparison to the unsteeped WGK of all the samples (Table VIII), the L values of the steeped kernels were lower irrespective of the steeping medium. This may have been due to non-enzymatic browning reactions that may have occurred during oven drying of the steeped kernels.

The L, a, b values for the flours from the steeped whole kernels (Table XII) showed a similar trend to those of the whole kernels (Table XI). However, the L values were relatively higher because the pigments were distributed more in the flours.

Table XI. Effect of steeping on the colour values of grain from Namibian pearl millet varieties after steeping in distilled water (Control) and a lactic acid solution of pH 3.5

Colour	Kangara (WGK)		Kangara (ADK)		Kantana (WGK)		Okashana 2 (WGK)	
	Distilled water control	Lactic acid solution	Distilled water control	Lactic acid solution	Distilled water control	Lactic acid solution	Distilled water control	Lactic acid solution
L	37.2bc ±0.6	40.6e[1,2] ±0.6	43.3f ±2.5	53.1g ±1.7	36.0b ±1.1	38.4cd ±0.7	33.9a ±0.9	37.7c ±1.8
a	3.4d ±0.2	3.3d ±0.1	1.1a ±0.3	1.5b ±0.2	4.0f ±0.7	3.8e ±0.2	2.1c ±0.1	2.2c ±0.2
b	12.2 c ±0.2	13.8e ±0.2	10.1a ±0.2	12.9d ±0.3	13.0d ±0.4	13.9e ±0.4	10.4a ±0.4	11.5 b ±0.6

1. “L” measures colour intensity with L = 0 for darkness, and L = 100 for lightness.
+ a = increase in red colour, - a = increase in green colour, + b = increase in yellow colour, -b = increase in blue colour.

2. Values are means and standard deviations for 3 replicates, means with different letters within a row differ significantly ($p<0.05$)

Table XII. Effect of steeping on the colour values of flour from Namibian pearl millet varieties after steeping in distilled water (Control) and a lactic acid solution of pH 3.5

Colour	Kangara (WGF)		Kangara (ADF)		Kantana (WGF)		Okashana 2 (WGF)	
	Distilled water control	Lactic acid solution	Distilled water control	Lactic acid solution	Distilled water control	Lactic acid solution	Distilled water control	Lactic acid solution
L	64.5b ±0.8	68.2d ±0.1	66.1c ±0.4	73.7e ±0.8	64.2b ±0.1	66.0c ±0.6	61.5a ±0.4	65.8c ±1.0
a	0.3d ±0.1	0.1c ±0.0	-0.5a ±0.1	-0.5a ±0.1	0.8f ±0.0	0.6e ±0.1	0.1c ±0.1	0.0b ±0.1
b	11.5f ±0.2	11.2e ±0.1	10.6c ±0.2	11.0d ±0.2	12.0g ±0.1	11.7f ±0.1	9.8a ±0.3	10.2b ±0.2

1. “L” measures colour intensity with L = 0 for darkness, and L = 100 for lightness.
+ a = increase in red colour, - a = increase in green colour, + b = increase in yellow colour, -b = increase in blue colour.

2. Values are means and standard deviations for 3 replicates; means with different letters within a row differ significantly ($p<0.05$)

2.2.4 Conclusions

Decortication can improve the lightness of pearl millet products. The step of steeping in the traditional pearl millet milling process can lower the levels of polyphenols, which improves the colour of the flour. Steeping in lactic acid gives a slightly lighter flour than steeping in water but apparently reduces the levels of polyphenols rather less. Thus, it seems that acid steeping is preferred over the water steeping for the acidic taste it gives to the flour. It may also render the flour more microbiologically safe due to its lowered pH.

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CHAPTER 3

3. DISCUSSION

This chapter will first address the methodologies used in this study, with reference to the determination and quantification of total phenols, condensed tannins and c-glycosyl flavones from pearl millet fractions. Specifically, the appropriateness of the methods used in measuring the desired effects, particularly what could have been done to improve their performance will also be stated. The observations and limitations of the field experiments conducted in this study are also discussed. This discussion will also focus on the effects of the traditional Namibian and industrial "dry milling" processes on the physical and nutritional composition of pearl millet grain. Specifically, the advantages and disadvantages of the two milling processes on the nutritional quality of pearl millet will be compared. Lastly, some potential methods for reducing the adverse effects on the nutritional quality of pearl millet grain of the two milling processes are explored.

3.1.1 Determination and quantification of total polyphenols

The ferric ammonium citrate assay, (International Organization for Standardization (ISO), 1988) was used to estimate the total phenolic content of pearl millet. This method is based on the interaction between phenols and ferric ions from ferric ammonium citrate in the presence of ethanolamine to give a coloured complex. The method is advantageous in the sense that an instantaneous reaction resulting in a stable colour which lasts for several hours (Mole & Waterman, 1987). The assay measures most phenols. Essentially it is not selective to specific phenolic compounds (Mole & Waterman, 1987). In this research, the total polyphenol values obtained for the pearl millet samples were near the lower detection limit. Thus, the protocol should have been modified so that a larger sample size was used to obtain absorbance readings within the desirable concentration range for the calibration curve.

Compared to other methods of polyphenol analysis such as the Folin Ciocalteu, Folin Denis, Prussian blue, the ferric ammonium citrate method has the disadvantage that it can not be used for samples with low concentrations of polyphenols (Mole & Waterman,

1987). Thus, the ferric ammonium citrate method alone is not efficient to unequivocally establish the presence of tannins in pearl millet without verification of other methods such as the Chlorox bleach test (Waniska et al., 1992) and the Vanillin-HCl method (Burns, 1971).

3.1.2 Determination and quantification of condensed tannins

The Chlorox bleach test, as described by Waniska et al. (1992), was used to confirm the absence of condensed tannins in the pearl millet varieties used in this research. In the Chlorox bleach test the pearl millet grain was soaked in a sodium hypochlorite solution (bleach) containing sodium hydroxide. The solution breaks down the outer pericarp layer of the pearl millet kernels to reveal the presence of a black-pigmented testa layer if condensed tannins are present (Price and Butler, 1977; Waniska et al., 1992). The black-pigmented testa layer is a result of the reaction of sodium hypochlorite and the components of the testa forming black-coloured pigments. However, since no black-pigmented testa layer was found, it is apparent that the pearl millet cultivars used in this study do not contain condensed tannins.

The Vanillin-HCl method was further used to confirm the absence of tannins obtained qualitatively through the Chlorox bleach test. This assay is primarily used to quantify condensed tannins. The principle of the method is based on the reaction of leucoanthocyanidins (catechin) and proanthocyanidins (tannins) with vanillin in the presence of HCl resulting in an intense red colour (Schofield, Mbugua and Pell, 2001). The negligible values that were obtained confirmed the absence of condensed tannins in the pearl millet samples. Elyas et al. (2002) detected 0.12% and 0.24% condensed tannins expressed as catechin equivalent (CE) in pearl millet but it is likely that this is just background noise. The findings of this study are consistent with those of McDonough et al. (1986) and McDonough and Rooney (1989) who concluded that pearl millet did not contain tannins.

3.1.3 Determination and quantification of c-glycosyl flavones

A method of Akingbala (1991) which is based on the original method of Reichert (1979), was used to quantify the c-glycosyl flavone content in the pearl millet as glucosylvitexin equivalents. The assay comprises a defatting process with hexane, extraction of flavonoids with methanol at 75ºC, addition of sodium methoxide and reading at 387 nm. The addition of the reagent sodium methoxide reacts with the extracted c-glycosyl flavones to give a yellow colour (Ms. Silke Rügheimer, Chemist, Analytical Laboratory Service in Namibia, personal communication). In the presence of sodium methoxide it is suggested that there is a change in chemical structure whereby more conjugated bonds are formed causing more light absorption.

The c-glycosyl flavone extract is colourless (because the A and B rings are isolated and are absorbed independently) and is stable due to the hydrogen bond between the carbonyl group on position 4 and the hydroxyl group on position 5 (Ms. Silke Rügheimer, Chemist, Analytical Laboratory Service in Namibia, personal communication). In an alkaline medium, for example when sodium methoxide (MeOH) is added, the hydrogen bond is broken. A chalcone structure is formed and colour intensity depends on pH of the medium and the auxiliary groups of the compound. Thus, in alkaline medium both the A and B rings become conjugated and the yellow colour is formed.

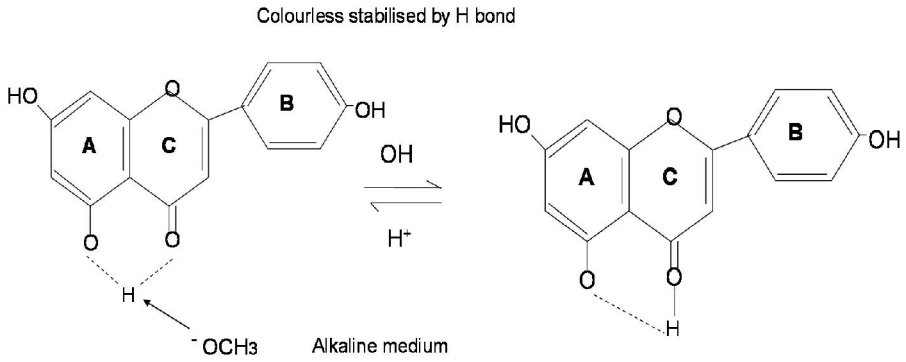

Fig. 10. Model showing the proposed mechanism of the reaction of sodium methoxide and c-glycosyl flavones causing more conjugated bonds in a conjugated system producing intense yellow colour.

3.2.1Observations on field experiments and limitations of the study

It would have been better to conduct the milling processes in the laboratory under controlled conditions. This would have enabled monitoring of the changes in pH over time to determine the length of time required to reach pH 4, where spoilage and pathogenic microorganisms are inhibited (Adams and Nicolaides, 1997) and similarly to improve the flavour and lighten the colour of flour. Under laboratory conditions it would have been possible to determine the effect of steeping and fermentation for different time intervals on colour changes in the flour. In addition, it would have enabled one to determine the time taken to develop the optimum amount of acid required to give the sour taste that is preferred by consumers. Furthermore, under laboratory conditions it would have been possible to determine the time intervals required to drop the pH to 4 to 4.5 to cause the lightening of flour by the corresponding changes in c-glycosyl flavones and changes in flavour. This was not possible in the field. It would have also been ideal to conduct the experiments in the laboratory where there is better control of conditions and easy access to analytical equipment. Nonetheless, the great advantage of having done the milling in the field is that it was the real process and not merely a simulation, as would have been in a laboratory.

The flour produced in the field was prone to rancidity because it was not practical to store it immediately under appropriate conditions so that it could not deteriorate. Lastly, it may have been easier to perform quantititative measurements in the laboratory rather than in the field.

3.2.2 Comparison of the traditional Namibian and industrial dry milling processes

This study set out to compare the effects of the traditional Namibian and industrial ”dry” milling processes on the nutritional composition, polyphenolic content and physical characteristics of pearl millet grain. It has been reported that milling processes result in the loss of protein, insoluble dietary fibre, fat and ash (Serna-Saldivar, Clegg and Rooney, 1994). This is due to the removal of pericarp and germ in the milling process. The traditional steep then mill Namibian milling process was carried out according to the milling procedure which is used for preparing the traditional flour that the people are

used to and prefer. The industrial milling process was included in the research so as to compare its effect on flour nutritional quality with those of the traditional milling process. The major effects of the two milling processes on pearl millet quality are given in Table XIII.

3.2.3. Effects of major unit operations in the traditional Namibian and industrial "dry milling" processes on the physical and nutritional composition of pearl millet grain.

The unit operations which have major effects on the nutritional content of flour from the traditional Namibian and industrial "dry milling" processes include decortication and fermentation. The effects of these operations are described below.

3.2.3.1 Effect of Decortication

As stated, the purpose of decortication is to improve the palatability and storage quality of the flour (Taylor, 2004). In this study, the traditional Namibian milling process resulted in lower protein, ash and fat contents in the flour compared to the industrial milling process. This is due to the greater extent to which the outermost layers such as the pericarp, aleurone and germ of the grains were removed during pestle and mortar decortication. It is noteworthy that a second decortication step was used (Fig. 6). After conditioning, grains were first decorticated and winnowed. The first decorticated and winnowed grains (semi-decorticated grain) were further subjected to a second decortication step followed by winnowing. This differed from the traditional type pearl millet milling process described by Taylor (2004) where there was a single decortication step. The second decortication step further increased nutrient losses. The industrial dry milling process resulted in flour with higher protein, ash and fat contents. This is due to less pericarp, aleurone and germ being removed by abrasion. Consequently, the industrial milled flour had a higher nutrient content than the traditionally milled flour. However, the shelf stability of the industrial milled flour with a higher total fat content (Table VI), is susceptible to rancidity (Reddy, Faubion and Hoseney, 1986). Moreover, industrial milled flour had more bran, which may be nutritionally desirable but is undesirable in terms of palatability of the product.

Table XIII. Advantages and disadvantages of the traditional Namibian and industrial dry milling processes

Type of Milling Process	Advantages	Disadvantages
Traditional steep then mill Namibian process	Lighter coloured flour preferred by consumers	Lower protein, ash and fat
	Less c-glycosyl flavones	Lower Vitamin B complexes
	Preferred sour taste for consumers	More labour
	More starch content	More water used for steeping
	Less energy required (just power supply for hammer mill)	Lower throughput
		Uncontrolled conditions in terms of steeping and drying
		Prone to contamination
		More time involved in processing
Industrial milling process	More Vitamin B complex	More unit operations Darker coloured flour less preferred by consumers
	More protein, ash and fat	More c-glycosyl flavones (potential goitrogens)
	Less labour intensive	Lower starch content
	High throughput	Energy intensive (power supply)
	Less prone to contamination	
	Less water involved in steeping	
	Less time taken to process	

Furthermore, the traditional Namibian milling process resulted in flour with higher starch content compared to flour from the industrial milling process. This is probably due to the proportionally higher loss of the other components such as ash and fat, whereas the starch, which is present in the endosperm was concentrated, during the traditional milling process. The traditional milled flour produced food products such as porridge which is more acceptable to consumers in terms of texture.

The traditional milling process, using a pestle and mortar was thought to remove less c-glycosyl flavones compared to that of a mechanical decortication. Mechanical decortication of the grain was thought to remove not only the c-glycosyl flavones present in the pericarp tissue and the aleurone, but also those in the germ and part of the peripheral endosperm because of the progressive abrasion of the outer layers of the kernel. However, the results of this study showed that less c-glycosyl flavones were found in the traditional milled flour compared to the industrial flour. This may be due to a more precise removal of the pericarp containing to the c-glycosyl flavones using a pestle and mortar. These findings are in agreement with the lower protein, ash and fat contents of the traditionally milled flour. Decortication using a pestle and mortar resulted in the removal of more pericarp, which contains the coloured pigments such as the c-glycosyl flavones. This contributed to the lighter colour found in traditional milled flour compared to the industrial milled flour which improved its appearance.

3.2.3.2 Effect of Fermentation

In this study, steeping in the traditional Namibian milling process which is actually lactic acid fermentation (Taylor, 2004) probably affected the vitamin B complex, protein contents, pH and colour of the flour. The traditional milled flour probably had less vitamin B complex compared to the industrial milled flour because these vitamins may have leached out of the grain into the steeping media. As is the case with the protein, ash and fat content the industrial milled flour probably had a higher vitamin B complex content since no steeping was involved.

Fermentation also caused a decrease in protein content which may have been due to leaching, and to the action of enzymes such as proteases and deaminases (Abdalla et al.,

1998a). Moulds and anaerobic bacteria that degrade protein may convert it to ammonia during the steeping (lactic acid fermentation) process. Fermentation was found to lighten the colour of the traditional milled flour. This is probably because c-glycosyl flavones responsible for the pigmentation of pearl millet leached out. These factors as in the case of pestle and mortar decortication improved the appearance and colour of the traditional milled flour, which was not the case for the industrial milled flour. The low pH in the fermentation process also gives the flour a lighter colour and sour taste which is preferred by the Namibian consumers.

Light microscopy showed that the grains contained cracks after fermentation which may have made it easier in the production of flour during the traditional milling process. This is of significance as it may reduce the effort required in milling with a pestle and mortar which is used in most households.

3.2.3.3 Factors that influence pearl millet consumer preference in Namibia

In general, whole grain pearl millet flour has a dark brown to greyish colour (Klopfenstein et al., 1991), which is not desired by the consumer. There is therefore a need to remove this colour by reducing the level of pigments. This is done by decortication, steeping and fermentation. Thus, the lighter colour of the fermented flour is more attractive to the consumer compared to the industrial milled flour. Despite the fact that the industrial milled flour is more nutritious than the traditional milled flour, people prefer the traditional milled flour which is lighter in colour and has the desired sour taste.

3.2.3.4 Disadvantages of the traditional Namibian milling process

A disadvantage of the traditional process is that it is labour intensive and its throughput is lower than the industrial process. Traditional flour is produced at a rate of approximately 700 g per hour (Dendy, 1995), whereas the industrial process using a typical roller mill can process several tons of grain per day. The traditional process is also time consuming. The steeping time is long (24 hours) excluding the drying process, which is affected by the season of the year. For example, during the rainy season when pearl millet is ready for harvesting, the humidity in the air is high making the drying process longer. This causes a mousy odour in the flour due to poor drying (Reddy et al., 1985). The traditional

method of drying is done on polyethylene sheets in the open air. A drawback of this is that flour may be contaminated with dust, sand, bird droppings and pests. In addition, if the traditional process was industrialized more energy would be required particularly for drying the steeped kernels and clean water would be needed for the process. Moreover, such a process would result in large volumes of effluent which would require disposal.

3.2.3.5 Suggested modifications to current technologies and alternative technologies to ensure nutrient retention

To produce flour that maintains both its nutritional value and consumer acceptance, some modifications to the traditional Namibian process are suggested (Figs. 11 and 12). It is recommended that decorticated kernels are mixed with decorticated and fermented kernels prior to hammer milling in a ratio of 2:3 respectively (Fig. 11). The advantage of this is that it would reduce the time required for sun drying because decorticated kernels which are much drier, would absorb moisture from the pestle and mortar decorticated and fermented kernels. The nutritional value of the flour from this blend would be higher. This is achieved as decorticated kernels have a higher nutrient content than decorticated, fermented and sun dried kernels, which would supplement and compensate for the losses such as Vitamin B complexes presumed to be lost during the steeping and fermentation processes. During the application of this technology it is important to ensure that the resulting water activity is kept low, after mixing relatively high moisture particles with dry particles. This is in order to prevent the growth of pathogenic fungal organisms.

Fortification of the traditional milled flour with vitamin A and B complexes, iron, zinc and folate is another method that could restore nutrients lost through steeping and fermentation (Fig. 12). As a matter of fact it may even increase its nutrient levels since food fortification actually means the addition of nutrients to levels higher than those found in its original state (whole grain flour) For example, vitamin A can be added in the form of vitamin palmitate at ~139 g/kg of flour as applied to maize meal (Department of Health of the Republic of South Africa, 2002). Thiamine mononitrate, folic acid and riboflavin can be added at about 14, 11 and 8 g/ kg of flour, respectively. Iron can be added in the form of elemental iron powder at about 180 g/ kg and zinc in the form of zinc oxide at ~ 94 g/kg. These fortification levels have been recommended to ensure that

staple cereal products such as bread and maize meal would contain at least 33% of the Recommended Dietary Allowance of these nutrients in them.

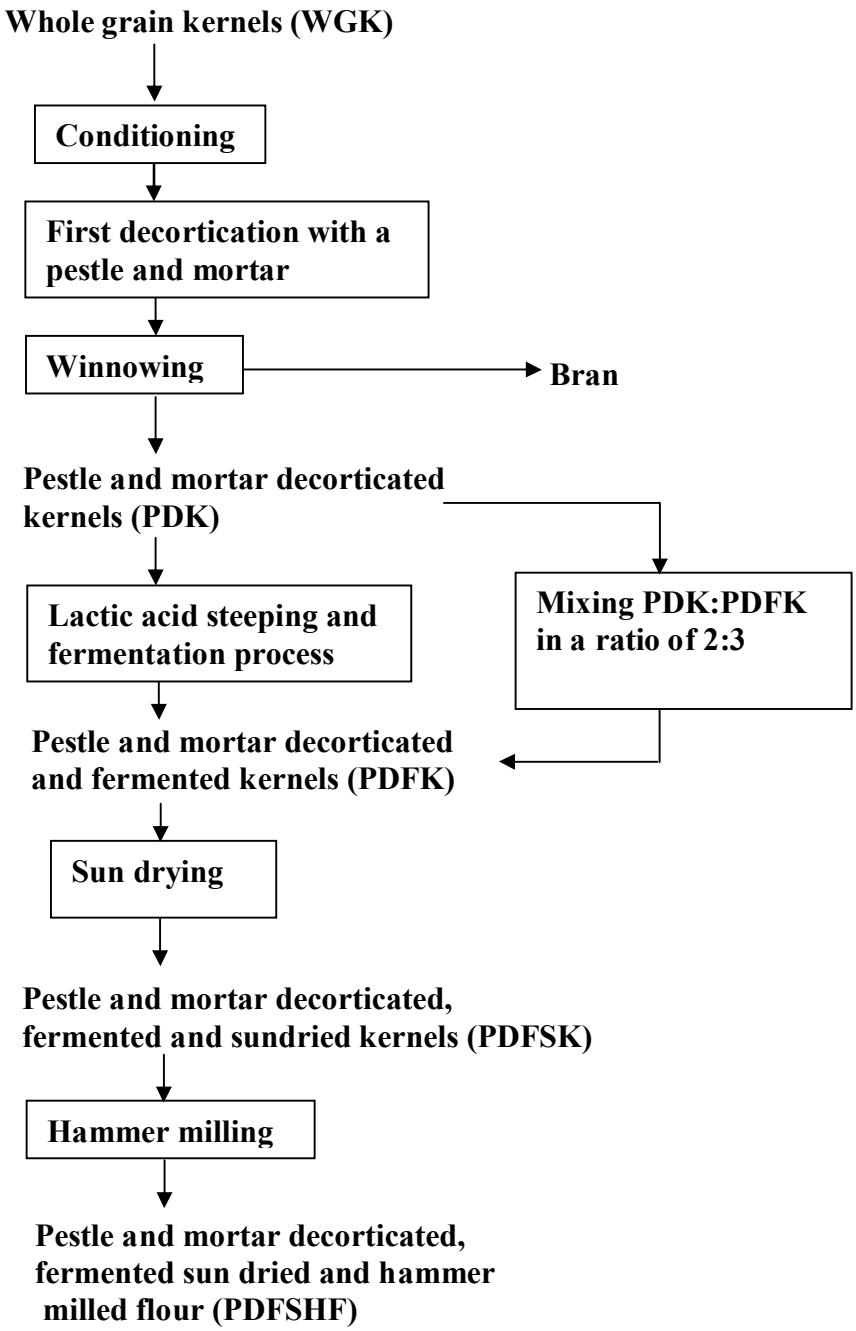

Fig. 11. Flow diagram of mixing PDK and PDFSK as a method to improve the traditional Namibian milling process.

A fortification programme at centres where hammer mills are installed should be put in place. The advantage of such a programme when undertaken may address deficiencies in the diet of pearl millet consumers. However, a problem is that fortification programmes require trained and knowledgeable people to ensure correct product dosing and formulation. Moreover, such programmes have significant financial implications.

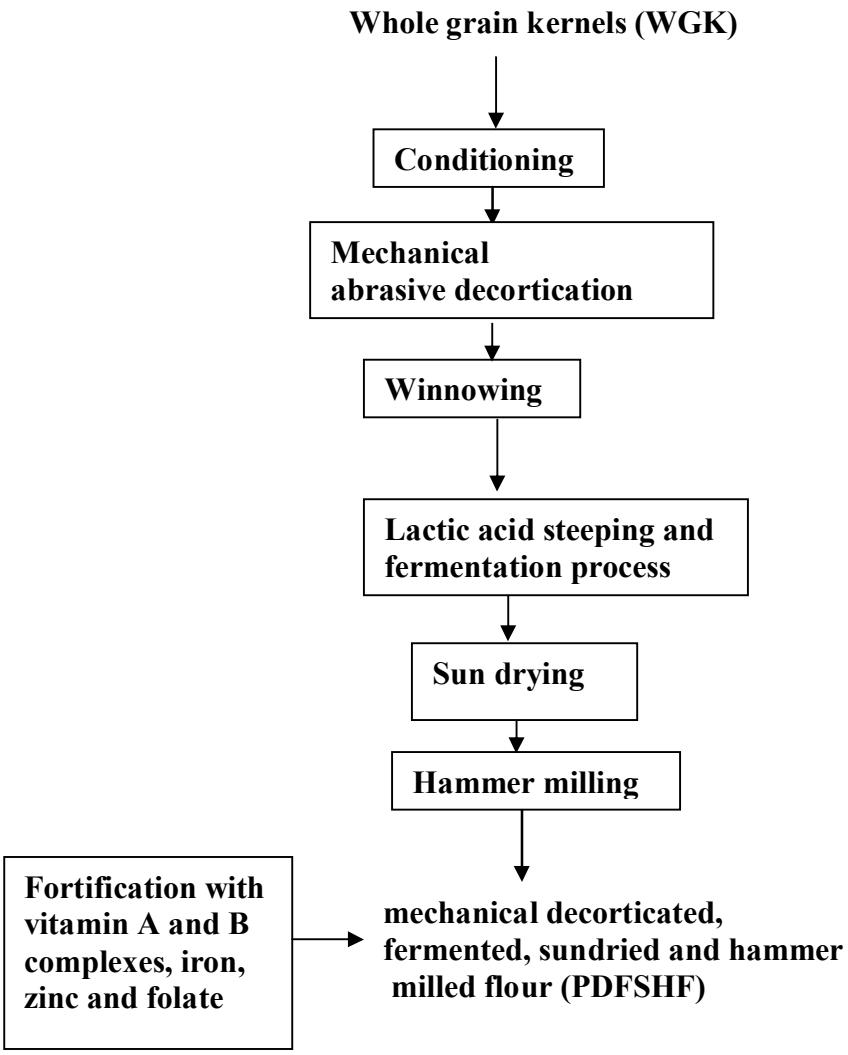

Fig. 12. Flow diagram of fortification to improve the nutritional content of traditional flour.

A method that is proposed to retain the nutritional value of the flour and still mimic the quality of flour made by the traditional process includes the sprinkling or moistening of kernels with water and allowing it to ferment for twenty four hours (Figure 13 a). This

will ensure less of the water soluble nutrients such as the vitamin B complex, proteins and carbohydrates are lost compared to the steeping and fermentation processes. Furthermore, it will also reduce the time involved during drying since grains are only moistened and not soaked. An alternative method would be to introduce food grade lactic acid to lighten the colour of the industrial milled flour and to give the sour taste (Figure 13 b). As shown in section 2.2, lactic acid bleaches pearl millet kernels and when milled a lighter coloured flour is produced. In addition, Descriptive and Consumer sensory tests can be performed to determine whether the addition of food grade lactic acid imparts the lactic acid flavour which consumers of pearl millet find appealing.

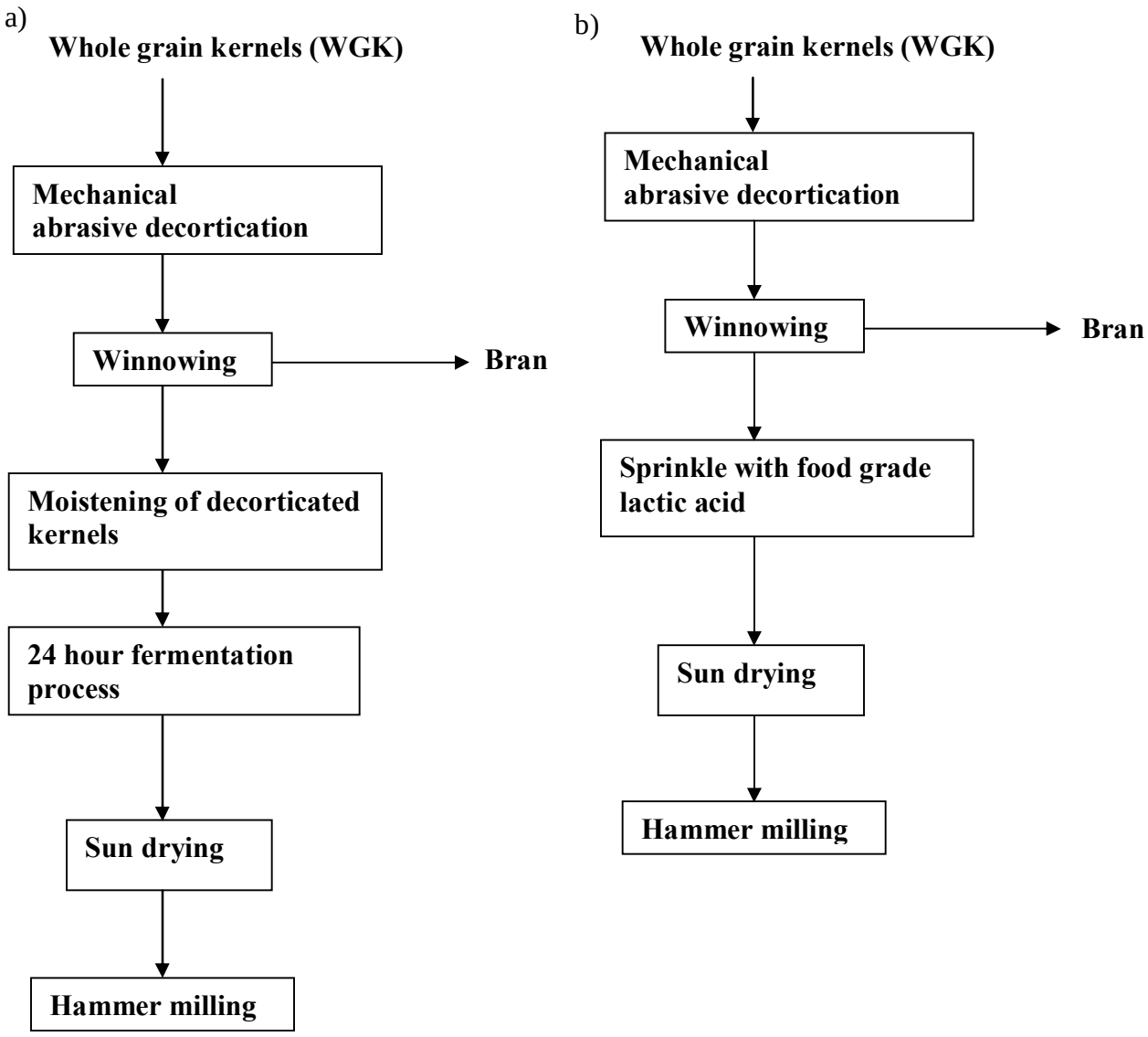

Fig. 13. Flow diagrams of alternative technologies to ensure nutrient retention
a) Moistening and fermentation of kernels b) Sprinkling with food grade lactic acid.

CHAPTER 4

4 CONCLUSIONS AND RECOMMENDATIONS

This research has established the effects of Namibian industrial and traditional dry milling processes on the colour, nutritional composition and phenolic content of pearl millet grain. Key differences between the milling processes affect the quality of flour. The traditional milling process can produce flour lighter in colour than the industrial milling process, which may be a reason for the popularity of traditional milled flour among consumers. Steeping in water allows fermentation to take place resulting in the production of lactic acid, which lowers the pH of the flour. The low pH of the flour bleaches colour pigments such as the c-glycosyl flavones resulting in a whiter flour. The reduction of the c-glycosyl flavones content is beneficial since they are considered goitrogenic. Thus, lactic acid steeping can improve the sensory properties of kernels and flour compared to steeping in water.

Industrial milled flour, however, is more nutritious than traditional milled flour in terms of protein, fat and mineral content. This is because the industrial milling process removes less of the pericarp and germ, resulting in flour with a higher nutritional content as compared to the flour produced through traditional Namibian milling processes.

The knowledge generated can be directed towards the design of an industrial milling process that may include sprinkling the grain with food grade lactic acid to give the sour taste and to leach out the colour pigments, particularly the c-glycosyl flavones, hence lightening the colour of the industrial milled flour. In this design the product will have a high nutritional content, be lighter in colour and have the sour taste, which consumers find appealing.

Further research should involve an in depth consumer study to determine the effects of colour of pearl millet flour and taste on consumer preference and acceptance.

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