ENZYMIC DECORTICATION OF SORGHUM GRAIN

BY

MUTHULISI SIWELA

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DECLARATION

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

[Signature]
ABSTRACT

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By

Muthulisi Siwela

Supervisor: Professor J.R.N. Taylor
Co-Supervisor: Doctor J. Dewar
Department: Food Science
Degree: Master of Science (Food Science)

Sorghum is a drought resistant indigenous African cereal and traditional food crop. Unfortunately, milling sorghum, as is normally done using a Prairie Research Laboratory (PRL)-type abrasive dehuller is not effective. Because in sorghum the germ is integral in the grain, the process of removing the outer bran layers by abrasion leads to high losses, up to as much as 40% if a flour of less than 1% fat is to be produced. The pericarp of sorghum is rich in cellulose and hemicelluloses, and the germ, lipids. Specific hydrolytic enzymes should degrade these chemical components and thus remove the bran and germ with minimal loss of grain material.

In this work, four industrial enzymes, endo-β-glucanase, xylanase, pectinase and lipase were applied either singly or in combination to two different commercial batches of sorghum to determine whether hydrolytic enzymes could be used to decorticate sorghum grain.

Sorghum grain was either de-waxed with hexane or scraped to remove the waxy material on the pericarp and seed coats. The grain was then soaked in a 10% (w/w) solution of either single or combined enzymes in flasks which were then incubated at 50°C in a water bath and left overnight with shaking. SEM showed that endo-β-glucanase caused decortication of the sorghum grain at the seed coat/aleurone layer interface with both the hexane de-waxed grain and the scraped one. The germ was not removed. The results suggested that endo-β-glucanase
hydrolysed the exposed pericarp cell wall material and thus loosened the pericarp, and hence its removal. The germ could not be removed perhaps due to the fact that, in sorghum grain the germ/endosperm interface is at right angles to the surface of the grain, thus enzymes have only a small area to act on and all the underlying interface is not accessible.

Different endo-β-glucanase concentrations were applied to partially deorticdicated sorghum grain to establish the optimum concentration to be used to remove the pericarp material to an acceptable level. The lowest level when there was a good deorticication effect, as indicated by grain ash content and colour, was at 0.01 % endo-β-glucanase concentration (ml/100 g grain).

The effectiveness of enzymic and mechanical deorticication was compared in terms of the relationships between deorticication yield and grain ash content, and between deorticication yield and grain colour. To reduce the grain ash to an acceptable level (about 1.1%), enzyme deorticication resulted in about 10% saving in grain material relative to the mechanical process. However, at the same deorticication yield, the mechanical process gave a lighter product than the enzymic process.

It appears that endo-β-glucanase can remove the pericarp from sorghum grain at an economically feasible concentration (0.01%), and that there is significant reduction of grain material loss relative to the mechanical process. However, more work needs to be done to improve the colour of the product, and to find a way to remove the germ.
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DEDICATION

To my brothers. Richard and Bekithemba, in memory of our late father Mr James Siwela and our brother Mthokozisi.
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CHAPTER 1

INTRODUCTION

Sorghum (Sorghum bicolor (L) Moench) is an indigenous African cereal and traditional food crop (Doggett, 1988). Sorghum ranks fifth in importance among the world’s cereals after wheat, rice, maize and barley respectively. After maize, sorghum is Africa’s most important cereal crop (Dendy, 1995).

Because sorghum is indigenous to Africa, it is agronomically much better suited to cultivation in the semi-arid and sub-tropical regions prevailing over much of the African continent than temperate cereals such as wheat and barley (Doggett, 1988). In fact, sorghum can survive both drought and heavy rainfall accompanied by some waterlogging.

Sorghum-based foods include porridges, alcoholic and non-alcoholic beverages and gruels (Murty and Kumar, 1995).

Although production of sorghum, in the developing countries, has been intensified since the 1960s, its processing technologies, particularly milling, are still not satisfactory (Sooliman, 1993). On the other hand, technologies for milling of other competing cereals such as maize, wheat and barley have already been well developed because they are associated with developed countries (Taylor, 1989).

Research and development of milling technologies for sorghum and millets intensified in the early 1970s through the work of national research organisations (for example in Nigeria and Botswana) supported by the International Development Research Centre (IDRC) of Canada (Bassey and Schmidt, 1989; Munck, 1995). The research resulted in the development of abrasive disk dehullers. Different types of abrasive disk dehullers
include the Prairie Research Laboratory (PRL) dehuller, Rural Industries Innovation Centre (RIIC) (Bassey and Schmidt, 1989).

Although considerable progress has been made in advancing sorghum milling technology, milling sorghum, as is normally done using a PRL-type dehuller, is not effective. The main problems cited are poor product quality (grittiness, speckiness and low colour) and low extraction rates (Cecil, 1992). Gomez (1993) stated that there was still no standard procedure for milling sorghum using abrasive disk dehullers.

The anatomical structure of sorghum grain affects its milling properties (Munck and Knudsen, 1982). In sorghum, the germ is integral in the grain (Rooney and Miller (1982), the process of removing the outer bran layers by abrasion leads to grain material losses, up to as much as 40% if flour of less than 1% fat is to be produced (Bassey and Schmidt, 1989; Sooliman, 1993). In South Africa, where about 60 000 ton of sorghum grain is milled annually, some 4.2 million Rands may be lost due to ineffective milling.

Some workers tried to mill sorghum on roller mills, but separation of the constituents of the grain was poor, an unattractive flour was obtained and the yields were low (Munck, 1995). Cecil (1986) reported better product quality especially in milling of high-tannin sorghum through a semiwet process on rollers but extraction rates (56%) of flour were lower than usually commercially acceptable in milling low-tannin sorghum and millets.

Hydrolytic enzymes may decorticate sorghum more effectively than conventional milling. They (hydrolytic enzymes) act on the polymers of sorghum grain such as the cell wall material (Verbruggen, Voragen and Hollemans, 1992; Taylor, Orovian and Dewar, 1994), proteins (Evans and Taylor, 1990a, b), starch (Taylor and Robbins, 1993; Taylor and Dewar, 1994) and lipids (Kent and Evers, 1994). Hence it is believed that hydrolytic enzymes can be used to specifically separate the non-starch
 polysaccharide-rich pericarp and lipid-rich germ from the starchy portion of the
sorghum grain (Serna-saldivar and Rooney, 1995).

1.1 Objectives

The overall objective of this work was to determine whether industrial hydrolytic
enzymes could be used to decorticate sorghum grain.

1.1.1 Specific Objectives

- To determine whether hydrolytic enzymes can remove the pericarp and germ from
  sorghum grain
- To optimise the enzymatic process
- To compare the effectiveness of enzymic decortication with mechanical
decortication of sorghum grain
CHAPTER 2

LITERATURE REVIEW

2.1 Cereal grain morphology and chemical composition
with particular reference to sorghum

Cereals are members of the monocotyledonous grass family Poaceae. The principal cereals mostly grown are wheat, maize, rice, barley, oats, rye, sorghum and millets (Hoseney, 1994). Cereal grains are often referred to as seeds (Kent and Evers, 1994) but strictly speaking, they are dry, one-seeded fruits (Hoseney, 1994). This type of fruit, precisely, is a caryopsis but is frequently referred to as a kernel or grain.

2.1.1 Grain morphology

Grain structure may affect grain processing. For example, because sorghum grain is small, spherical and the germ is generally integral in the grain, decortication is considered the most appropriate milling technology (Bassey and Schmidt, 1989). The relative positions of the different anatomical parts of sorghum may affect enzyme access to target anatomical parts. Factors affecting the milling quality of sorghum are dealt with in 2.3.1.

According to Kent and Evers (1994) the basic structural form of cereal caryopsis is strikingly consistent, to the extent that a generalised cereal grain can be described (Figure 1).
Figure 1. Generalised structure of a cereal grain (Kent and Evers, 1994)

The caryopsis consists of a fruit coat or pericarp, which surrounds the seed and adheres tightly to the seed coat (Hoseney, 1994). The seed comprises the embryonic axis, scutellum, endosperm, nucellus, testa or seed coat (Figure 1). The caryopsis of all cereals develops within modified leaves called the chaff parts or glumes. In rice and most cultivars of barley and oats, the glumes are persistent and are still present after threshing of the grain and constitute the hull (Hoseney, 1994). In wheat, rye, maize, sorghum and pearl millet, the grain and hull separate readily during threshing, and the grains are said to be naked because they have an uncovered caryopsis (Hoseney, 1994).

They are generally spherical, range from 20-30 mg in weight and may be white, red, yellow, or brown (Hoseney, 1994). Rooney and Miller (1982) stated that kernel size, shape, and details of germ placement inside the kernel affect milling properties, water uptake, and mould susceptibility. The anatomical parts of the sorghum grain are shown in Figure 2.
Figure 2. Longitudinal cross section of sorghum grain. S.A., stylar area; E., endosperm; S., scutellum; E.A., embryonic axis. (Hoseney, 1994)

2.1.1.1 The pericarp or fruit coat

This is the outermost layer of the caryopsis. The pericarp (or fruit coat) is a multilayered structure consisting of several complete and incomplete layers (Kent and Evers, 1994). In all cereal grains the pericarp is dry at maturity, largely consisting of empty cells. During development the pericarp serves to protect and support the growing endosperm and embryo.

The outer layers of a sorghum kernel reveal a thick pericarp, in most varieties, consisting of three layers: the epicarp, the mesocarp (Figure 2), and the endocarp. The epicarp is the outermost portion of the sorghum kernel and is often divided into epidermis and hypodermis. The first cell layer is the epidermis consisting of thick-walled rectangular cells coated by a waxy cutin layer and often contains pigments (Rooney and Miller.
1982). The chemical nature of the waxes and cutin are dealt with in 2.2.1.5. The middle portion is the mesocarp that may be a few cells thick or several layers of cells. The sorghum mesocarp is unique in that its cells contain starch granules. The innermost layer of the pericarp is the endocarp that is composed of cross and tube cell layers. The cross cells are long and narrow with their long axis perpendicular to the long axis of the kernel and the tube cells have their long axis parallel to the long axis of the kernel. The cross and tube cells function to transport water throughout the grain.

Kent and Evers (1994) stated that the chemical components of the pericarp are predominantly the structural non-starch polysaccharides, cellulose and hemicelluloses, and their chemical nature is described in 2.2.1.1 and 2.2.1.2 respectively. The pericarp also contains some proteins, lipids and ash.

2.1.1.2 The seed coats (testa) and nucellar epidermis

The seed coat (testa) is the outer-most tissue of the seed (Kent and Evers, 1994) and the nucellar epidermis, or hyaline layer, is closely united to both the seed coat and the aleurone layer (Hoseney, 1994). In sorghum, the seed coat may or may not contain a pigmented inner integument (Rooney and Miller, 1982). The pigmented inner integument often contains high levels of condensed tannins, which due to their bitter taste confer resistance to predation by birds (Rooney and Miller, 1982). The condensed tannins have been found to inhibit enzymatic activity in milled sorghum malt (Daiber, 1975), and exhibit anti-nutritional activity due to their interaction with proteins (Butler, 1982).

The testa may consist of one or two cellular layers. The testa frequently accumulates corky substances in its cells during ripening which may contribute to grain colour and regulate permeability of the testa (Kent and Evers, 1994). A waxy cuticle, thicker than that of the nucellar epidermis, is typical, and also plays a role in regulating water and gaseous exchange (Kent and Evers, 1994).
2.1.1.3 The embryo or germ

The embryonic axis (plant of the next generation) and the scutellum together constitute the embryo (Kent and Evers. 1994). The embryonic axis consists of primordial roots and shoot with leaf initials. It is connected to and couched in the shield-like scutellum, which lies between it and the endosperm. The scutellum behaves as a secretory and absorptive organ, serving the requirements of the embryonic axis when germination occurs. It consists mainly of parenchymatous cells but an epithelium of elongated column cells adjacent to the endosperm is also present.

The germ in sorghum is generally about 9.8-10% of the whole kernel (Rooney and Miller. 1982; Hoseney. 1994). However, Rooney and Miller (1982) noted that many sorghum varieties with very small germs and thin pericarps exist that would probably have up to 90% endosperm with 3-5% bran and 5-7% germ.

The germ of some sorghum cultivars is more deeply embedded inside the endosperm and is extremely difficult to remove (Aldelraman and Farrell. 1981; Rooney and Miller. 1982; Sooliman. 1993: Serna-Saldívar and Rooney. 1995). However, in some sorghum varieties, the germ protrudes from the kernel.

Hoseney (1994) stated that the germ contains proteins, lipids, sugar and ash. Taylor and Schussler (1986) found that about 16% of the sorghum proteins were in the germ, and the proteins were mainly albumins and globulins, and glutelins. Rooney (1978) found that the grain of sorghum contained about 3.5% ether-extractable material and pointed out that the lipids were concentrated in the germ. The chemical nature of cereal lipids is described in 2.2.1.4.

2.1.1.4 The aleurone layer

The aleurone layer is generally one cell thick. It completely surrounds the kernel, covering both the starchy endosperm and germ. The average size of the aleurone layer
cells is 50 μm and the cell walls are 3-4 μm and have been reported to be largely cellulose (Hoseney, 1994). The aleurone layer along with seed coat, nucellar epidermis and pericarp are removed to form what the miller calls bran. Aleurone cells contain a large nucleus and a large number of aleurone granules (Hoseney, 1994; Kent and Evers, 1994).

2.1.1.5 The starchy endosperm

The endosperm is the largest tissue of the grain (Hoseney, 1994). The starchy endosperm is composed of three types of cells: peripheral, prismatic and central cells. The endosperm cell walls are composed of pentosans, other hemicelluloses, and β-glucans but not cellulose.

Like maize, the sorghum starchy endosperm may be divided into two: the peripheral translucent, corneous/glassy/steely/horny endosperm and the inner, chalky/floury endosperm (Rooney and Miller, 1982; Hoseney, 1994). Taylor, Novellie and Liebenberg (1984) reported that the corneous endosperm of sorghum is composed of starch granules with protein bodies on their surface and the starch-protein body system is embedded in a protein matrix. The floury endosperm contains starch granules interspaced by air. The starchy endosperm is composed of cells packed with nutrients that can be mobilised to support growth of the embryonic axis at the onset of germination. The major nutrient is insoluble starch followed by protein. Protein is concentrated in the cells that are just beneath the aleurone layer (Hoseney, 1994). Protein quantity per unit mass of endosperm tissue increases towards the periphery (Kent and Evers, 1994).

2.1.1.6 The stylar area and hilum

The stylar area is the point at which the style was attached during pollination of the seed. The hilum is the scar tissue resulting from detachment of the seed from the funiculus (Rooney and Miller, 1982).
2.2 Chemical components of sorghum bran and germ

The chemical components of a cereal grain determine its functional properties (Hoseney, 1994). For example, the association between protein and starch may affect the milling properties of a cereal grain (Hoseney, 1994). Because enzymes are specific (Palmer, 1995), the choice of enzymes used for cereal processing should depend on the chemical nature of the cereal grain. Kent and Evers (1994) noted that, unlike grain morphology the chemical composition of cereals varies widely. Cereal protein, for example, varies widely and is influenced by both environmental and genetic factors (Hoseney, 1994). Accordingly, sorghum proximate composition varies significantly due to genetics and environment, and protein content is usually the most variable (Serna-Saldivar and Rooney, 1995).

2.2.1 Non-starch polysaccharides

Non-starch polysaccharides comprise hemicelluloses, pectin and cellulose. Non-starch polysaccharides are found mainly in the plant cell walls where they have a structural function (Coulitate, 1989). The non-starch polysaccharides form the largest portion of dietary fibre (Theander, Westerlund and Aman, 1993). Dietary fibre consists of endogenous components of plant materials that are resistant to digestion by enzymes in the monogastric stomach and upper gastrointestinal tract.

The amount of fibre in a cereal flour is reduced during processing because whole grain flour with a high fibre content and products made from it have inferior texture, colour, and digestibility (Pushpamma and Vogel, 1982).

Serna-Saldivar and Rooney (1995) stated that whole grain flour of sorghum contains about 9.3% fibre, 7.9% is insoluble and is primarily composed of non-starch polysaccharides, cellulose and hemicelluloses. Small amounts of lignin, a
non-starch polysaccharide is also present. Verbruggen, Beldman, Voragen and Hollemans (1993) reported the presence of pectin-like substances in the pericarp of the sorghum grain.

2.2.1.1 Cellulose

Cellulose is the major structural polysaccharide of plants and the most abundant biopolymer (Coffey, Bell and Henderson, 1995). Cellulose is an isotactic β-1,4-polyacetal of 4-O-β-D-glucopyranosyl-D-glucose (cellobiose), as the basic unit consists of two units of glucose by β-1, 4 linked (Figure 3a). The β-1,4 configuration results in a rigid and linear structure of cellulose that may contain up to 10 000 β-D-(1-4)-linked gluco-pyranosyl units (Coultae, 1989). The molecular conformation obtained with this linkage strongly favours the formation of H-bonds between sugar units in the chain and between adjacent chains. This leads to crystallinity of the cellulose because the chains are tightly packed in compact aggregates (microfibrils) (Coffey, Bell and Henderson, 1995) that are surrounded by a matrix of other cell constituents (Figure 3b). Therefore, cellulose has low solubility in water and is resistant to enzymic degradation. In the solid state, highly ordered crystalline areas are interspersed between less ordered amorphous zones. These amorphous zones are regions in which the OH groups are more readily available for reaction than in the more ordered crystalline areas, which are less reactive (Coffey, Bell and Henderson, 1995). The amorphous areas of cellulose normally hold large quantities of water and other cell wall components like proteins and hemicelluloses (Coughlan, 1985).

In sorghum grain, cellulose is confined to the cell walls of the pericarp (Serna-Saldívar and Rooney, 1995).
2.2.1.2 Hemicelluloses

The collective term "hemicellulose" usually refers to those polysaccharide constituents of plant cell walls that are soluble in alkali (Dekker. 1979: 1985). Bigelis (1993) defined hemicelluloses as non-starch polysaccharides found in plant cell walls exclusive of pectic and cellulosic substances. The hemicelluloses are present in all layers of the plant cell wall, but are concentrated mainly in the primary and secondary layers where they occur closely associated with cellulose and lignin (Dekker. 1979: 1985). The hemicelluloses are classified according to their structure and chemical composition: L-arabinan is a polymer of L-arabinose units, D-galactan of D-galactose units, D-mannans of D-mannose
units. and D-xylans of D-xylose units. According to Dekker (1979, 1985), Coultate (1989), (Theander, Westerlund and Aman (1993) and Chesson (1995), hemicellulose molecules are heteropolysaccharides consisting of highly substituted sugar monomers. The substituents include methyl, O-methyl ether, phenolic and other groups. Fig 4(a) shows some of the commonly occurring hemicelluloses.

![Chemical structure of glucuronoarabinoxylans and mixed-linked β-glucans](image)

**Figure 4.** (a) Commonly occurring hemicelluloses (Theander Westerlund and Aman 1995). (b) Arabinoxylan isolated from sorghum pericarp (Verbruggen, Voragen and Hollemans. 1992)
Although “hemicellulose” and “pentosan” are sometimes used interchangeably (Hoseney, 1994), pentosan specifically refers to a hemicellulose which is built on a 5-C backbone which is usually xylose (Coultate, 1989. Theander. Westerlund and Aman (1993) stated that the common structural feature of cereal arabinoxylans is a chain of β-1, 4 linked xylose units to which single units of L-arabinose and D-glucuronic acid or its 4-O-methyl-ester are attached. In some arabinoxylans, the xylose units are partially acetylated, and phenolic acids also may be found linked to arabinose units. Pentosans can be soluble or insoluble in water (Hoseney, 1994).

β-glucans are also a specific group of hemicelluloses and consist of mixed β-1,3 and β-1,4-glicosidic links and their structure is included in Figure 4 (a). They are generally found in the cereal endosperm cell walls (Theander, Westerlund and Aman. 1993).

Hemicelluloses found in the pericarp cell walls of sorghum grain are predominantly predominantly arabinoxylan (Karim and Rooney. 1972). Arabinoxylan is highly substituted (arabinose/xylose = 0.9) and contains the acidic sugars D-glucuronic, 4-O-methylated D-glucuronic and D-galacturonic acid (Figure 4) (Woolard, Rathbone and Novellie 1976; Bach Knudsen and Munck, 1985; Verbruggen, Voragen and Hollemans. 1992; Verbruggen, Beldman. Voragen and Hollemans, 1993; Verbruggen, Beldman and Voragen. 1995).

The endosperm cell wall of sorghum contains predominantly mixed linkage1,3 and 1,4 β-glucans and cellulose is absent (Serna-Saldivar and Rooney. 1995). Results obtained by Earp. Doherty and Rooney (1983) and Earp. Doherty. Fulcher and Rooney (1983) suggest that mixed linkage1,3 and 1,4 β-glucans may be present in the pericarp, aleurone layer and endosperm cell walls. Ramesh and Tharanathan (1999) found that in sorghum, the yield of mixed β- (1,3. 1,4)-D-glucans found in the endosperm cell wall was greatly improved by extraction at elevated temperatures (100°C gave the highest yield).
2.2.1.3 Pectins

Pectic substances are a group of closely associated polysaccharides from the primary cell walls and intercellular regions of higher plants (Voragen, Pilnik, Thibault Axelos and Renard. 1995). They cement cells together (Roberts. 1990). The dominant feature of pectins is a linear chain of α-(1,4)-linked D-galacturonic acid units in which varying proportions of the acid groups are present as methoxyl (methyl) esters. Other constituent sugars are attached in side chains, the most being, L-arabinose, and D-xylose (Whistler and Daniel. 1985; Voragen, Pilnik, Thibault Axelos and Renard. 1995) (Figure 5). The term pectic substances is commonly used to encompass the methoxyl ester, pectin, deesterified pectic acid, and its salts, pectates, and certain neutral polysaccharides (arabinans, arabinogalactans, galactans) lacking the galacturonic acid backbone often found in association with pectin (Voragen, Pilnik, Thibault Axelos and Renard. 1995).

Figure 5. Representative structure of pectin (Whistler and Daniel. 1985)

Although Serna-Saldivar and Rooney (1995) stated that no pectin was found in sorghum. Verbruggen. Beldman, Voragen and Hollemans (1993) found a relatively high content of uronic acid in sorghum after extraction with sodium dodecyl sulphate and suggested that it could be due to the presence of pectin-like substances.
2.2.1.4 Sorghum lipids

Hoseney (1994) stated that lipids are loosely defined as materials soluble in organic solvents. In cereal literature, lipids are often defined as “free” or “bound”; this distinction is based upon solubility. If the lipid is soluble in a non-polar solvent such as petroleum ether, it is considered free; if it requires a polar solvent for extraction, it is considered bound (Hoseney, 1994). According to Kent and Evers (1994) the term “lipid” covers a wide range of compounds including long-chain hydrocarbons, alcohols, aldehydes and fatty acids, and derivatives such as glycerides, wax esters, phospholipids, glycolipids and sulpholipids. However, plants usually store lipids as triacylglycerols, and cereal grains conform to this plant characteristic (Kent and Evers, 1994). Glycerides are compounds formed by esterification of the tertiary alcohol glycerol, and one to three fatty acids (Figure 6).

![Chemical structure of triglycerides]

Fatty Acids + Glycerol → Triglyceride

\[
\begin{align*}
R_1\text{-C-} &\text{OH} & \text{HO-C-H}_2 & \text{R}_1\text{-C-O-C-} &\text{H}_2 \\
R_2\text{-C-} &\text{OH} & \text{HO-C-H} & \text{R}_2\text{-C-O-C-} &\text{H} \\
R_3\text{-C-} &\text{OH} & \text{HO-C-H}_2 & \text{R}_3\text{-C-O-C-} &\text{H}_2
\end{align*}
\]

\[3\text{H}_2\text{O}\]

**Figure 6.** Structure and formation of triglycerides (Staufer, 1996)

According to Hoseney (1994) and Kent and Evers (1994), the highest triacylglycerol levels occur in the aleurone and scutellar tissue of cereal grains, but there are appreciable quantities in the embryonic axis and in the endosperm of oats.
Salisbury and Ross (1992) stated that plant lipids/fats are always stored in specialised bodies in the cytoplasm and the bodies have been called lipid bodies, spherosomes, and oleosomes.

Sorghum contains 2.1-5.0% lipids (Hoseney, 1994). Osagie (1987) reported that sorghum lipids extracted with hexane-ether (4:1) consist mainly of non-polar or neutral lipids (93.2%). Non-polar lipids were mainly triglycerides (85%) followed by diglycerides. The polar lipids were mainly glycolipids (5.9%) and phospholipids (0.9%). Sorghum germ oil is similar to maize oil in fatty acid composition and properties (Rooney, 1978), and the dominant fatty acids are linoleic and oleic acids (Serna-Saldivar and Rooney, 1995).

2.2.1.5 *Sorghum cutin and waxes*

A cuticle slows down water loss from the parts of a herbaceous plant, including leaves, stems, flowers, fruits and seeds, and covers the entire shoot system. The cuticle also controls gaseous and solute movement, and provides protection against some plant pathogens and against mechanical damage (Salisbury and Ross, 1992). Much of the cuticle is reported to be composed of a heterogeneous mixture of components collectively called cutin, while the remainder consist of overlying waxes and pectic substances that are attached to the cell wall (Kolattukudy and Espelie, 1985; Salisbury and Ross, 1992; Roberts, 1990).

Cutin is a heterogeneous polymer consisting largely of various combinations of members in two groups of fatty acids, a group having 16 carbons and one having 18 carbons. The fatty acids exist as hydroxy and hydroxy-epoxy fatty acids (Kolattukudy and Espelie, 1985). The polymeric nature of cutin arises from ester bonds uniting the various fatty acids (Kolattukudy and Espelie, 1985; Salisbury and Ross, 1992). Small amounts of phenolic acids are also present in cutin.
According to Baker (1982), and Salisbury and Ross (1992), many plant waxes contain long-chain fatty acids esterified with long-chain monohydric alcohols, but they also contain free long-chain alcohols, aldehydes, and ketones ranging from 22-32 carbons and even true hydrocarbons containing up to 37 carbons.

A waxy cuticle covers the pericarp and the seed coats of cereals (Kent and Evers, 1994). The cuticle on the seed coats, especially in sorghum is thicker than that on the pericarp.

The chemical nature of the waxes of the sorghum kernel could not be found in literature. However, Bianchi, Avato, Bertorelli and Mariani (1978), and Atkin, Hamilton, Mitchell and Sewell (1982) reported the presence of predominantly free fatty acids, and small amounts of esters, aldehydes, alcohols, n-alkanes and sterols on the leaves of sorghum. The typical chain lengths of aldehydes free alcohols and free fatty acids were C_{28} and C_{30}. Hubbard et al. (1950), according to Freeman and Watson (1969) extracted sorghum wax by dipping the grain in hot benzene and found that the wax content ranged from 0.24-0.44%. The mentioned workers substituted hexane for benzene and got similar results.

2.3 Processing of sorghum for human food-Milling

Cereal grain milling aims at separating the anatomical parts of the kernel to obtain a clean endosperm which is then ground into a meal/flour (Hahn, 1969; Hoseney, 1994; Kent and Evers, 1994). Grain milling is important in that it improves the palatability of the product through the removal of the fibre-rich outer layers (pericarp and aleurone layer) and improves the storage properties of the meal through reduction of its fat content which would result in rancidity. Decortication of sorghum improves visual appearance and consumer acceptability due to the removal of the outer layers that are usually coloured, waxy and bitter. Antinutritional factors such as phytate and polyphenols are also greatly reduced (Hahn, 1969; Desikachar, 1982).
The technologies used to mill sorghum can be broadly divided into traditional milling and industrial milling (Munck, 1995). Various factors affect the milling quality of the sorghum grain.

2.3.1 Factors affecting the milling quality of sorghum

The efficiency of decortication is influenced by the physical characteristics of the sorghum being milled (Munck and Bach Knudsen, 1982).

2.3.1.1 Grain texture and hardness

Grain hardness is caused by the genetically controlled strength of the bond between protein and starch in the endosperm (Hoseney, 1994). Milling quality is most influenced by grain hardness (Maxson, Fryar and Rooney, 1977; Fransisco, 1982; Shepherd, 1982). Harder grains generally give a higher milling yield because it is easier to remove the bran cleanly from the endosperm without breaking it while softer grains tend to be crumbly making it difficult to separate the anatomical parts.

Grain texture defines the proportions of the two portions of the endosperm—the outer portion called the vitreous, corneous, or pearly endosperm, and the inner called the floury, starchy, or chalky endosperm. Vitreousness is usually related with grain hardness (Rooney and Miller, 1982; Hoseney, 1994) and hence could affect the milling yield.

2.3.1.2 Grain size and percent size fractions

Hahn (1969) stated that one of the most pressing problems for the sorghum dry miller is that of obtaining an adequate supply of clean and uniformly high quality grain. A sample with a high proportion of small grain will have a low milling yield because the smaller kernels will be removed with the bran. The sorghum grain usually contains broken kernels, foreign material, and other grains. In addition, sorghum grains are known to vary
quite widely in composition and properties depending on variety, location of growth, climate, crop year and genetics (Hahn, 1969; Rooney and Miller, 1982).

2.3.1.3 Grain colour

Grain colour influences the colour of any product made from that grain. For example, if sorghum is to be milled for porridge meal, a white or light colour is generally preferred (DeSikachar, 1982).

2.3.1.4 Pericarp thickness

Grain with a thin pericarp needs shorter decortication time than thick-pericarp grain (Gomez. Obilana. Martin, Madzvamuse and Monyo. 1997). Shepherd (1981) examined the unique flaking phenomenon of sorghum when it is peeled. Shepherd observed that, during decortication, the sorghum pericarp peeled smoothly producing large flakes down to the mesocarp and thereafter further milling would result in breakage of the kernel producing fine pieces that of meal contaminated with bran. In all sorghum types, flakes appear to result from cleavage in the mesocarp. The results suggested that the design of decortication mills should take cognisance of pericarp thickness, particularly whether the mesocarp is thin or thick.

2.3.1.5 Relative position of germ to the whole grain

In many sorghum varieties the germ is deeply interred in the grain making it difficult to remove it through decortication (Rooney and Miller, 1982).

2.3.2 Traditional milling of sorghum

The traditional equipment is the pestle and mortar, and the stone hand mill (quern) consisting of a flat stationary stone and a moveable stone. The most simple type of processing is to grind the whole grain in a stone hand mill, a village stone mill, or a hammer mill driven by a diesel engine. after which the most coarse bran particles are sieved away, giving about 95% extraction. In rural areas of both India and Africa, home
decoration is often preferred because it gives a cooked product of higher quality (Perten, 1983; Munck, 1995). Munck (1995) further noted that in other countries in Africa such as Tanzania and Nigeria, sorghum is regularly decorticated in a wooden mortar with a pestle in a "semiwet" process, followed by winnowing in a basket, and finally by hand-milling in a stone mill. Extraction rates are usually between 72 and 86% after the two milling steps.

The principles underlying stone milling and winnowing are that when the grain is rubbed between two rough surfaces or in a pestle and mortar the bran is sheared off the endosperm. Winnowing brings the less dense bran to the surface where it is removed from the meal. A conditioning (adding little water) step toughens the bran and thus eases its separation from the endosperm. Uncontrolled conditioning tends to reduce the shelf life of the flour due to microbial growth and rancidity. Grinding reduces endosperm size to flour (Munck, 1995).

Because sorghum grain which is milled is generally hard, a considerable amount of energy and drudgery is involved in producing products that are suitable for cooking and for human consumption by hand decortication (Munck, 1995). If high-tannin sorghums are used even more energy has to be put in if a lighter flour is to be obtained and yields can be as low as 50%.

2.3.3 Industrial milling of sorghum

Industrial milling methods include those that have been adapted from wheat milling and specifically designed abrasive methods. Hahn (1969), Kent and Evers (1994) and Munck (1995) note that most of these techniques begin with a decortication step using mills with abrasive discs, or carborundum stones. A decortication rate of about 20% was recommended for good consumer acceptance. Reduction to flour particle size may be achieved by roller milling, impaction or pin milling (Hoseney, 1994). Degerming may or may not be part of the process since grains are too small.
2.3.3.1 Roller milling

Roller milling generally involves shearing and crushing grain between a series of steel rollers that rotate in opposite directions and at a differential speed and then sifting off the bran with a series of sieves (Hoseney, 1994).

Perten (1976), according to Munck (1995) considered plain roller milling unfit for sorghum because of the relatively high processing cost, low extraction rates and poor product quality. It was found that the sorghum grain was brittle even after conditioning and therefore inspite of having a favourable smooth spherical shape it could not be roller milled.

Cecil (1986) found that sorghum milled better on roller mills if it was pre-steeped (maximum 6 h) at 60-70°C to a moisture content of 20-27%. Extraction rates were around 65%. Gomez (1993) optimised the semi-wet process proposed by Cecil (1986) and obtained a product of better quality and higher extraction rates (about 60%) than dry roller milling of sorghum. However, the semi-wet process was criticised for the possibility of microbial proliferation due to the moist conditions, and for the extra expenses of drying the meal to lower the moisture content.

2.3.3.2. Decortication milling of sorghum

Decortication is the removal of the outer layers of the grain (Hoseney, 1994). The decortication/pearling device has an abrasive surface, and the outer layers are essentially sanded off the grain. The clean endosperm thus obtained can then be ground into a meal/flour using a grinding mill such as a burr mill, pin mill and hammer mill. According to Hoseney (1994), decortication appears suited to sorghum and millets because the grains are round and do not have a crease, and that the process is similar to the traditional hand pounding with a wooden pestle in a mortar. Two types of decortication mills, attrition type decorticator and Abrasion type decorticator are recognised (Munck, 1995).
**Attrition-type decorticator**

The operating elements of this type of dehuller are several, such as a cylinder which is often in the form of sieves, that can be pressurised with tightly packed seeds fed by a screw. Another element is a rotating metal disk with grinding elements, most often one stationary and one moving, tuned for coarse milling. Decortication is generally brought about by passing grains under pressure through narrow openings that are bounded by rough surfaces of moving and stationary screws, plates or discs. The distances between the abrasive surfaces are adjustable, providing variable ratios between coarse (partly decorticated grains) and finer particles (bran plus broken endosperm). The bran and endosperm fragments are sucked off with cyclones or are discharged through screens by an air current at high pressure, usually the endosperms are simultaneously ground to flour and collected (Kent and Evers, 1994; Munck, 1995). Some examples of attrition type decorticators are Palyi Compact mill (Figure 7), DHA 400 and 600, and Squires rice mill. The grain is usually conditioned before milling.

Attrition milling of sorghum also has the draw-back in that its effectiveness largely depends on the milling qualities of the sorghum grain, especially the position of the germ in relation to the whole grain (Serna-Saldivar and Rooney, 1995) and uniformity of kernel size (Sooliman, 1993).
**Figure 7.** Schematic diagram of the front view (left) and side view (right) of the Palyi compact mill (Munck, 1995)

**Abrasion-type deorticator**

Abrasion milling utilises carborundum or other abrasive surfaces mounted on a vertical or horizontal rotor in order to abrade, by friction grinding, the outer layers of the grain (Munck, 1995). The abrasion principle is applied to design a variety of dehullers that can be used to deorticte sorghum, rice, barley (Hahn, 1969). Rice pearlers and whiteners of the abrasion-type can also be used to deorticte sorghum (Munck, 1995). The features of a PRL-dehuller are well described in the literature, for example Morei (1988), Bassey and Schmidt (1989) and Munck (1995).
Figure 8 shows the general features of the PRL-type dehuller. In the PRL type dehuller, the abrasive action is provided by 13 carborundum stones or 27 resinoid discs mounted on horizontal axis. The grains also rub against each other. When the feed gate is opened, the grain flows from the hopper into the body of the machine. Fines generated by the action of the abrasive surface are taken off by the fan and bagged at a cyclone. Decorticated grains leave the machine via an overflow outlet (Bassey and Schmidt. 1989; Munck. 1995). According to Munck (1995) the extraction rates may be from 70 to 85%. In order to avoid clogging of the mill, the grain is not conditioned. PRL-type dehullers and a wide range of its modified versions are traded amongst SADC countries and are used both at village and industrial level (Munck. 1995).

Figure 8. A PRL-type disk dehuller (Munck. 1995)
2.4 Use of industrial enzymes in processing plant-based foods and their possible use to decorticate sorghum

Enzymes are complex, globular protein catalysts that accelerate chemical reaction rates by factors of $10^{12}$-10$^{20}$ (Richardson and Hyslop, 1985) over that of uncatalysed reactions at temperatures around 25-90°C, without themselves suffering any overall change (Palmer, 1995).

2.4.1 General enzyme characteristics and properties

According to Lehninger, Nelson and Cox (1993), the distinguishing feature of an enzyme-catalysed reaction is that it occurs within the confines of a pocket called the active site. The molecule that is bound by the active site and acted upon by the enzyme is called the substrate. The enzyme-substrate complex is central to the action of enzymes, and it is the starting point for mathematical treatments defining the kinetic behaviour of enzyme-catalysed reactions and for theoretical descriptions of enzyme mechanisms, such as the classical Michaelis-Menten equation proposed in 1913 (Lehninger et al. 1993). According to the enzyme-substrate complex theory, the enzyme and substrate concentrations are the prime determinants of the reaction rate under controlled environmental conditions. Enzymic activity is influenced by the conditions prevailing within and outside the substrate. The conditions have to be controlled so as to control enzymic activity. The main factors affecting enzymatic activity are temperature, pH, moisture, ions and ionic strength, ionising radiation, shearing, pressure and interfacial effects (Richardson and Hyslop, 1985). Enzymes can be reversibly and irreversibly inactivated, and they normally require other substances (cofactors) to activate them (Palmer, 1995).

According to Palmer (1995), the Enzyme Commission divided enzymes into six classes according to the type of reaction catalysed. The classes are Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases.
2.4.2 Industrial enzymes for processing plant-based foods

Enzymes used in food processing are often called “commercial enzymes” (Reed, 1993), or “industrial enzymes” (Taylor, 1991). They may be from plant, animal or microbial sources. Commercial or industrial enzymes are not highly refined and are often sold in bulk. The enzyme preparation contains one main enzyme and smaller amounts of other enzymes (Reed, 1993). Taylor (1991) states that most industrial enzymes are hydrolases. Hydrolases catalyse the hydrolytic cleavage of C-C, C-O, C-N, and some other bonds (Perham, 1990). Reed (1993) states that most industrial enzymes are obtained from micro-organisms because micro-organisms are relatively easy to manipulate genetically.

Application of industrial enzymes in food processing is widely dealt with in the literature, for example (Richardson and Hyslop, 1985; Sheppard, 1986; Linko, 1987; Pilnik and Voragen, 1990; Whitaker, 1990, Taylor, 1991).

Sheppard (1986) states that the most attractive feature of enzymes with regard to their use in food processing is that they generally work under mild conditions. With few exceptions, industrial enzymes work at atmospheric pressure, within the pH range 3-9 and at temperatures 25-90°C (many at 45-55°C). In addition to operating under mild conditions, enzyme reactions are specific producing the desired product with minimal side reactions (Sheppard, 1986). The enzymic process is thus predictable and can be optimised. A third advantage from the use of enzymes within the food industry is that enzymatic processes tend to have lower effluent treatment costs (Sheppard, 1986; Whitaker, 1990).

Carbohydrases are probably the most used enzymes in processing plant-foods (Taylor, 1991). Carbohydrases break down complex polysaccharide molecules to simpler molecules. Carbohydrases are applied in baking and brewing (Taylor, 1991), and in fruits and vegetable processing (Pilnik, Voragen and Vos. 1975; Voragen, Geerest and Pilnik 1982; Adams and Kirk 1991; Baker and Grohmann, 1995)(Table 1).
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sources</th>
<th>Conversions catalysed</th>
<th>Food use</th>
</tr>
</thead>
</table>
2. *Bacillus subtilis*, var.  
3. Barley malt       | β-D-Glucans + H₂O → Oligosaccharides + glucose (β-1,3 and β-1,4 bonds)                 | Degums mash in brewing; hydrolyses β-glucan gums in barley used as brewing adjunct to facilitate filtrate filtration during brewing; increase yield of extract in coffee substitute manufacture |
| Cellulase (s)        | 1. *Aspergillus niger*, var.  
2. *Trichoderma reesei*, var. | Cellulose + H₂O → β-dextrins (β-1,4-glucan bonds)                                      | Complex enzyme system; aids in juice clarification; increase yield in extraction of essential oils and spices; improves beer “body”; improves cookability and rehydration of dehydrated vegetables; acids in increasing available proteins in seeds; forms fermentable sugar in waste grape and apple pomace; potential for producing glucose from cellulosic waste |
| Hemicellulase        | *Aspergillus niger*, var                     | Hemicellulose + H₂O → β-dextrins (β-1,4-glucan bonds of gums - locust bean, guar, etc.) | Aids dehusking seed coats of coffee beans; allows controlled degradation of food gums; removes pentosans from bread; facilitates degeneration of corn; improves nutritional availability of plant proteins; facilitates mashing in brewing |
| Pectinase (contains polygalacturonase pectin methylsterase, pectate lyse) | 1. *Aspergillus niger*, var  
2. *Rhizopus oryzae*, var. | Pectin methylesterase  
Demethylates pectin  
Polygalacturonase hydrolyses β-D-1,4-galacturonide. | Aids in clarification and filtration of fruit juices and wines; prevents gelling in fruit juice concentrates and puree; controls cloud retention in juices; controls pectin levels in jelly; facilitates manufacture of glazes fruit, separation of mandarin orange segments |
2.4.3 The potential of enzymes to decorticate sorghum

Although the pericarp of cereal grains contains non-starch polysaccharides, which are predominantly cellulose and hemicelluloses that are similar to those found in fruits and vegetables, there is very little literature in the area of application of enzymes to decorticate the cereal grains. According to Dekker (1979), the possibility of employing enzymes to decorticate cereal grains was advocated by Fukumoto et al. in 1972. Richardson and Hyshop (1985) stated that hemicellulases are used to facilitate the degemination of maize. The application of enzymes to decorticate sorghum, in particular, could not be found in the literature.

Dewar, Orovan and Taylor (1997) found that alkali steeping of sorghum grain resulted in increased permeability of the sorghum grain. That was interpreted as being due to the alkali degrading the non-starch polysaccharide cell wall material. Hydrolytic enzymes have been found to act on macromolecules in the sorghum grain such as the cell wall material (Aisien, 1982; Dale, Young and Omole, 1990; Taylor, Orovan and Dewar, 1994). starch (Glennie, Harris and Liebenberg, 1983; Taylor and Robbins, 1993; Taylor and Dewar, 1994) and proteins (Evans and Taylor, 1990a, b) in a similar way to strong chemicals such as alkali. Fullbrook (1983) found that lipases could partially hydrolyse rapeseed and soybean oil which facilitated oil extraction with hexane. Therefore, it should be possible to use hydrolytic enzymes such as cellulases, hemicellulases, pectinases and lipases to cleanly separate sorghum grain endosperm (meal) from the non-starch polysaccharide-rich pericarp and the lipid-rich germ.

2.4.3.1 Cellulases

Cellulolytic enzymes are synthesised by a large number of micro-organisms which include fungi, antinomyetes, gliding bacteria (myxobacteria) and true bacteria. However, only fungi appear to excrete large amounts of cellulase (Wood, 1985).
According to Mandels (1982), the most active cellulases are derived from *Trichoderma reesei*, *T. viride*, *T. lignorum*, *T. koningii*, *T. pseudokoningii*, and *T. longibrachiatum*. The enzymatic hydrolysis of crystalline cellulose is a complex process requiring the participation of several enzymes (Enari, 1983). Native cellulose is enzymically hydrolysed by the combined action of endo-β-glucanases, exo-β-glucanases and β-glucosidases (Emert, Gum. Lang, Liu and Brown, 1974; Goksoyr and Eriksen, 1980; Mandels, 1982; Enari, 1983; Coughlan, 1985; Eriksen and Wood, 1985; Wood, 1985). Each enzyme is generally found to exist in several forms and these forms may differ in substrate specificity, ability to absorb the substrate and in their capacity to interact synergistically with other enzymes in the system. The three main cellulolytic enzymes mentioned form the “cellulase complex” or “cellulase system” (Wood, 1985). The chemical nature of cellulose is dealt with in 2.2.1.1.

**Endoglucanases**

Endoglucanases attack amorphous cellulose in a random fashion, resulting in a rapid decrease in chain length and water-soluble cello-oligosaccharides are the intermediate products (Wood, 1985).

**Cellbiohydrolases**

Cellbiohydrolases degrade cellulose by splitting off cellbiose units from the non-reducing end of the chain. “Swollen” partially degraded amorphous celluloses and soluble cello-oligosaccharides, triose to hexose, are readily degraded (Goksoyr and Eriksen, 1980, Eriksen and Wood, 1985).

**β-Glucosidases**

β-Glucosidases hydrolyse cellbiose and soluble cello-oligosaccharides to glucose, but cellulose is not degraded. β-Glucosidase is distinguishable by its transferase activity.
retention of $\beta$-configuration on hydrolysis, inhibition by low concentrations of gluconolactone, and a capacity for hydrolysis of cello-oligosaccharides with a low degree of polymerisation (Wood, 1985).

**Mode of action of the cellulase system/complex**

The mode of action of the cellulase system is complex and has been a subject of debate for many years (Enari, 1983). According to Enari (1983), the first hypothesis was probably that proposed by Reese *et al.* (1950). These authors proposed a two-step process with an initial "activation" step followed by the action of a non-hydrolytic enzyme called $C_1$. The hydrolysis of the activated cellulose is then effected by hydrolytic $C_x$ enzymes, and completed by a $\beta$-glucosidases (Figure 9a). However, research findings have resulted in abandonment of Reese’s hypothesis, and the adoption of a more accepted model for the enzymatic hydrolysis of cellulose (Mandels, 1982; Enari, 1983).

According to this modern view, endoglucanase acts on amorphous regions in the cellulose fibres. This opens up new chain ends for attack by cellobiohydrolase, which then removes glucose units from the non-reducing ends of the chains. $\beta$-glucosidases further enhances the total hydrolysis by removing cellobiose, the end product inhibitor of cellobiohydrolase and endoglucanase (Figure 9b). In this scheme, endo-glucanase is believed to be rate-limiting. Endoglucanase alone hydrolyses amorphous regions of glucan chains on the surface of the cellulose fibrils since it can only act on exposed glucan chains. Its random cleaving action does not lead to removal of surface layers or the exposure of new glucan chains. It is then followed by cellobiohydrolase which splits-off cellobiose from the exposed non-reducing chains-ends. But when all nicked chain-ends have been removed, cellobiohydrolase cannot attack the newly exposed glucan chains. When endoglucanase is present it can again hydrolyse the exposed glucan chains. In this way endoglucanase and cellobiohydrolase act synergistically creating sites of action for each other.
Figure 9. Mode of hydrolysis of cellulose by the cellulase system: (a) old model, C₁ and C₂ cellulase hypothesis (Emert, Gum, Lang, Liu and Brown, 1974). (b) Modern model (Enari, 1983)

Mandels (1982, 1985) and Coughlan (1985) commented that the economic utilisation of cellulates to degrade cellulosic material is greatly limited by the crystalline nature of cellulose. Mandels (1983) also remarked that the specific activity of cellulase is very low on insoluble cellulose. 0.6-0.7 filter paper cellulase units/mg of protein. Glucoamylase has a specific activity 100 times greater, i.e. 69 units/mg protein, with starch as substrate. To overcome the crystallinity problem of native cellulose, many pretreatments such as milling, alkali swelling, or dissolving and reprecipitating are suggested (Goksoyr and Eriksen, 1980; Mandels, 1985).

2.4.3.2 Hemicellulases

Hemicellulases specifically degrade those glycans that make up the backbone chain of the hemicelluloses (Dekker, 1979, 1985). Hemicelluloses are dealt with in 2.2.1.2.
Typical hemicellulases are β-D-galactanases, β-D-mannases, and β-D-xylanases. Hemicellulase, like most other polysaccharide-degrading enzymes, attack their substrates in two ways: exo and endo hydrolytic attack. An exo enzyme degrades the polysaccharide by successive removal of terminal glucose or oligosaccharides units and proceeds in a stepwise manner, usually from non-reducing end of the polysaccharide chain. Endo enzymes attack polysaccharides in a random manner, causing multiple scission that is accompanied by a marked decrease in the degree of polymerisation of the substrate (Dekker, 1985). Hemicellulases of the endo type constitute the most common group of the hemicellulases.

The occurrence of hemicellulases and especially the xylanase is widespread. Various bacteria, fungi and yeast secrete the enzymes extracellularly (Biely, 1985; Wong, Tan and Saddler, 1988). The mode of action of xylanases is shown on Figure 10.

![Figure 10](image)

**Figure 10.** A hypothetical plant xylan and the sites of attack by microbial xylanotic enzymes. The fragment comprising five D-xylose units is presented in the upper part of the figure. Ac. acetyl group: Araf, L-arabinofuranosyl. MeGlcA, 4-O-methyl-D-glucuronic acid. Xyl, D-xylose (Biely, 1985)
2.4.3.3 Pectinases

Commercial pectinases are fungal preparations, mainly from *Aspergillus* sp. (Pilnik and Voragen. 1993). According to Fogarty and Kelly (1983), Lea (1991) and Pilnik and Voragen. (1993), there are three main types of pectolytic enzymes, pectin methylesterase, polygalacturonase and pectate lyase. Substrates of pectinases enzymes are pectic substances that occur as structural polysaccharides in the middle lamella and the primary cell walls of higher plants and are predominant in the parenchymous tissues (Pilnik and Rombouts. 1979. Pilnik and Voragen. 1993). The basic structure of pectin has been described in section 2.2.1.3 and the points of attack by the named pectic enzymes are shown on Figure 11.

![Diagram of pectin hydrolysis](image)

**Figure 11.** Hydrolysis of pectin by pectinases (Pilnik and Voragen. 1993)
2.4.3.4 Lipases

Lipases comprise a group of water-soluble enzymes whose biological function is to catalyse the hydrolysis of triacylglycerols to give free fatty acids, diacylglycerols, monoacylglycerols and glycerol (Brockerhoff, 1974). The chemical nature of lipids is discussed in section 2.2.1.4.

Due to their biological role in hydrolytic degradation of natural fats and oils, lipases are ubiquitous in practically all types of organisms, such as plants, mammals and microorganisms. Because of their widespread industrial application, commercial lipases are usually sold as crude mixtures in which the desired biocatalyst represents only a small fraction (Andersch, Berger, Hermann, Laumen, Lobell, Seemayer, Waldinger and Schneider, 1997).

According to Macrae and Hammond (1985) and Nielsen (1985), lipases exhibit position specificity (are generally 1,3 specific) and specificity towards short chain triglycerides versus long chain triglycerides. Lipases are generally characterised by the ability to catalyse the hydrolysis of ester bonds at the interface between an insoluble substrate phase and an aqueous phase in which the enzyme is dissolved (Cygler and Schrag, 1997). Although lipases are primarily responsible for the hydrolysis of acylglycerides, a number of other low- and high-molecular weight esters, thiol esters, amides and polyol/poly acid esters are accepted as substrates, and hence this unique group of enzymes can be used in various industrial processes (Gandhi, 1997). The generalised hydrolysis reaction of the lipases is shown on Figure 12.

\[
\text{RCOOR}^1 + \text{H}_2\text{O} \rightleftharpoons \text{RCOOH} + \text{R}^1 \text{OH}
\]

**Figure 12.** Lipase hydrolysis of an acylglyceride (Gandhi, 1997)

The industrial uses of lipases may be divided into lipid hydrolysis, and lipid synthesis and modification (Gandhi, 1997). Oils and fats are hydrolysed industrially to produce
free fatty acids, soaps and glycerol which find wide applications especially in soaps and
detergents, cosmetics, pharmaceuticals and food (Andree, Muller and Schmid, 1980;

2.5 General remarks

Sorghum grain needs to be processed into a palatable product through the removal of the
fibre-rich pericarp layer and its shelf-life needs to be increased through removal of the
lipid-rich germ. Milling is the method currently used. Generally, abrasive decortication
appears to be the most suitable method and is indeed the most used commercially.
However, because the germ is integral in the grain, milling sorghum through abrasive
decortication results in a significant loss of the endosperm and this is uneconomical.
Non-starch polysaccharide degrading enzymes, cellulases, hemicellulases and pectinases,
are used in the processing of plant-based foods that have similar cell wall chemical
composition as sorghum. Moreover, some hydrolytic enzymes have been shown to
degrade the cell wall material in the outer bran layers of sorghum. Therefore, it appears
possible to use specific hydrolytic enzymes to decorticate sorghum. An enzymic process
would have the advantages of being potentially cheaper, safer and because enzymes are
specific, less loss should occur when compared with conventional milling. Therefore, the
potential of enzymes to decorticate sorghum should be tested.
CHAPTER 3

MATERIALS AND METHODS

3.1 Grain

Two batches of red condensed tannin-free commercial sorghums were kindly supplied by Nola (Pty) Ltd (Randfontein, South Africa). The two sorghums were randomly named SORG 1 and SORG 2. The grain was stored at 9-10°C.

3.2 Enzymes

Enzymes SA (Benmore, Republic of South Africa) kindly donated four enzymes. The enzymes were as follows: Endo-β-glucanase (Denimax® Acid Conc., Novo Nordisk, Denmark), Xylanase (Pentopan Mono BG, Novo Nordisk), Pectinase (Pectinex® 100 L, Novo Nordisk) and Lipase (Lipolase™, Novo Nordisk). The enzymes were stored at 4°C.

3.3 Grain cleaning

Before performing the various analyses, the grain samples were sub-sampled into smaller portions (about 5 kg). The sub-samples were cleaned using a Bates laboratory aspirator (HTM, Mcgill, Houston, USA). Large foreign material was hand picked and the broken kernels were removed by sieving the samples through a 1.28 mm screen. The cleaned grain was stored in closed containers at 9-10°C.
3.4 Characterisation of grain

Because the two sorghums were not pure cultivars, the following analyses were performed on the cleaned grain in order to characterise them:

3.4.1 Physical characteristics

Visual examination

A sample was drawn from a cleaned sub-sample of each sorghum and examined with the aid of a magnifying glass on a white filter paper. Kernel colour and any other distinguishing features were assessed.

The two sorghums appeared to have the same kernel size and their glumes were darkish and similar. However, sorghum SORG 2 had a deeper red colour than sorghum SORG 1.

Hectolitre mass

Hectolitre mass is the mass of 100 L of grain. A hectolitre mass instrument, whose receiver capacity was 0.5 L, was used to determine the hectolitre mass. The moisture content of whole grains was measured using AACC method 44-15A (American Association of Cereal Chemists, 1983) to express the hectolitre mass on a dry basis. The determination was done in triplicate.

1 000 kernel mass

1 000 kernel mass is the mass of 1 000 grains. A Numigral (Tripette and Renand, Villeuve La Garenne, France) was used to determine 1 000 kernel mass. Determination was in triplicate.
Kernel hardness

Kernel hardness was measured in terms of abrasive hardness index (AHI) (Oomah, Reichert and Youngs. 1981). AHI was determined according to Taylor, Dewar, Taylor and Von Ascheraden (1997), thus: A TADD (TADD Model 4E-230, Venables Machine Work Ltd, Saskatoon, Canada) was used to progressively decorticate the grain. Abrasion was brought about by sandpaper (60 grit, Norton type R284 metalite). For each sample, 5 x 50 g aliquots of grain were weighed out. An aliquot was distributed into the TADD cups. Abrasion was performed for 2 min. The abraded grain was then cleaned with an aspirator, which was named previously, weighed and then discarded. The procedure was repeated for 4, 6, 8 and 10 min of abrasion and the data plotted graphically (Fig. 13). AHI was calculated as the time in seconds to abrade off 1% by mass of the grain.
Figure 13. Grain harness measured as abrasive hardness index (AHI) using a tangential abrasive disk dehuller (TADD)

It can be seen from Figure 13 that it took a shorter time to achieve 1% abrasion loss when SORG 2 was milled. the regression line is steeper than that of SORG 1.

Endosperm texture

Endosperm texture was determined using the method described by Rooney and Miller (1982). Ten sound kernels of each sorghum were cut into symmetrical halves and examined with the aid of a magnifying glass. By reference to photographs of samples that had been previously rated (Figure 14), endosperm texture was rated from 1 to 5. A rating of 1 represented an endosperm that was almost completely glassy/vitreous and 5 represented a virtually floury/chalky endosperm. Mean rating scores were determined.
Figure 14. Endosperm texture of sorghum: median longitudinal half kernels (in cross section) of sorghum showing the range from almost entirely corneous/vitreous to entirely chalky/floury kernels. Endosperm texture ratings are A and B-5, C-4, D-3, E and F, 2, and G-1. (Rooney and Miller, 1982)
The physical characteristics of the two sorghums are summarised on Table 2.

**Table 2. Physical characteristics of the two sorghums**

<table>
<thead>
<tr>
<th>Sorghum</th>
<th>Hectolitre mass (kg dry basis)</th>
<th>1 000 kernel mass (g dry basis)</th>
<th>AHI</th>
<th>Endosperm texture (rating)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SORG 1</td>
<td>77.3 a</td>
<td>23.9 a</td>
<td>45</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SORG 2</td>
<td>77.8 a</td>
<td>27.0 b</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>(0.5)</td>
<td>(0.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean values in the same column with different letters differ significantly from each other (p<0.05).

* Standard deviation is given in parentheses.

* A rating of 1 represented an endosperm that was almost completely glassy/vitreous and 5 represented a virtually floury/chalky endosperm.

AHI, abrasive harness index-time in seconds to abrade off 1% of grain.

Table 2 shows that the sorghums differed in 1 000 kernel mass, kernel hardness and endosperm texture but had the same hectolitre mass. SORG 2 had bigger grains (higher 1 000 kernel mass), softer (lower AHI) and less vitreous (higher endosperm texture rating) than SORG 1.

**3.4.2 Chemical characteristics**

The grain was milled to fine flour with a laboratory hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 0.8 mm screen. The following determinations were made in triplicate:
Moisture

Moisture content was determined using AOAC official method 925.10 (air oven) (Association of Official Analytical Chemists, 1995a). Approx. 2 g of sorghum grain was weighed accurately into a cooled, weighed dish, which had been heated to 130°C. The samples were heated at 130°C in a forced-air oven for 1 h. The samples were then cooled in a dessicator and weighed. Loss in weight was determined as moisture using the following formula:

\[
\% \text{ Moisture} = \frac{\text{Fresh mass - Dry mass}}{\text{Fresh mass}} \times 100
\]

Fat

Fat was measured using AACC Method 30-20 (Soxhlet extraction) (American Association of Cereal Chemists, 1983). The principle is that petroleum ether dissolves (extraction) all the lipid material in the sample. The petroleum ether is then evaporated over a boiling water bath leaving the lipid material as a sediment in the container and the amount of lipid (fat) extracted is calculated by mass difference.

Six clean 250 ml beakers were dried in an oven for 1.5 h at 103°C. The beakers were weighed accurately and then stored in a dessicator.

Approx. 3-5 g of sorghum grain was weighed accurately and then transferred to a Soxhlet extractor. Approx. 250 ml 30-60°C boiling range petroleum ether was placed in a 500 ml flat bottomed flask along with four anti-bumping granules. The flask, Soxhlet extractor and condenser were assembled on a heating mantle. The condenser water was turned on and then the heating mantle. Extraction was allowed to continue for 4 h. The heating mantle was then turned off and the contents of the flask transferred to a pre-weighed beaker. The contents of the beaker were evaporated to dryness on a boiling water bath in
a fume cupboard. The beaker was then placed in an oven at 103°C for a further 30 min. After this time, the beaker was reweighed.

Fat was calculated using the following formula:

\[
\% \text{ Fat} = \frac{(\text{Mass of beaker} + \text{flask}) - (\text{Mass of beaker}) \times 100}{\text{Mass of sample}}
\]

Ash

Ash was measured according to AOAC Official Method 923.03 (Association of Official Analytical Chemists, 1995b). The principle is that combustion (550°C) converts all the organic matter in the sample into gaseous carbon and the minerals sediment on the dishes. The mineral content (ash) is then calculated by mass difference.

Approx. 3 g sample was weighed accurately into an ashing crucible which had been previous ignited, cooled in a dessicator and weighed. The samples were combusted in a furnace at approx. 550°C for 6 h. The samples were then cooled in a dessicator, and the resulting light grey ash was weighed. Ash content was calculated using the following formula:

\[
\% \text{ Ash} = \frac{(\text{Mass of crucible + ash}) - \text{Mass of empty crucible} \times 100}{\text{Mass of sample}}
\]

Crude protein (N x 6.25)

Protein was measured using a Kjeldahl method (Chang, 1994). The Kjeldahl method is based on the wet combustion of the sample by heating with conc. H\text{2}SO\text{4} in the presence of metallic and other catalysts to effect the reduction of organic nitrogen in the sample to ammonia, which is retained as ammonium sulphate. The digest, having been made alkaline, is distilled or steam distilled to release the ammonia which is trapped and titrated with HCL.
About 1 g (accurately weighed) samples were measured into Kjeldahl digestion flasks. Kjeltab (catalyst) (Thompson and Capper, Cheshire, UK) were added followed by 20 ml conc. H₂SO₄. The samples were digested in a Buchi 430 Digestor (Buchi, Flawil, Switzerland) for about 2 h, and then let to cool. A Buchi 322 Distillation Unit (Buchi, Flawil, Switzerland) was used for ammonia distillation, reaction with 4% boric acid and titration with 0.1 M HCL.

Table 3 gives a summary of the chemical characteristics of the two sorghums.

**Table 3. Chemical characteristics of the two sorghums**

<table>
<thead>
<tr>
<th>Sorghum</th>
<th>Moisture (%)</th>
<th>Fat (% dry basis)</th>
<th>Ash (% dry basis)</th>
<th>Protein (% dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SORG 1</td>
<td>11.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(0.0)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>(0.1)</td>
<td>(0.0)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>SORG 2</td>
<td>12.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.1)</td>
<td>(0.0)</td>
<td>(0.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean values in the same column with different letters differ significantly from each other (p<0.05).

<sup>*</sup> Standard deviation is given in parentheses.

Table 3 shows that sorghum SORG 2 contained more protein and fat than SORG 1 but had slightly less ash. The moisture contents of the two sorghums were not significantly different.
3.5 Determination of whether hydrolytic enzymes can be used to decorticate sorghum grain

A series of experiments were carried out to determine whether hydrolytic enzymes could be used to decorticate sorghum grain. The experiments were replicated at least twice and duplicated in all cases.

3.5.1 Determination of whether hydrolytic enzymes can be used to decorticate sorghum grain under semiwet conditions

Experiment 1

About 14 ml 10% (w/w) solution of endo-β-glucanase, xylanase or lipase was added to 100 g of sorghum grain in a 1 L beaker. A 10% (w/w) solution of a (1:1:1) mixture of endo-β-glucanase, xylanase and lipase was also applied to the sorghum grain. The amount of enzyme added was such that the moisture content of enzyme with grain was 26%. That was similar to the moisture content of steeped grain in a semiwet milling process for sorghum described by Cecil (1992). The beaker was covered with polythene paper. The stirring blades of a Kenwood Power-Five mixer (Kenwood, Newlane, England) were placed in the grain through a puncture in the polythene. A control in which the live enzyme solution was replaced by a boiled enzyme solution was included. The enzyme was boiled in a 250 ml conical flask which was covered with a watch glass to avoid loss of moisture due to evaporation on a hot plate. The enzyme was boiled to inactivate it. The beakers were incubated at 50°C overnight with stirring at a steady speed.

The hypothesis was that abrasive action due to mixing would render the pericarp and germ accessible to the enzymes that would then hydrolyse the non-starch polysaccharides and lipids in the pericarp and germ respectively. The grain was visually examined with the aid of a magnifying glass to observe if there was a decortication effect.
Experiment 2

Determination of the effect of increasing grain abrasion and limiting grain mobility

It was believed that a more powerful stirrer was needed and that movement of the kernels needed to be restricted in order to partially abrade the grain and thus enable enzyme access to the pericarp and germ tissues.

A laboratory stirrer (Gallenkamp, England) was used. The stirrer comprised four flat stainless steel blades mounted on a stainless steel rod (about 30 cm long) which was mounted to an electric motor (15 A/240V). A laboratory wet mill screen holder made of stainless steel was placed in the grain such that the kernels were squeezed between the walls of the beaker and the screen holder. The screen holder comprised a solid (10 cm diameter, 0.5 cm thickness) stainless steel spherical base on whose circumference were 24 vertical (2.5 cm long) stainless steel rods that were spaced by 1 cm. Otherwise the rest of the experimental design was the same as in Experiment 1.

Experiment 3

Use of undiluted enzymes

It was hypothesised that the enzyme concentration needed to be increased to effect decortication of sorghum grain. Another enzyme, pectinase was also introduced. The enzymes, endo-β-glucanase, xylanase, pectinase and lipase were applied without dilution. About 14 g of either a single enzyme or combined enzymes were added to 100 g of each sorghum. The rest of the experimental design was as in Experiment 2.
Experiment 4

Determination of the effect of soaking sorghum grain in diluted enzymes in combination with either stirring or sand addition combined with shaking in a water bath on enzyme decortication of sorghum grain

It was believed that because in the previous experiment the undiluted enzyme had set to a thick paste, enzyme diffusion into the grain tissues was greatly reduced. The grain needed to be soaked in liquid to increase enzyme mobility.

Exactly 100 g of sorghum grain was soaked in 100 ml of a 10% solution of the 4 enzymes, endo-β-glucanase, xylanase, pectinase and lipase, which were applied either singly or in combinations. The rest of the experimental design was as in Experiment 2.

Additionally, 25 ml of the same single or combined enzymes were added to a 250 ml conical flask that contained 25 g of grain and 25 g of sea sand. It was believed that including sand would result in partial abrasion of the grain. Controls were as follows: grain+boiled enzyme, grain+sand+boiled enzyme, and grain+live enzyme. The conical flasks were incubated at 50°C in a water bath and left overnight shaking.

Experiment 5

Total solids (TS)

The objective was to test the hypothesis that live endo-β-glucanase had caused hydrolysis of the non-starch polysaccharides in the pericarp resulting in lighter grains and release of soluble sugars that resulted in stickiness of the liquid in the flasks where it had been added.
Experiment 4 was repeated but only endo-β-glucanase was applied to the sorghum grain. Total solids (TS) of the liquid from the enzyme-treated grain were determined. TS were determined by evaporating off a known mass of sample and weighing the dry residue. The procedure was as follows: A clean evaporating basin was weighed and the mass recorded as $W_1$, to the nearest 0.002 g. The contents of the flasks were thoroughly mixed by inversion and then approx. 10 ml sample was drawn immediately and transferred to the evaporating basin and the mass was recorded as $W_2$. The evaporating water basins were placed on a boiling water and allowed to evaporate until the residue appeared to be perfectly dry. The water bath was switched off and the basins transferred to the central area of a hot (103°C) air oven. After 3 h heating, the evaporating basins were removed from the oven and allowed to cool to room temperature in a desiccator. The evaporating basin was reweighed and the mass recorded as $W_3$. TS were calculated as follows:

$$\% \ \text{Total solids (TS)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Experiment 6

**Determination of the effect of either de-waxing or scraping on enzyme decortication of sorghum grain**

It was believed that the waxy layer on the grain outer surface acted as a barrier to enzyme access to the germ and pericarp. The following experiments were performed to test this hypothesis:

**De-waxing experiments**

Exactly 50 g of each sorghum was immersed in 100 ml hexane in a 250 ml conical flask (in duplicate) which had been dried and weighed. The flasks were stoppered. A control in which there was no grain in the flask was included. The flasks were incubated at 50°C
for 3 h with shaking. The hexane was collected into a clean, dry, pre-weighed flask and evaporated over a boiling water bath. The grain was rinsed with fresh hexane, which was then transferred to a flask and evaporated as has been described. The grain was rinsed 3 times, then transferred to a evaporating dish and dried overnight in a forced-air oven at 50°C. The flasks were dried in a forced-air oven at 100°C for 1 h, cooled and weighed. The amount of hexane-soluble substances dissolved from the grain was calculated by mass difference.

Five de-waxed grains were treated with 25 ml of a 10% solution of either single or combined enzymes (Table 4) in stoppered 250 ml conical flasks. The conical flasks were incubated overnight at 50°C in a shaking water bath.

The samples were rinsed with tap water, dried at room temperature, and examined by scanning electron microscopy (SEM). The procedure for SEM was as follows: Samples were frozen in liquid nitrogen. Each grain was fractured with a scalpel and the fractured grains were freeze dried in a vacuum coater. Samples were then mounted on aluminium stubs, sputter coated with gold and viewed in a JOEL JSM 840 (Joel, Tokyo, Japan) SEM at 5 kV according to Goldstein, Newbury, Echlin, Joy, Romig, Lyman, Fiori and Lifshin (1992).

**Scraping experiments**

About half the kernel surface was slightly scraped with a scalpel such that the pericarp was partly removed. Five scraped grains were treated with 25 ml of a 10% solution of single or combined enzymes (Table 4) in stoppered 250 ml conical flasks. Incubation with enzymes, drying of grains and SEM was as with the hexane de-waxed grains.
### Table 4. Different treatments and enzymes applied on sorghum grain

<table>
<thead>
<tr>
<th>Enzyme(s)</th>
<th>Test sample</th>
<th>Control samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endo-β-glucanase</td>
<td>De-waxed grain+Live enzyme</td>
<td>Grain not de-waxed+Live enzyme; Grain de-waxed+Boiled enzyme; Grain not de-waxed+Boiled enzyme</td>
</tr>
<tr>
<td>Xylanase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pectinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase/Xylanase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase/Pectinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase/Lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylanase/Pectinase/Lipase</td>
<td>Scraped grain+Live enzyme</td>
<td>Grain not scraped+Live enzyme; Grain scraped+Boiled enzyme; Grain not scraped+Boiled enzyme</td>
</tr>
<tr>
<td>Endo-β-glucanase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylanase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pectinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase/Xylanase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase/Pectinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase/Lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylanase/Pectinase/Lipase</td>
<td></td>
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</tr>
</tbody>
</table>

### Experiment 7

**Determination of the effect of de-waxing the grain twice or scraping the germ area on enzyme hydrolysis of the germ of sorghum grain**

Two experiments were performed to test the hypothesis that a waxy layer on the seed coats prevented enzyme access to the germ:

**De-wax the grain twice**

The sorghums were de-waxed as described previously and decorticated with 10% endo-β-glucanase as described in Experiment 6. Reasonably decorticated grains were chosen, rinsed with tap water, dried at room temperature and then de-waxed again with
hexane. The amount of hexane-soluble substances dissolved was determined as in Experiment 6.

Five grains that had been decorticated with endo-β-glucanase and de-waxed twice were treated with 25 ml of 10% solution of either single or combined enzymes (Table 4) in 250 ml conical flasks. Incubation with enzymes, drying of grains and SEM was done as in Experiment 6.

**Scrape the germ area**

Hexane de-waxed grains were decorticated with 10% endo-β-glucanase and then scraped with a scalpel in the germ area in order to expose the germ.

Five scraped grains were treated with enzymes as has been described in Experiment 6. Incubation with enzymes, drying of grains and SEM was as in Experiment 6.

**3.6 Process optimisation**

The objective was to establish the optimal enzyme: grain ratio required to achieve an acceptable decortication effect.

Because it would be undesirable to use a chemical that is highly inflammmable and toxic as hexane in an industrial process, the grain was instead partially decorticated with a Rice Pearler (Miag. Braunschweig, Germany). About 500 g grain of each sorghum was passed through the Rice Pearler. By trial and error the gap setting was adjusting such that approximately 5% decortication loss was achieved. The grain was cleaned and the broken kernels removed as described previously.

Exactly 100 g of partially decorticated sorghum grain was placed in 250 ml conical flasks. Exactly 100 ml of different concentrations of endo-β-glucanase were added to differently
labelled flasks to achieve the following enzyme concentrations: 0%, 0.001%, 0.01%, 0.1%, 1%, 10%, 20% and 50% (ml enzyme/100 g grain). The flasks were incubated at 50°C overnight with shaking.

The grain was washed with tap water. Each grain sample was transferred to a clean nylon bag and rubbed between hands to remove any loose pericarp. Approximately, an equal amount of rubbing was given to each sample. The grain samples were then transferred to large crucibles and left in a fume-board overnight to dry at room temperature.

Ash content and colour of the grain were determined to indicate the extent of enzyme decortication.

Ash content was measured according to the AOAC Official Method 923.03 referred to previously.

A Hunterlab (Hunter Associates Laboratory, Reston, USA) was used to measure grain colour. The machine was standardised with a white and a black tile. About 100 g samples were placed in a cuvette which was then placed into the machine and the grain colour was read as L, a and b values. Analysis was in duplicate.

Enzyme decortication yield was determined by measuring the 1 000 kernel mass of the samples using the Numigral as described previously. The moisture content of whole grains was measured using the AACC method 44-15A, previously referred to, to express 1 000 kernel on weight dry basis. The yield was expressed as:

\[
\text{Yield} = \frac{1 \text{ 000 kernel mass of enzyme treated grain}}{1 \text{ 000 kernel mass of enzyme-untreated grain}} \times 100\% 
\]
3.7 Comparison of the effectiveness of enzyme decortication with machine
decortication

The objective was to compare the yield obtained through enzyme decortication with
machine decortication at the same ash and colour levels.

Machine decortication

About 1 000 g grain of each sorghum was passed through the Rice Pearler. After each
passage about 200 g of grain was collected and packed in a labelled polythene bag. The
rest of the grain was passed through the Pearler. The process was repeated a total of 4.
About 200 g of grain that had not been passed through the Rice Pearler was also packed;
it was used as the control.

Yield was determined by measuring the 1000 kernel mass as described previously. The
moisture content of whole grain at each decortication was determined in order to express
yield on a dry basis.

Yield was expressed as:

\[
\text{Yield} = \frac{\text{1 000 kernel mass of partially decorticated grain}}{\text{1 000 kernel mass of undecorticated grain}} \times 100\%
\]

Ash content and colour of the grain were determined to indicate the extent of machine
decortication.

Ash was measured according to the AOAC Official Method 923.03. Grain colour was
measured on the Hunter L a b scale as described previously.
Enzyme decortication

Ash content, colour and enzyme decortication yield at various endo-β-glucanase concentrations which were obtained in “process optimisation” (Section 3.6) were used.

Decortication effectiveness of the two processes was compared graphically (Figures 22, 23 and 24).

3.8 Statistical analysis

Statistica for Windows (1995) (Statsoft, Inc., Tulsa, USA) was used for statistical analysis. The t-test was performed to test whether there was a significant difference between any 2 means. Means were calculated from replicate data. Analysis was at the 5% significance level.
CHAPTER 4

RESULTS

4.1 Determination of whether hydrolytic enzymes can be used to decorticate sorghum grain

4.1.1 Effect of semiwet conditions on enzyme decortication of sorghum grain

A 10% (w/w) solution of a (1:1:1) mixture of endo-β-glucanase, xylanase and lipase with grain to give a total moisture content of 26% (semiwet conditions) had no effect on the pericarp and germ of either sorghums (Data not shown). Each of the mentioned enzymes also had no effect on the grain when it was applied singly at 10% concentration (w/w).

Effect of increasing grain abrasion

Replacing the Kenwood Power-Five mixer with a laboratory stirrer which was more powerful in combination with application of a wet mill screen holder for limiting grain movement did not result in the removal of the pericarp and germ of both sorghums by endo-β-glucanase, xylanase and lipase, which were applied as described above, under semiwet conditions.

Effect of undiluted enzymes on sorghum grain

Undiluted single enzymes (endo-β-glucanase, lipase and pectinase) or combined enzymes had no effect on sorghum grain under semiwet conditions. The mixture of endo-β-glucanase, xylanase and lipase formed a thick paste on the grain.

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4.1.2. Effect of soaking sorghum grain in water in combination with either stirring or sand addition combined with shaking on enzyme decortication of sorghum grain

Soaking the grain in a 10% (w/w) solution of single enzymes (endo-β-glucanase, lipase and pectinase) or combined enzymes in stirred conditions had no effect on the pericarp or germ of either sorghums (Data not shown).

However, a 10% (w/w) solution of endo-β-glucanase alone appeared to have an effect on the sorghum grain that had been soaked and incubated in a shaking water bath. The grain in the flasks where live endo-β-glucanase had been added appeared lighter and the pericarp could be peeled easily with a scalpel. The liquid was sticky. The total solids content (TS) of the sticky liquid is shown on Table 5.
Table 5. Effect of soaking sorghum grain in 10% (w/w) endo-β-glucanase and sand addition on the total solids content of treatment liquid

<table>
<thead>
<tr>
<th>Sorghum</th>
<th>Treatment</th>
<th>Total solids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SORG 1</td>
<td>Live endo-β-glucanase plus sand</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Live endo-β-glucanase without sand</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Boiled endo-β-glucanase plus sand</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Boiled endo-β-glucanase minus sand</td>
<td>1.8</td>
</tr>
<tr>
<td>SORG 2</td>
<td>Live endo-β-glucanase plus sand</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Live endo-β-glucanase without sand</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Boiled endo-β-glucanase plus sand</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Boiled endo-β-glucanase minus sand</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Table 5 shows that there was a difference in the total solids content of the test sample (Live endo-β-glucanase plus sand) and the control samples “Boiled endo-β-glucanase plus sand” and “Boiled endo-β-glucanase minus sand”), but no difference between the test sample and the control sample “Live endo-β-glucanase without sand”. Live endo-β-glucanase had an effect on the grain as shown by higher total solids. Application of sand had no effect on enzyme decortication of sorghum grain.
4.1.3 Effect of de-waxing and scraping on enzyme decortication of sorghum grain

Figure 15. Effect of de-waxing and scraping on enzyme decortication of sorghum grain. 1. Endo-β-glucanase; 2. Xylanase; 3. Xylanase/Endo-β-glucanase. a, de-waxed grain; b, scraped; c, whole grain. T, live enzyme; C, boiled enzyme. (Grain was soaked in 10% enzyme).

Figure 15 shows that live endo-β-glucanase caused removal of the pericarp of both the de-waxed and scraped grains and hence the grains looked lighter. Xylanase alone or in combination with endo-β-glucanase had no effect on the grain as the grains, which were, either whole, de-waxed or scraped retained their original colour.
Figure 16. Representative scanning electron micrographs showing the effects of de-waxing and scraping on enzyme decortication of sorghum grain. (A). Grain de-waxed and endo-β-glucanase applied: Endosperm (e) separated from pericarp at seedcoat/aleurone layer (sc/al) interface. (B), (control) same treatment as (A) except that boiled endo-β-glucanase was applied: No effect
Figure 16. Representative scanning electron micrographs showing the effects of de-waxing and scraping on enzyme decortication of sorghum grain. (C), grain scraped and endo-β-glucanase applied: Endosperm (e) separated from pericarp as in (A) but germ (g) not removed. (D), (control) same treatment as (C) except that boiled endo-β-glucanase was applied: No effect. (E), Grain de-waxed, xylanase/endo-β-glucanase applied: No effect. p, pericarp.
Figure 16 shows the microstructure of some of the grains. Endo-β-glucanase caused decortication of the sorghum grain at the seed coat/aleurone layer interface in both the hexane de-waxed and the scraped samples (Figure 16A and C, respectively). The germ, however, was not removed (Figure 16C). A combination of enzymes did not remove the pericarp or the germ (Figures 16 E).

**Table 6.** Effect of various treatments on enzyme decortication of sorghum grain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pericarp</th>
<th>Germ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) De-wax grain then apply:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Endo-β-glucanase and pectinase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endo-β-glucanase, lipase and pectinase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase and pectinase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase and lipase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase, endo-β-glucanase, lipase and pectinase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lipase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2) Scrape grain then apply:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase and lipase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endo-β-glucanase and pectinase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endo-β-glucanase, lipase and pectinase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endo-β-glucanase and xylanase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase and Pectinase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase and Lipase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase, endo-β-glucanase, lipase and pectinase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lipase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pectinase</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Effect  - No effect

Table 6 shows that endo-β-glucanase alone had an effect on decortication of sorghum grain. Other single enzymes and all enzyme combinations had no effect.
4.1.4 Effect of de-waxing, enzyme treatment and de-waxing again or scraping the germ area on enzyme hydrolysis of the germ

Figure 17. Representative scanning electron micrographs showing the effect of de-waxing, enzyme treatment and de-waxing again or scraping the germ area on enzyme hydrolysis of the germ. (a) Grain de-waxed, decorticated with 10% (w/w) endo-\(\beta\)-glucanase, de-waxed again and 10% (w/w) endo-\(\beta\)-glucanase applied: No effect. (b), 10% (w/w) endo-\(\beta\)-glucanase applied on whole grain: No effect. (c), treated as (a), then germ area scraped and 10% (w/w) endo-\(\beta\)-glucanase/lipase applied: Evidence of very slight hydrolysis of germ shown by arrow.
Table 7. Effect of different treatments and enzymes on hydrolysis of the germ

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on Germ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) De-wax, de corticate with endo-β-glucanase, de-wax, then apply:</td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase</td>
<td>-</td>
</tr>
<tr>
<td>Endo-β-glucanase and lipase</td>
<td>+</td>
</tr>
<tr>
<td>Endo-β-glucanase and pectinase</td>
<td>+</td>
</tr>
<tr>
<td>Endo-β-glucanase, lipase and pectinase</td>
<td>+</td>
</tr>
<tr>
<td>Endo-β-glucanase and xylanase</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase and pectinase</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase and lipase</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase, endo-β-glucanase, lipase and pectinase</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase</td>
<td>-</td>
</tr>
<tr>
<td>Lipase</td>
<td>+</td>
</tr>
<tr>
<td>Pectinase</td>
<td>-</td>
</tr>
<tr>
<td>2) Scrape germ area and apply:</td>
<td></td>
</tr>
<tr>
<td>Endo-β-glucanase</td>
<td>-</td>
</tr>
<tr>
<td>Endo-β-glucanase and lipase</td>
<td>+</td>
</tr>
<tr>
<td>Endo-β-glucanase and pectinase</td>
<td>+</td>
</tr>
<tr>
<td>Endo-β-glucanase, lipase and pectinase</td>
<td>+</td>
</tr>
<tr>
<td>Endo-β-glucanase and xylanase</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase and pectinase</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase and lipase</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase, endo-β-glucanase, lipase and pectinase</td>
<td>-</td>
</tr>
<tr>
<td>Xylanase</td>
<td>-</td>
</tr>
<tr>
<td>Lipase</td>
<td>+</td>
</tr>
<tr>
<td>Pectinase</td>
<td>-</td>
</tr>
</tbody>
</table>

- Very slight hydrolysis  - No hydrolysis

Figure 17 and Table 7 show that single enzyme or combined enzymes either had no effect on the germ or there was only very slight hydrolysis.
Table 8 shows the amount of hexane-soluble substances dissolved from sorghum grain on single and de-waxing and double de-waxing following endo-β-glucanase treatment, which was determined to establish whether hexane dissolved away the waxy material on the pericarp and seed coats, respectively.

Table 8. Effect of single and double de-waxing on the removal of the waxy material (hexane-soluble substances) on the sorghum grain (% dry basis)

<table>
<thead>
<tr>
<th></th>
<th>SORG 1</th>
<th>SORG 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>First de-waxing</td>
<td>0.33^a</td>
<td>0.37^a</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>Second de-waxing</td>
<td>0.07^b</td>
<td>0.11^b</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.02)</td>
</tr>
</tbody>
</table>

^a Mean values in the same column with the same letters did not differ significantly from each other (p<0.05).

* Standard deviation is given in parentheses.

Table 8 shows that hexane dissolved away some hexane-soluble substances from both sorghums on both the first and second de-waxing processes. More hexane-soluble substances were dissolved away on the first de-waxing process than the second one. The amount of hexane-soluble substances dissolved away was not significantly different between the two sorghums on first and second de-waxing, respectively.
4.2 Process optimisation

Process optimisation was done to establish the optimum enzyme (endo-β-glucanase): substrate (sorghum grain) ratio that would result in removal of the pericarp from partially decorticated sorghum to an acceptable level.

Figure 18. Effect of Endo-β-glucanase concentration on decortication of partially de-waxed sorghum grain. 1. SORG 1; 2. SORG 2. S, 0%; T, 0.001%; U, 0.01%; V, 0.1%; W, 1%; X, 10%; Y, 20%; Z, 50% endo-β-glucanase (ml/100 g grain).
Figure 18 shows that the grain became progressively lighter as endo-β-glucanase concentration was increased. It could be seen that more pericarp was removed at higher endo-β-glucanase concentrations. It appeared that the pericarp was persistent at the hilum area.

Figures 19, 20 and 21 show the effect of Endo-β-glucanase concentration on ash content yield and colour of partially decorticated sorghum grain.

![Graph showing ash content vs endo-β-glucanase concentration]

**Figure 19.** Effect of Endo-β-glucanase concentration on ash content of partially decorticated sorghum grain

Figure 19 shows that the ash content of both sorghums decreased progressively as the enzyme concentration increased and leveled off at about 1% enzyme.
Figure 20. Effect of Endo-β-glucanase concentration on decortication yield of partially decorticated sorghum grain.

Figure 20 shows that the amount of grain material removed increased progressively as the enzyme concentration was increased for both sorghums.
Figure 21. Effect of Endo-β-glucanase concentration on colour (Hunter L a b scale) of partially decorticated sorghum grain. (A). SORG 1; (B). SORG 2.
Figure 21 shows that increasing enzyme concentration resulted in an increase in lightness (Hunter L value) of both sorghums. There was a progressive decrease in the redness (Hunter a value) of both sorghums as the enzyme concentration was increased. The Hunter b values remained almost constant as the enzyme concentration was increased for both sorghums.

4.3 Comparison of the effectiveness of enzyme decortication with machine decortication of sorghum grain

The relationships between yield and ash, and yield and colour of enzyme and machine decorticated grain were analysed (Figures 22, 23 and 24) to establish which method of decortication was more effective.
Figure 22. Relationship between ash content and decorticating yield for enzyme and machine decorticating of partially decorticating sorghum grain. (A). SORG 1; (B). SORG 2. M. machine; E. enzyme.
Figure 22 shows that to achieve an acceptable ash content (about 1.1%) there was less loss of grain material when the enzyme was used compared to machine decortication. About 0.01% (ml/100 g grain) enzyme was used to reduce the ash content of the grain to about 1.1% and about 15% grain material was lost. To achieve the same ash level using a machine, about 25% grain material was lost for both sorghums.
Figure 23. Relationship between grain lightness and decortication yield for enzyme and machine decortication of partially decorticated sorghum grain.

(A). SORG 1; (B). SORG 2. M. machine; E. enzyme.

Figure 23 shows that at the same decortication yield, machine decortication produced a lighter (higher Hunter L value) grain than enzyme decortication for both sorghums.
Figure 24. Relationship between grain redness and decortication yield for enzyme and machine decortication of partially decorticated sorghum grain.

Figure 24 shows that at the same decortication yield, machine decortication gave a less red grain than enzyme decortication for both sorghums.
CHAPTER 5

DISCUSSION

The physical and chemical characteristics of the two sorghums show that the sorghum batches (SORG 1 and SORG 2) were different. SORG 1 had a lower endosperm texture rating (rated 3) than SORG 2 (rated 4) indicating that its endosperm was more vitreous than of SORG 2 (Rooney and Miller, 1982). The endosperm texture ratings of the two sorghums were related to kernel hardness (given as abrasion hardness index (AHI)). A grain that has a vitreous endosperm is usually harder (Rooney and Miller, 1982; Hoseney, 1994). Thus SORG 1 was more vitreous and harder (AHI=45) while SORG 2 had an intermediate endosperm texture and was soft (AHI=30). However, according to Hoseney (1994), the causes of hardness and vitreousness are different, and the two do not always go together. Hardness is caused by the genetically controlled strength of the bond between protein and starch in the endosperm. Vitreousness, on the other hand, results from lack of air spaces in the kernel. Because SORG 2 was softer, it took less time (30 s) to abrade off 1% material from it using the TADD than SORG 1 for which it took 45 s. SORG 2 would be harder to mill because softer grains have a crumbly endosperm making it difficult to separate the endosperm from the bran (Maxson, Fryar and Rooney, 1977). However, the same sorghum (SORG 2) may be easier to decorticate using enzymes than SORG 1 because the outer layers would be easier to peel off as shown by its lower AHI.

SORG 2 had a higher 1 000 kernel mass suggesting that its grains were bigger than those of SORG 1. Genetic and environment factors are known to cause variation in kernel size (Rooney and Miller, 1982). However, the hectolitre mass of the two sorghums was not significantly different indicating that their density was the same (Gomez, Obilana, Martin, Madzvamuse and Monyo, 1997).
The chemical components of the two sorghums generally agree with those cited in the literature: moisture, 10-12%; fat, 2.1-5% and ash, 1.6-1.7% (Serna-Saldívar and Rooney, 1995). However, SORG 1 and SORG 2 had 7.0% and 7.8% protein respectively, which were both much lower than 11.5-12.3%, the range given by Serna-Saldívar and Rooney, (1995). The difference may be due to environmental and genetic factors, which are reported to affect sorghum protein content (Serna-Saldívar and Rooney, 1995). There was no big difference in the chemical components between the two sorghums except that SORG 2 had a higher fat content, which indicates that it has a larger germ.

It appears that enzymic decortication of sorghum grain under semiwet conditions (26% total liquid in grain) was not possible as shown by the negative results when either single or combined enzymes were applied. The semiwet process was not successful despite increasing grain abrasion through the use of a more powerful stirrer. The results suggest that enzymes should be diluted to increase their mobility. Enzyme mobility is necessary because enzyme molecules should collide with substrate molecules in order for a reaction to occur (Palmer, 1995). It would also be necessary to hydrate the grain so that the enzymes can gain access to the substrate by diffusion.

Undiluted single enzymes (endo-β-glucanase, lipase and pectinase) or combined enzymes had no effect on sorghum grain probably due to limited mobility of the undiluted enzymes. Also, because the grain was not fully hydrated (semwet conditions), the enzymes had no access to the substrate (non-starch polysaccharides and lipids) by diffusion. The enzyme solution set into a thick paste in the grain, which was likely to be due to evaporation of water.

It appears that stirring did not produce an effective abrasive force which would break down the cell wall structure and thus allow enzyme access to the non-starch polysaccharides in the pericarp and lipids in the germ. It appears that live endo-β-glucanase had a slight effect on sorghum grain that was incubated in a shaking water bath as indicated by slight increase in the total solids (Table 5). The results on Table 5 suggest that live endo-β-glucanase
hydrolysed cellulose in the pericarp cell walls (Serna-Saldívar and Rooney, 1995) and mixed linkage 1.3 and 1.4 β-glucans in the pericarp, aleurone layer and endosperm cell walls (Earp, Doherty and Rooney, 1983; Earp, Doherty, Fulcher and Rooney, 1983; Glennie, Harris and Liebenberg, 1983) releasing glucose and intermediate molecular weight oligosaccharides (Wood, 1985). The mentioned products of cellulose and β-glucan hydrolysis appear to have caused an increase in total solids. The solids in the solution from the control, flask where “Boiled endo-β-glucanase” was added, should have originated from the enzyme or any washable solid material on the sorghum grain. It appears that the sand did not produce an effective abrasive force since the total solids in the flasks where it was either included or excluded were the same.

The total solids from the flasks where the live enzyme was added were not much greater than the total solids in the samples where a boiled enzyme was added suggesting that a slight hydrolysis of the cellulose and β-glucans had occurred. Limited hydrolysis of the cellulose and β-glucans is likely to be due to the fact that endo-β-glucanase had limited access to the pericarp, aleurone layer and endosperm cell walls. A waxy cuticle on the pericarp and seed coats (Kent and Evers, 1994) could have been a barrier to enzyme access to the cell walls. Plant cuticles are composed of cutin, waxes and some pectic substances (Kolattukudy and Espelie, 1985). Salisbury and Ross (1992) stated that a plant cuticle functions as a barrier to diffusion of substances in and out of plant organs. The crystallinity of cellulose might also have contributed to limited degradation of the pericarp. Mandels (1982; 1985) and Coughlan (1985) noted that crystalline cellulose is compact and insoluble and therefore is resistant to enzymic hydrolysis. The association between cellulose and other polymers such as hemicelluloses and proteins renders it more resistant to enzymic attack. However, it was expected in this work that partial decortication of the sorghum grain would make the cellulose more susceptible to enzymic attack. According to Mandels (1985), mechanical action such as milling breaks down cell wall structure and disrupts cellulose crystallinity, thus exposing the cellulose to enzymic attack.

Use of a shaking water bath seems to have been more effective than stirring since some
degradation of the cell walls seems to have occurred. Shaking is likely to have promoted enzymic action through both keeping the enzyme in contact with the substrate and removing the products of hydrolysis, and producing an abrasive force.

Figure 16 suggests that applying hexane or scraping the grain enabled endo-β-glucanase to remove the pericarp from the grain. Hubbard et al. (1950), according to Freeman and Watson (1969) used hexane to extract wax from sorghum grain and found that sorghum contained 0.24-0.44% wax. Table 8 shows that first de-waxing removed 0.33% and 0.37% hexane-soluble substances, which should include wax (Kolattukudy and Espelie, 1985), from SORG 1 and SORG 2 respectively. There was a large decrease in the amount of hexane-soluble substances when hexane was applied to the grain for the second time, which was a clear indication that hexane dissolved the waxy material which overlies the pericarp (Kent and Evers. 1994). Thus it appears that de-waxing allowed enzyme access to the pericarp cell walls. Scraping the grain exposed the inner grain tissues (Figure 16) and hence had the same effect as de-waxing. Because the pericarp walls were exposed either due to de-waxing or scraping the grain, endo-β-glucanase hydrolysed the cellulose and mixed linkage 1,3 and 1,4 β-glucans in the pericarp cell walls, and thus caused disruption of the integrity of the pericarp and hence its removal from the grain.

The result that xylanase had no effect on sorghum grain that had either been scraped or de-waxed (Figure 15 and Table 6) was surprising. According to Serna-Saldívar and Rooney (1995), hemicelluloses (predominantly arabinoxylans) are second to cellulose in quantity in the cell wall of sorghum grain. In this work, it was expected that xylanase would hydrolyse the arabinoxylans and thus contribute to the degradation of the cell walls of the bran layers. Dale, Harris and Liebenberg (1990) reported that a mixture of industrial cellulases and hemicellulases caused reduction of mash viscosity when the enzyme solution was added to mash whose grits contained a high proportion of raw sorghum. The results suggested that hemicellulases acting in combination with cellulases degraded the non-starch polysaccharides in the cell wall of sorghum grain. Elsewhere in the literature (for example Taylor. 1991; Bigelis. 1993), it is stated that hemicellulases are used in food processing to
hydrolyse hemicelluloses. The ineffectiveness of xylanase in this work was perhaps due to the fact that this particular xylanase was specifically developed for use in bread making (Van Aswegen, Technical Director, Enzymes SA, personal communication, February 1999). According to Hoseney (1994), hemicelluloses of coarse cereals (maize, sorghum, and millets) are chemically more complicated than hemicelluloses found in wheat. Verbruggen Beldman, Voragen and Hollemans (1993) reported that alkali-extractable arabinoxylans of sorghum were highly substituted and were composed mainly of arabinose, xylose, glucose and uronic acids. Verbruggen, Beldman and Voragen (1995) found that water-unextractable hemicelluloses in sorghum contained phenolic acids such as coumaric acid and ferulic acid, which may be involved in oxidative cross-linking. Chemical complexity might contribute to resistance to enzymic hydrolysis.

It was also surprising that enzymes had no effect on the germ. It was expected that, because a structural lipoprotein membrane surrounds the germ (Salisbury and Ross, 1992), a lipase would hydrolyse the lipid-rich membrane releasing some fatty acids, which would exude out. Endo-β-glucanase would hydrolyse the non-starch polysaccharides (cellulose and β-glucans) and xylanase, arabinoxylans, in the structural cell walls, and thus cause loosening of the germ. The germ would then fall off the grain. The negative results were perhaps due to the fact that a thick waxy cuticle on the seed coats of sorghum grains (Kent and Evers, 1994) acted as a barrier to enzyme access to the germ. Most likely, the main reason for the germ not to be removed from the grain is its relative position in the grain. The germ/endosperm interface is at right angles to the surface of the grain, thus the enzymes have only a small area to act on and much of the underlying interface is not accessible.

It was also important to note that combined enzymes appeared to somehow inhibit each other (Table 6). For example a combination of xylanase with any of the other enzymes always gave a negative result. Substances such as surfactants applied to each enzyme could be incompatible with the other enzymes (Van Aswegen, Technical Director.
Enzymes SA, personal communication, February 1999).

It appears that because of the relative position of the germ as described previously, de-waxing, decortication with endo-β-glucanase followed by a second de-waxing step, or scraping the germ area of a grain that had been decorticated with endo-β-glucanase, could not result in removal of the germ of both sorghums. However, it was interesting to note that some enzymes and enzyme combinations caused slight hydrolysis of the germ (Table 7). Lipase was the only enzyme that slightly hydrolysed the germ when it was applied singly. That can be attributed to the fact that the germ is predominantly lipid (Serna-Saldívar and Rooney, 1995) and thus there was a high substrate concentration for the lipase. An enzymic reaction is enhanced by a high substrate concentration (Palmer, 1995). A combination of endo-β-glucanase and pectinase caused slight hydrolysis of the germ and that should have been due to their concerted hydrolytic action on cellulose and possibly β-glucans. and pectic substances in the cell walls of the germ. The results show that neither of the two enzymes (endo-β-glucanase and pectinase) could hydrolyse the germ alone probably due to the integral association of the mentioned structural macromolecules. Combinations of endo-β-glucanase and lipase, and endo-β-glucanase, lipase and pectinase also caused slight hydrolysis of the germ, perhaps because of the same reason that the enzymes complemented one another in degrading the structural polymers in the germ. However, it appears that xylanase somehow inhibited other enzymes.

The goal of this work was to develop an enzymic process that would remove the outer bran layers and the germ from sorghum grain in a similar manner as milling, but more effectively. The results obtained, however, show that only the pericarp can be removed using endo-β-glucanase. Enzymic removal of the pericarp from sorghum grain would be beneficial especially in the processing of soft sorghum grain. As mentioned earlier. soft grain is hard to mill because the meal obtained thereof is contaminated with bran. On the
other hand it would be easier to cleanly separate the pericarp from the endosperm of a soft grain. In soft grain, the pericarp would peel off easily when the enzyme is applied.

To make the enzymic removal of the pericarp from sorghum grain economical, it would be necessary to establish the minimum enzyme: substrate (grain) ratio required to remove the pericarp to an acceptable level while there is as minimum as possible endosperm (meal) loss. An enzyme driven process is economical at about 0.1-1% enzyme dosage (Sheppard, 1986).

According to Kent and Evers (1994), ash content and colour of a pearled grain or its flour can be used to measure the effectiveness of the milling process because, for example in sorghum about 80% of the grain ash is located in the germ and pericarp (Serna-Saldivar and Rooney. 1995) and consumers of sorghum generally prefer products that are white or light (DeSikachar, 1982). Similarly, ash and colour may be used to indicate that the pericarp has been removed from sorghum grain to an acceptable level by the enzyme.

Figure 19 shows that, for both sorghums, it was beneficial to increase endo-β-glucanase concentration to about 1% because more pericarp was removed as the enzyme concentration was increased as indicated by a progressive decrease in the ash content of the grain. It appears that it would not be economical to increase enzyme concentration above 1% because there would be a negligible removal of the pericarp as indicated by an almost constant ash content of the grain.

Figure 20 shows that increasing enzyme concentration had an adverse effect in that more grain material was lost as indicated by a progressive decrease in yield. The grain material lost would include the unwanted pericarp plus some endosperm associated with it. It appears that the maximum loss in grain material was about 15% at about 1%
endo-β-glucanase concentration for both sorghums. That maximum loss in grain material would be greatly smaller than about 40% loss (Bassey and Schmidt, 1989) due to mechanical decortication.

Figure 21 shows that increasing enzyme concentration was beneficial in that the grain became progressively lighter and less red. An increase in the Hunter L value is indicative of an increase in lightness whilst decreasing Hunter a values show a decrease in redness (Deman, 1980). The grain became progressively lighter because more pericarp, which was red, was removed as the enzyme concentration was increased. The Hunter values also suggest that it would not be economical to increase enzyme concentration above 1% because the redness of the grain would remain more or less constant for both sorghums. Increasing enzyme concentration had no effect on the Hunter b values because sorghum grain is neither yellow nor blue.

The results shown on Figures 19, 20 and 21 indicate that more pericarp was removed as endo-β-glucanase concentration was increased and that agrees with the accepted principle that an increase in enzyme concentration tends to enhance an enzymic reaction (Palmer, 1995). The pericarp was persistent at the hilum area (Figure 18) probably because it was associated with some complex substances that are involved in attachment of the grain to the funiculus (Serna-Saldivar and Rooney, 1995).

The results in Figures 19, 20 and 21 show that a good decortication effect was achieved at an endo-β-glucanase concentration of about 0.01% (ml/100 g grain). If ash content is used as an indicator of the effectiveness of enzymic decortication, the ash content (about 1.1%) (Figure 19) of the enzyme-decorticated sorghums obtained at about 0.01% enzyme is acceptable because Kent and Evers (1994) stated that the ash content of pearled sorghum should be about 1.2%. Therefore, the results suggest that 0.01% enzyme would be optimal for enzyme removal of the pericarp. That converts to 100 ml enzyme/ton grain. Interestingly, a dosage of 0.01% enzyme is considered economical (Sheppard,
Figure 22 shows that, for both sorghums, enzymic decortication was more effective than mechanical decortication because to achieve about 1.1% grain ash (dry basis), there was less loss in grain material, indicated by higher yields, when the enzymic decortication was used. As has been stated earlier, the accepted ash level in pearled sorghum is 1.2% (dry basis) (Kent and Evers, 1994). For example, to reduce the ash content of SORG 1 to about 1.1% ash (dry basis) about 15% and 25% loss in grain material was incurred when the enzymic and the mechanical processes were applied, respectively. It appears, therefore, that enzymic decortication of sorghum grain would result in about 10% saving in grain loss relative to mechanical decortication. The apparent effectiveness of the enzymic process may be attributed to the way in which it removes the bran material from the grain. Figure 16A shows that endo-β-glucanase caused decortication of the grain at the seed coat/aleurone layer interface. It appears that because endo-β-glucanase is substrate-specific, it causes a clean separation of the endosperm and the pericarp and hence there is a lower loss in grain material. On the other hand, mechanical decortication results in poor separation of the bran from the endosperm (Hoseney, 1994).

Figures 23 and 24 show that the product obtained when mechanical decortication was applied was of better sensory quality because it was lighter and less red. DeSikachar (1982) stated that consumers of sorghum generally prefer products that are white or light. For example, Figure 23 shows that, for both sorghums, to obtain about 85% decortication yield, the approximate yield of the optimised enzymic process, the Hunter L values were about 35 and 45 for enzyme and mechanical decortication, respectively. The relationship between grain colour and decortication yield seems not to agree with the relationship between grain ash content and decortication yield. As stated earlier the relationship between grain ash content and decortication yield suggests that the enzymic decortication removed more pericarp than the mechanical decortication. On the contrary, the relationship between grain colour and decortication yield suggests that the mechanical decortication removed more pericarp than the enzymic decortication. However, the
contradiction may be accounted for by the colour of the enzyme. The endo-β-glucanase used was pigmented. Its brownish colour was persistent after rinsing the enzyme-treated grain. The lowest Hunter L values (Figure 23) for both methods of decortication are for the controls. The control for the enzymic process was sorghum grain that had been partially decorticated but without enzymic treatment. The control for mechanical decortication was whole sorghum grain. It was expected therefore that the control for the enzymic process would give a higher Hunter L value, due to partial removal of the pericarp, than the control for the mechanical process. However, because of the pigmenting effect of the enzyme, the control for the mechanical process was lighter.

Although enzymic decortication yields were economically feasible at an endo-β-glucanase concentration of about 0.01% (ml/100 g grain), the product would probably not be acceptable to the consumers because of its inferior colour.
CONCLUSIONS AND RECOMMENDATIONS

Hydrolytic enzymes (endo-β-glucanase, xylanase, lipase and pectinase), when applied either singly or in combination, have no effect on the pericarp and germ of sorghum grain under semiwet conditions. It appears that the enzymes must be diluted to increase their mobility and that the grain needs to be hydrated to allow enzyme access to the substrate by diffusion.

Endo-β-glucanase causes decortication of sorghum grain at the seed coat/aleurone layer interface. The result is obtained when the grain is either de-waxed or scraped, the conditions necessary are incubation in a shaking water bath at 50°C overnight. Either de-waxing or scraping the grain are necessary to remove the waxy cuticle on the pericarp and seed coats which acts as a barrier to enzyme access to the cell walls of the pericarp. Other enzymes (xylanase, lipase and pectinase) have no effect on the sorghum grain, and it is likely that the chemical complexity of arabinoxylans which are found in the pericarp cell walls contributes to resistance to hydrolysis by xylanase. Generally, combined enzymes appear to somehow inhibit each other and hence have no effect on the sorghum grain under the described experimental conditions. Either single or combined enzymes (endo-β-glucanase, xylanase, lipase and pectinase) do not remove the germ from the sorghum grain. The germ can not be removed perhaps due to the fact that, in sorghum grain the germ/endsosperm interface is at right angles to the surface of the grain, thus enzymes have only a small area to act on and all the underlying interface is not accessible.

Increasing endo-β-glucanase concentration from 0.1% (ml/100 g grain) results in a progressive decrease in the pericarp content of partially decorticated sorghum grain as shown by a progressive decrease in grain ash, grain redness and an increase in grain lightness. The ash content of the two sorghums drops to just below 1.2% (dry basis) at
0.01% endo-\( \beta \)-glucanase concentration (ml/100 g grain), the maximum allowed for pearled sorghum, suggesting that that concentration is optimal for the enzymic removal of the pericarp.

If grain ash is used to indicate the effectiveness of decortication, enzyme decortication appears more effective than machine decortication because in order to reduce grain ash to an acceptable level (about 1.1%, dry basis) the enzymic process saves about 10% loss in grain material relative to the mechanical process. Enzymic decorticating is more effective than the mechanical process because the enzyme cleanly separates the pericarp and endosperm at the seed coat/aleurone layer interface. However, at the same decortication yield, the machine-decorticated sorghum is lighter and less red than the enzyme-decorticated sorghum. The inferior colour of the enzyme-decorticated product was perhaps due to that the endo-\( \beta \)-glucanase used was pigmented. A non-pigmented enzyme should be used.

It appears that endo-\( \beta \)-glucanase can remove the pericarp from sorghum grain at an economically feasible concentration (0.01%), and that there is significant reduction of grain material loss relative to the mechanical process. However, more work needs to be done to improve the colour of the product, and to find a way to remove the germ.
CHAPTER 7

REFERENCES


