CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

1.1 INTRODUCTION

Maize (*Zea mays)(L)* is the staple food of many African countries and many types of maize are grown around the world. Maize is indigenous to the Americas. It is an annual plant belonging to the grass family and it is a warm season crop requiring warmer growing temperatures than the small grains (for example wheat). The United States is the biggest producer of maize in the world with 200 million tons per annum, followed by China with 92 million tons per annum (FAO 1999). In Africa, South Africa is the biggest producer of maize with an annual production of approximately 10 million tons, but depending on the rainfall, it can vary from as little as 2.9 million tons in the 1991/92 season (a severe drought year) to as high as 14.4 million tons in the 1980/81 season (National Department of Agriculture 2001). Of the production, an average of 3.2 million tons is milled by the dry milling industry. The milled products are mainly used for human consumption with maize meal (super and special maize meal) being the largest product (National Department of Agriculture 2001).

There are five general classes of maize, namely flint, popcorn, flour, dent and sweet corn. This classification is based on kernel characteristics (Benson and Pearce 1987). Dent maize was developed by hybridization between flint and flour types. It is the predominant type used in the South African dry maize milling industry (Maree and Bruwer 1998).

The maize kernel contains two major fractions of endosperm, namely a vitreous or semi-transparent fraction usually of greater “strength” or resistance to breakage and an opaque, floury fraction that disintegrates easily due to shear forces during milling (Chandrashekar and Mazhar 1999). The difference between the two types of endosperm is attributed to differences in their cellular and biochemical structures. The starch granules are compact and polygonal in the vitreous endosperm, giving rise to a “flinty” appearance, also described as semi-transparent or more accurately, translucent, allowing the transmittance of light. In the floury endosperm, the granules are spherical with intervening air spaces causing diffraction of light and an opaque appearance (Watson 1987a).

The end-use quality of maize cultivars can be influenced by many factors that induce variation. Some of these factors are the effects of the harvest conditions, soil conditions, cultivar, mechanical conditions during transport. There are many others. Variation is also caused by factors such as heterogeneity in maize within a cultivar and even on the same ear (Wolf, Buzan, MacMasters and Rist 1952; Watson 1987a). In industrial milling, the cultivars are usually mixed as well causing further variation.

In South Africa, where porridge made from dry milled white maize meal is a staple food, maize millers optimize for maximum yield of clean vitreous endosperm during milling (Fowler 1993). Near Infrared Reflectance (NIR) correlated with milling resistance has been used as a guide to select maize types suitable for milling. However, it did not produce consistent results in terms of milling performance and extraction of vitreous endosperm. The results were specifically inconsistent over more than one season. This resulted in a new effort to develop methods for predicting the performance of South African maize (personal communication, Randall, P.G., Director, P Cubed). The need for a better understanding of the endosperm properties of South African maize was identified by the South African milling industry, along with the need for development of a rapid, non-destructive, on-line detection method suitable for characterization of maize for milling-milling in terms of clean vitreous endosperm yield (personal communication, Viljoen, A., Research and Development Manager, Tiger Milling and Baking). Currently, work is also done to recalibrate the Near Infra-red Transmittance (NIT) method used in South Africa against a small-scale milling test to determine the milling performance of maize instead of using a milling resistance test such as the test described by Vorwerck and Miecke (1973), which was used for the initial calibrations (personal communication, Randall, P.G., Director, P Cubed).

Worldwide, several types of methods have been investigated for quantitatively predicting the milling performance of maize. These methods can be divided into five categories namely:

- Methods measuring the resistance to milling or crushing
- Milling simulation tests on a small or larger-scale using grinding, sieving and weighing of the various fractions
- Methods measuring a physical property, such as kernel density or translucency and correlating the method with milling yield
- Estimation of the vitreous/floury ratio by hand-dissection
- Estimation of the vitreous/floury ratio by visual examination on cut kernel surfaces (including the use of machine vision technology) (Watson 1987a).

Few of the methods investigated so far conform to the criteria of being rapid, non-destructive and on-line. Near Infrared Transmittance is one of a few non-destructive tests, but it is difficult to calibrate especially within a industrial milling environment. This is due to insufficient understanding of the actual relationship between the transmittance measurements and the desired quality specifications required in the milling industry (personal communication, Viljoen, A., Research and Development Manager, Tiger Milling and Baking).

Translucency is defined according to Sykes (1983) as "the transmittance of light, but not the same as transparency". The translucency of vitreous maize endosperm, although well known as a physical property, has not developed yet as an analytical tool for predicting milling yield. There are very few references in the literature and none of the research investigated the potential to correlate translucency with milling performance. The measurement of translucency has the potential to be developed as a rapid non-destructive method using Image Analysis (IA), with a potential to be used on-line if the image analyzer and associated systems can be incorporated into the grain mill stream.

The terminology describing maize milling properties is not well defined with the terms "hardness", "vitreousness", "horny", "translucency" and others used interchangeably throughout the literature. For clarity in this thesis, the term vitreousness will be defined as the yield of the visible vitreous or "glassy" endosperm after separation of the vitreous and opaque endosperm by hand-dissection, expressed as a mass percentage or as a surface area percentage in cut kernels. The term translucency will be used to describe the semi-transparent appearance of maize endosperm that permits the transmittance of light and that can be detected and quantified by a camera or light detector and image analyser. Maize kernels may have similar amounts of vitreous endosperm, but due to various other factors, their respective translucencies may differ, as translucency can also be influenced by the absorption of light inside the vitreous endosperm. Light can be absorbed by colour pigments, and also scattered due to small differences in the three-dimensional structures of the germ and opaque endosperm portions in relation to the vitreous portion. Biochemical differences such as small differences between the ratio of starch granules to protein matrix structures can also potentially influence the actual measured translucency. Translucency can, however, be detected on whole maize kernels and is a non-destructive physical property.

The term "hardness", although widely used also to refer to vitreousness, will be referred to in terms of "grain strength" according to the definition provided by Chandrashekar and Mazhar (1999). Grain strength was not tested in this study as it encompasses another research field relating to aspects such as milling resistance and other mechanical properties such as stress/strain relationships. Grain strength tests, such as the Stenvert Hardness Test described by Pomeranz, Czuchajowska, Martin and Lai (1985) are all destructive. They were excluded as possible candidates for evaluation to find a non-destructive methods for this study.

Milling yield will be defined in terms of the yield of the various classes of end-products such as "semolina", "super maize meal" and "flaking grits" that are derived from the vitreous portions of the maize kernel during milling. The vitreous portions constitute the higher priced products such as flaking grits

(Paulsen and Hill 1985). The terms used to describe the end products differ between countries for example “semolina” (Germany) and “super maize meal” (South Africa) are essentially the same products. It is preferred to define these products in terms of composition and particle size index, in order to compare data between mills and countries.

To develop a non-destructive Image Analysis (IA) test for translucency, fundamental research is needed to understand the variability of measurements among maize kernels and to develop a standardized method for evaluation. Factors that will influence the accuracy of translucency measurements may include:

- Kernel shape, sphericity and thickness
- Illumination strength and type of the light source
- Ratio of light source size to kernel size
- Kernel thickness
- Size of germ and tip cap
- Position of germ and tip cap during measurements
- Overexposure due to light passing around the sides of the kernels (requiring a need to block excess light to create sufficient contrast)
- Variations amongst cultivars
- Ratio of vitreous/opaque endosperm
- Colour of the endosperm and pericarp
- Damaged kernels (percentage).

1.2 PROBLEM STATEMENT

The following points summarise the problem:

- A need exists for a rapid, non-destructive test to characterize maize kernels in terms of milling performance
- The rapid test must be potentially suitable for on-line testing of large quantities of maize in a rapid accurate manner
- The test must preferably be non-destructive, in order to reduce human error and to allow seed breeders to use the test as a selection method for which only a tiny quantity of kernels are available during the breeding programme
- Aspects such as “hardness”, “vitreousness”, “translucency” and “strength” are often used as synonyms, while they are, in fact, different properties and the relationships between them are not well understood.
- The translucency of maize kernels can potentially be related to vitreousness and provides a possibility of being used as a quantitative, non-destructive analytical tool to predict milling performance.
- Data describing the correlation between translucency and milling performance or yield of endosperm as determined by dissection methods are not available.

1.3 OBJECTIVES

- To optimize the non-destructive measurement of translucency as a physical property of maize kernel
- To develop a non-destructive measurement technique for translucency using IA, preferably with regard to sample preparation other than cleaning (to remove damaged kernels), including taking into account the need to exclude light passing around the kernels causing a decrease in contrast during detection
- To correlate the translucency measurements with maize vitreousness (yield of vitreous endosperm) using hand dissection
- To develop correction factors for translucency, taking into account factors such as kernel size and thickness variations influencing exposure and detection levels (to be programmed into computer software for future applications)
- To apply the developed technique, including the application of the correction factors, to actual milling trials in order to verify its potential as a predictor of milling performance.

CHAPTER 2: LITERATURE REVIEW

2.1 MORPHOLOGY OF THE MAIZE KERNEL

The maize kernel is described by Barling (1963) as a large naked caryopsis with a broad apex and narrow base, often still attached to a short stalk (known today as the tip cap). The embryo can be seen through the fused pericarp and testa lying against one face. The rest of the maize kernel was described as being filled with endosperm that may be of varying colour and character.

Dent maize, which is commonly used in the milling industry, has a large flattened kernel. It is the largest of the common cereal kernels, weighing an average of 350 mg. The basic structure of the kernel is shown in Figure 2.1 (Hoseney 1994). The maize kernel is quite variable in colour, ranging from white to dark brown or purple. White and yellow are the most common colours. The pericarp and tip cap together constitutes about 5 to 6% of the kernel. The germ (which is relatively large) makes up 10 to 14% of the kernel and the remainder is the endosperm. Maize contains two types of endosperm in the same kernel. The cellular structures of the two endosperm types are shown in Figure 2.2 (Hoseney 1994).

The bond between the protein and starch in maize kernel endosperm is quite strong. Water alone will not allow for an adequate separation of protein and starch during wet milling. The endosperm cells are large, with thin cell walls and a noticeable difference exists between the two types, known as the vitreous (glassy in appearance) and opaque (mealy in appearance) endosperms (Hoseney 1994). Vitreous endosperm is also frequently referred to as “horny” (Hoseney 1994). While the vitreous endosperm contains tightly fitted polygonally shaped starch granules embedded in a rigid protein matrix, the opaque endosperm contains of loosely fitted spherical granules within thin papery filaments of protein with many air spaces between them and thereby causing opacity. Although the opaque endosperm part of dent maize

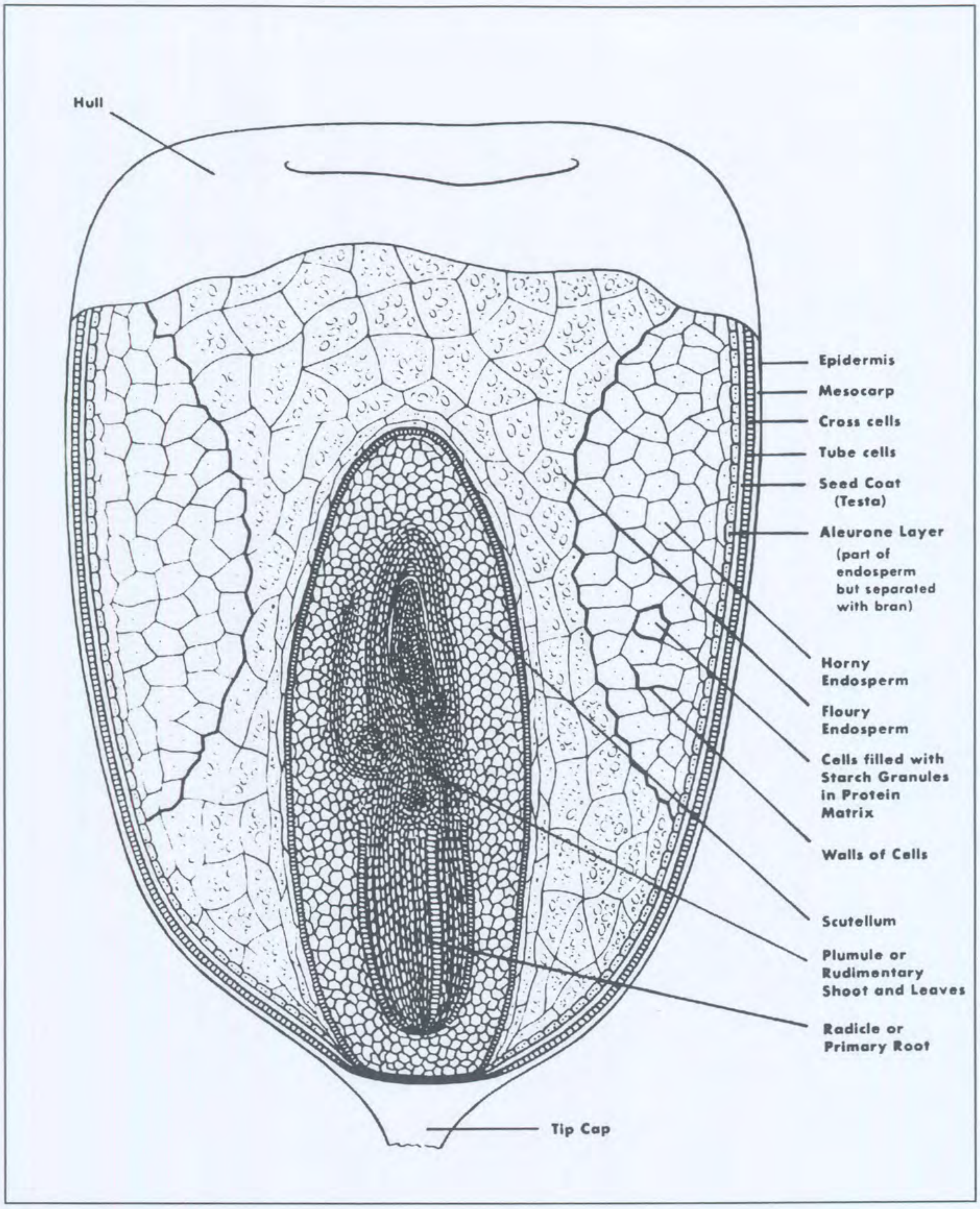

Figure 2.1 Longitudinal section of a dent maize kernel showing the morphology and the different endosperm types (Hoseney 1994)

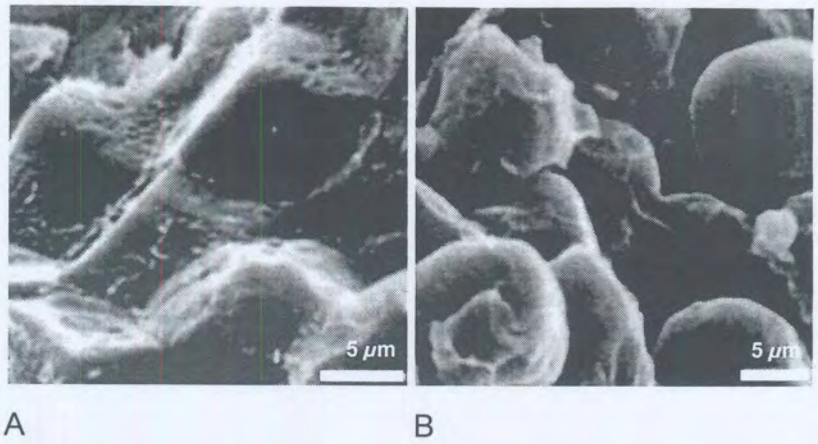

Figure 2.2 Scanning electron micrographs of maize vitreous (A) and opaque (B) endosperm. Note tightly packed polygonal starch granules (A) versus loose round granules (B) (Hoseney 1994)

appears to be similar to the endosperm of opaque floury maize mutants containing no vitreous endosperm, it is controversial to assume that these opaque endosperm types are similar (Hoseney 1994).

Maize prolamin proteins (zeins) are related to the structure of the two types of endosperm and many authors such as Pratt, Paulis, Miller, Nelsen and Bietz (1995), Mestres and Matencio (1996), Chandrashekar and Mazhar (1999), and Dombrink-Kurtzman and Bietz (1993) have reported relationships between vitreous and opaque endosperm and the proportions of zein types in each. It is generally agreed that the contents of α-zein and β-zein are both important in grain endosperm structure, both having an effect on the appearance of the endosperm and the grain strength (in terms of milling resistance) resulting from the way the starch granules are packed within the various protein matrixes (Chandrashekar and Mazhar, 1999). The role of γ-zein in grain strength or friability, as measured by starch damage was described by Mestres and Matencio (1996). According to these authors, it has been suggested that vitreousness is related to the proportion (%) of two isolated γ-zein fractions (27 kDa and 16 kDa). In the same work, kernel vitreousness was demonstrated to be specifically linked to the 16 kDa fraction and not the 27 kDa fraction. The 16 kDa protein fraction also correlated with coarse maize grit yield. Mestres and Matencio (1996) also demonstrated that α-zein did not have a significant correlation with vitreousness, but was positively correlated, along with the yield of salt extractable proteins, to the milling characteristics of the maize kernels in terms of friability. A strong inverse correlation was also found between damaged starch determined on the ground products passing through the 315 μm sieve and kernel friability. Friability was defined as the proportion (%) of milled maize kernels passing through a 315 μm sieve after samples were milled using a pilot roller mill.

In contrast with Mestres and Matencio (1996), Dombrink-Kurtzman and Bietz (1993) and Robutti, Borras and Eyherabide (1997) both showed that there was more α-zein (19 and 22 kDa) in vitreous endosperm fractions than in opaque endosperm fractions. Dombrink-Kurtzman and Bietz (1993) also showed that opaque endosperm contained nearly twice as much 27 kDa γ-zein than vitreous endosperm fractions. This observation is similar to the findings of Mestres and Matencio (1996). Dombrink-Kurtzman and Bietz (1993) also concluded that the distribution of the various types of zein was not uniform throughout the maize endosperm. The zeins and their distribution in the endosperm therefore determine the final shape of the starchy endosperm protein bodies, the organelles of zein protein storage. The protein bodies dictate the morphology of the starch granules. Two types exist, namely a tightly packed polygonal shape in the vitreous endosperm and a loosely packed round shape with interstitial air pockets in the opaque endosperm (Dombrink-Kurtzman and Bietz 1993; Chandrashekar and Mazhar 1999).

Vitreousness is a dominant genetic trait somehow linked to zein composition and structure of protein bodies, while floury endosperm is produced by recessive genes (Watson 1987b; Dombrink-Kurtzman and Bietz 1993). The exact nature of the genetic inheritance is also not clear. Chandrashekar and Mazhar (1999) has proposed the existence of a “master gene” which may control an array of seemingly unrelated biochemical changes in the kernels such as protein composition, protein body formation, cell wall structure and starch granule development. A combination of all these structural developments in the kernel may have one common goal, namely the development of a maize kernel with either vitreous or opaque endosperm depending on the genetic code of that cultivar of maize.

It seems as if consensus among various authors with regard to the exact role of the different prolamin fractions in relation to endosperm vitreousness has not been reached yet. Chandrashekar and Mazhar (1999) proposed that the γ-zeins are deposited first during kernel development and the α-zeins are then secreted into "pockets" of γ-zeins, which are rich in the sulphur-containing amino acid cysteine and is capable of forming disulphide bonds resulting in increased vitreousness of the endosperm. There is evidence showing that the vitreous portions of endosperm have more cell-wall matrix available for housing the protein bodies and the matrix protein around the protein bodies are more readily linked with disulphide bonds if they are in close proximity to each other, resulting in a flinty or vitreous endosperm (Chandrashekar and Mazhar 1999). However, on the basis of different findings from authors such as Dombrink-Kurtzman and Bietz (1993) and Mestres and Matencio (1993), the understanding of the biochemical basis for explaining maize endosperm morphology and its implications to processing properties such as the yield of a specific product during milling is not yet clear. When maize kernels are illuminated by putting them on top of a light box comprising a light source underneath a ground glass or Perspex screen, a system referred to as candling, a range of visible endosperm distribution patterns can be detected (Paez, Helm and Zuber 1968; Bauman 1971). Figure 2.3 (Watson 1987a) shows the appearance of candled maize when evaluating for stress cracks. The vitreous endosperm tends to be translucent, allowing light to pass through, while the opaque endosperm appears black as it does not allow light to pass through. The two different types of endosperm are clearly visible, as well as some stress cracks in this case. Stress cracks are a phenomenon generally occurring in vitreous endosperm when maize kernels are subjected to excessive stress during artificial drying (Watson 1987a).

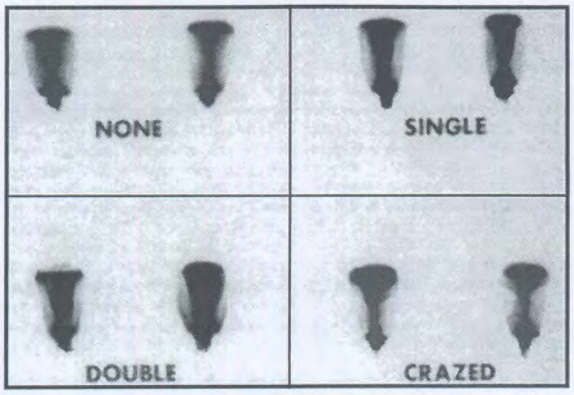

Figure 2.3 Maize kernels placed on top of a light box showing translucent and opaque parts. Maize kernels are also showing stress cracks which can be single, double or multiple (crazed) (Watson 1987a)

The term translucency is described as the passing of light through a material, but with diffusion in such a way that objects behind the translucent material cannot be seen. This differs from the term transparency, the passing of light through, but without diffusion so that it enables objects behind the material to be clearly visible. Window glass can be transparent when no light diffusion occurs or translucent when the surface is treated to cause light diffusion, for example by sand blasting.

The energy of light passing through any optical medium is partially absorbed, increasing the internal energy in the material and correspondingly decreasing the intensity of the light. The decrease in the intensity of the light is proportional to the initial light intensity and to the thickness of the material. The absorption coefficient can be calculated for a material according to Lambert's law (Sears, Zemansky and Young 1982). The absorption coefficient is also often wavelength dependent and can also be influenced by the polarization of the incident light. A beam of light passing through an optical medium may also be attenuated by scattering. In contrast to absorption, in which the energy is ordinarily converted to internal energy, scattering simply redirects some of the radiation into directions other than that of the beam. Scattering of light is wavelength dependent (Sears, Zemansky and Young 1982). As the maize kernel is a chemically and physically complex structure as shown in Figure 2.1, every morphological part will have an effect on the absorption and scattering of a light beam shining through the kernels.

The appearance of cereal grain vitreous endosperm shows similarity with true glass transition found in super cooled liquids exhibiting solid properties at temperatures below their melting points (for example in window panes and hard boiled sweets). In the glass transition state, molecules are randomly tightly packed, but not crystallized, because viscosity is sufficiently high to prevent crystallization (Atkins 1987). In the case of polymers, such as protein or starch, the glass transition state is reached when there is a step change in molecular mobility in the amorphous phase of the polymer. Material in the amorphous phase is rigid below the glass transition temperature and rubbery above it. Amorphous materials flow, they do not melt. The glass transition temperature or T_g is the temperature at which a supersaturated solution or amorphous liquid converts to a glass and it is observed in substances that contain significant regions of amorphous or partially amorphous material. This includes foods and food tissues (Fennema, 1996).

The vitreous endosperm has a structure consisting of tightly packed highly organized cells (Watson 1987a). However, inside the cells the prolamin proteins are tightly packed and in the rigid phase resembling the glassy state of the polymer.

During drying, the glass transition temperature of a food material increases as water is removed, approaching the glass transition temperature of the pure substance (Fennema, 1996). During the filling of maize kernel cells in the development stages with protein molecules, the protein will be in an amorphous state if the cells are tightly packed.

The glass transition temperature of zein protein is 30°C at a moisture content of 15%. The glass transition temperature increases exponentially with decreasing moisture content (Lawton, 1992). During slow drying of the developing kernels on the land, the protein will be in a glassy state if the glass transition temperature is above the environmental temperature when the protein is in an amorphous form. Therefore, the protein in the vitreous endosperm is in the glassy state and has a vitreous appearance. In the case of the opaque endosperm, less protein is present, giving rise to a less tight packing structure, allowing the protein to become organized and not amorphous. The protein will therefore not be in the glassy state.

Water can act as a plasticiser in food products thereby decreasing the T_g and increasing free volume. This action will result in increased molecular mobility both above and below T_g. Water must be absorbed in the amorphous regions to be effective as a plasticiser (Fennema, 1996). The increased mobility will result into a product that will become more rubbery and will not shatter easily, which is typically found after conditioning of dry maize kernels (adding water) before milling.

The selection of the term vitreousness to describe the state of the endosperm instead of using a mechanical property such as “hardness”, is therefore preferred as it describes the appearance of the endosperm in its glassy state.

2.2 OBJECTIVES OF MAIZE DRY MILLING

Kent (1984) described the objectives of dry maize milling as being: to obtain the maximum yield of maize grits with the least possible contamination with fat and black specks of tip cap and to recover as much as possible of the remainder of the endosperm as meal, while making the minimum amount of flour, and to recover the maximum amount of germ in the form of large clean particles with the maximum oil content.

The maize dry milling process has been described by Fowler (1993) as a complex series of repetitions of grinding and sieving operations designed to achieve the following objectives:

- To separate the primary raw material, which is the starchy endosperm, from the maize kernel while minimising contamination of this material by the germ and seed coat fractions. Maize germ and seed coat material consisting of the pericarp, mesocarp, aleurone layer and tip cap are by-products of maize milling and when combined, are referred to in the maize milling trade as "hominy chop".

- To reduce the pure endosperm material in size by grinding it to a predetermined granularity or fineness.
- To separate and isolate reduced endosperm material by sifting it into predetermined classes based on particle size.
- To maximise the yield of endosperm and minimise bran contamination. Bran refers to the pericarp-containing product of the dry milling process and it also includes tip cap, aleurone layer and some adhering pieces of starchy endosperm (Watson, 1987a). Adhering endosperm must be limited as much as possible as it results in product loss.

Gerstenkorn (1991) described the objective of maize dry milling as the maximising of the yield of grits from the endosperm, having a specific particle size and a fat content of less than 0.9% on a dry basis.

The various definitions describing the dry milling of maize all have one common main theme, that is maximisation of the yield of clean value-added products from the maize kernels. The main focus is to obtain clean endosperm and more specifically, clean vitreous endosperm. Vitreous endosperm is the primary product used for producing a whole range of other products, such as maize grits of various particle size distributions and maize meals.

Maize milling may or may not include de-germing as a preliminary step. Non de-germing dry milling is carried out in small grist mills or some modern larger roller mills using a combination of coarse-fluted rollers, specialized sifters, air classification and gravitational separation equipment (known as "purifiers") along with the refining stages containing a series of rollers (with finer flutes), plan sifters and aspirators. The maize is ground to make a coarse meal with some separation of the bran and germ, but the endosperm products are usually contaminated by oil at levels of 2% and higher.

In the de-germing process used in most large industrial mills, the first objective is to separate the germ and most of the bran (pericarp material) from the remainder of the grain with a minimum of contamination by oil and bran. Degerming is done after tempering (conditioning) of the maize using water addition. The added water is used to soften the pericarp and germ, but without softening the endosperm. After degerming, the dry milling system employs roller mills and plansifters to gradually reduce the particle size of the cleaned vitreous endosperm grits, accompanied by further cleaning (Kent 1984). Such a process results in endosperm-derived products of a low fat content, usually less than 1.5% (Fowler 1993).

The primary products derived from the tempering-degerming process are maize grits, maize meals and maize flours. An almost infinite number of products can be made as a result of the particle size reduction on the roller mills. Most of the products can, however, be classified into six classes based on their particle size distribution and composition. These classes are described in Table 2.1 (Kent 1984; Alexander 1987; Fowler 1993).

Table 2.1 Classes of maize products obtained from the tempering-degerming dry milling system

Product, (South African name)	**Description of product and synonyms in literature**	**Yield (weight %)**	**Particle size distribution (mm)**	**Fat (%)**	**Protein (%)**	**Starch (%)**	**Moisture (%)**
Samp	Flaking grits (large grits used for breakfast cereal manufacture)	12*	3.4–5.8	0.8	9.0	84.0	12.0
Maize rice	Coarse grits	15*	2.0–1.4	0.8	9.0	84.0	12.5
Grits	Regular grits, usually medium or fine (sometimes also called "semolina")	23*	medium: 1.0–1.4 fine: 0.65–1.0 **	0.7	9.0	82.0	12.0
Super maize meal	Maize meal or "cornmeal"	10*	0.3–0.65	1.0	9.0	80.0	13.0
Special maize meal	Fine meal or "coarse cones"	10***	0.17–0.3	2.3	9.2	80.0	13.5
Flour	Maize flour or "corn flour"	5	below 0.17	1.8	8.7	80.0	13.2
Germ	A mixture of bran and germ, also called "hominy feed"	24	0.5–6.7	7.8	9.0	43.8	14.0

* By adding together the yield of these four "super" products, a total yield of 60% is achieved. The composition of these products is similar because they are mainly derived from reducing the particle size of the primary product, which is the flaking grits. In some instances, companies will produce as much as possible flaking grits with a yield of more than 50%, without further reduction to produce flour, depending on the market. Therefore, the yield percentages of these four products will vary between mills, but if added up, will average at 60% (Fowler 1993).

** Also referred to in the literature as "semolina"

*** Depending on the market need, this fraction can also be added to the first four fractions to yield a meal with a slightly higher fat content.

In South Africa, where the staple food for a large part of the population consists of a porridge made from super or special maize meal, the grits are used mainly for the production of these meals. A typical yield of super maize meal for a South African mill is 58%. Super maize meal is manufactured by reducing the particle size of the three "primary" products namely samp, rice and grits obtained in the beginning stages of the milling process. The four products, comprising samp, rice, grits or super maize meal are also referred to as "super" or "primary" products (Fowler 1993). The term refers to products with a fat content of generally less than 1%, a uniform composition and the fact that they are derived mainly from the main portion of the clean maize endosperm (Alexander 1987).

Another important product from the process is a range of so-called "brewers grits". These are mainly derived from coarse and regular grits which are milled using the reduction rollers to specific particle size distributions required by brewers. The products are essentially the same as all "super" products in terms of composition and origin (Alexander 1987).

During the milling process, a fine fraction is also produced (smaller than 0.17 mm sieve opening) and is usually referred to as "break flour". In Table 2.1, this fraction is referred to as "flour". Break flour is derived mainly from the opaque portion of the maize endosperm, during the breaking action occuring during the degerming stage. A similar breaking action also takes place when the particle size of maize grits is reduced with roller mills. The actions of breaking and particle size reduction run in parallel and will simultaneously produce smaller grit particles as well as so called "break flour". The "break flour", which is very fine, is sieved out of the grit particles. When a maize kernel is subjected to shearing forces, the vitreous endosperm will break up into smaller pieces, but remain in a relatively larger particle size (> 0.17 mm). The opaque endosperm, on the other hand, becomes powdery under shear forces and immediately produces flour that is sieved out (as less than 0.17 mm in size). Terminology describing these fractions is used indiscriminately in the literature and for clarity, the following definitions will be used:

Flour or break flour – the fraction obtained from reducing the opaque endosperm into a powder during the various milling stages and which is sifted out through a 0.17 mm sieve opening.

Reduction flour – a term sometimes used to actually describe super and special maize meal and which is derived mainly from the vitreous portion of the endosperm. These fractions are produced by reducing the particle size of the vitreous portions of the endosperm by crushing between reduction rollers.

"Reduction flour" has the same composition as maize grits or "super" products (Alexander 1987, Fowler 1993).

To avoid confusion, the term "flour" in this thesis will only refer to "break flour", derived from the opaque portion of the maize endosperm. Products produced by reducing the particle size of vitreous endosperm pieces will be referred to as "meal" instead of "reduction flour", being either super maize meal or special maize meal, depending on their fat and fibre contents. As the term "reduction flour" can cause confusion as different millers define it differently, the term will not be used in this thesis.

Break flour is produced as a by-product during the production of meal. As maize grits consisting of vitreous endosperm is broken into smaller pieces by the milling action, a small portion of the grits will disintegrate into powder, which is then sifted out as flour. Also, as the initial separation of vitreous and opaque endosperm fractions is never ideal, small pieces of opaque endosperm usually adhere to the vitreous endosperm making up the grits and these opaque pieces also disintegrate into flour during further reduction rolling. Therefore, break flour can consist of many of these powder fractions and will not only be derived from the first break. After sieving out the powdery flour particles which are less than 0.17 mm in size, the cleaned vitreous endosperm particles will then make up the meal fraction.

The whole maize kernel consists of 82% endosperm, 5% bran and 13% germ and tip cap and therefore, a maximum yield of 82% clean endosperm on a total kernel weight basis is theoretically possible (Kent 1984). In practice, however, a yield of about 75% of endosperm material, calculated on a whole kernel weight, basis is the rule. This discrepancy is the result of many factors such as maize quality, milling practice, plant design and production control (Fowler, 1993). The endosperm material also contains a small amount of fat which gradually increases as the yield of the endosperm increases. It becomes more and more difficult to mechanically separate the mixture of endosperm and germ fractions during the reduction stages of the milling process and a cut-off point is reached where cost of separation outweighs the benefit of increasing yield with a few percentage points.

The term “total yield” can reflect different methods of calculation. Generally, it means adding up the weights of all the maize endosperm-derived products. These products may not exceed a certain maximum content of fat, ash and crude fibre, calculated as a percentage of the weight of the incoming maize. However, different mills and countries have different reference points. Total yield can be calculated based on incoming maize before cleaning, or incoming maize after cleaning, which will give different results. Also, some mills tend to calculate yield on an “as is” basis with the moisture contents of the products slightly more than the moisture contents of the incoming maize. Usually, a mill’s profit margin is made here by the addition of water to the total amount of products. Moisture content can, however, not exceed 14% as it will result in fungal growth in the end products (Fowler 1993). These products are also referred to as “white” products (Paulsen and Hill 1985).

To reduce confusion with the terminology, each factor measured in this thesis will be defined accordingly in terms of its respective reference points. As an almost infinite number of products are possible taking into account the number of variables possible in a mill, products can only be defined uniformly in terms of particle size and composition. Although similar trends in the yield of clean endosperm products may exist between different mills, extrapolation of one mill to another is dangerous due to the differences between mills. However, in general all mills strive to maximise the yield of clean endosperm products. The ability to predict the potential maximum yield of clean endosperm to be derived from a specific batch of maize is a common need among all millers. The most valuable products are also derived from the vitreous endosperm, which leads to the need for predicting vitreous endosperm yield as the primary objective.

2.3 ANALYTICAL TECHNIQUES USED FOR MAIZE KERNEL MILLING PROPERTY EVALUATION

2.3.1 Introduction

Kernel strength is the fundamental principle which many of the techniques attempt to measure. Kernel strength of cereals is a critical factor in relation to losses during milling and is often measured as and referred to in the literature as "hardness" (Chandrashekar and Mazhar 1999). "Soft" maize kernels will give smaller amounts of large grits as a final milling product than normal kernels, and they require a longer sieving time for good separation of the fine fractions from the coarser fractions. Unusually "hard" kernels, on the other hand, require more energy to mill and require more maintenance of the mill rolls. "Hard" grain is also less resilient and develops more stress cracks or broken kernels during handling (Tran, deMan and Rasper, 1981). In maize and also sorghum research literature, the terms hardness, strength and vitreousness are often used loosely and synonymously. Vitreousness is commonly associated with hardness and dry milling behaviour, (Paulsen and Hill 1985; Watson 1987a; Mestres, Louis-Alexandré, Matencio and Lahlou 1991), but a clear relationship does not always hold (Abdelrahman and Hoseney 1984; Chandrashekar and Mazhar 1999), mainly due to the problem that vitreousness itself is not understood with sufficient precision (Watson 1987a).

Several research studies have been done to investigate methods for measuring maize kernel strength. A suitable method must be reliable, simple and rapid enough for industrial operations. The resulting strength index should represent accurately the quality of the grain with respect to its performance in a mill and provide the means for an approximate estimation of milling costs. The same method must be suitable for use by plant breeders for routine quality control of maize genetic material in terms of the potential milling properties of new cultivars (Tran, deMan and Rasper 1981). Therefore it should not require excessive sample size. A selection of small-scale tests using grain strength determination as the underlying principle were evaluated by Tran, deMan and Rasper (1981). These authors evaluated a compression test using an Instron Universal Testing Machine, a breakage test using a Stein Breakage tester (McGinty 1970), a pearling test used for measuring the torque during milling using a Strong-Scott barley pearler and a grinding test using a disc grinding mill including measuring torque. They concluded that these small-scale kernel strength tests can discriminate between kernels of different moisture content (influencing the plasticity of the endosperm and bran). However, results for pearling resistance using the Strong-Scott barley pearler and small-scale milling did not correlate as kernels of higher moisture content were more difficult to pearl (needed more energy) in contrast with less energy needed when the kernels of lower moisture contents were milled.

Although these small-scale tests usually differentiate easily between different samples in terms of a measureable characteristic such as milling resistance, it is often very difficult to correlate the results with processing conditions. Sample sizes for analysis are often very small in relation to the total amount of product they represent. This produces large variability in results due to problems with homogenous and representative sampling methods (Tran, deMan and Rasper 1981). Most small-scale milling resistance tests are based on the principles of one of the abovementioned tests and usually measure some aspect of milling resistance such as torque or time to mill. Many tests have been modified specifically for use with maize, for example the Stein Breakage Tester as modified by Miller, Hughes, Rousser and Booth (1981), which is an improvement to the original test by McGinty (1970). These tests usually make use of small sample sizes and can be used either by plant breeders or by the industrial millers, but they are destructive.

Apart from grain strength, another important maize milling property is the potential yield of products such as flaking grits or small grits called “semolina”. As stated, these products will have a granular appearance and are derived mainly from the vitreous endosperm. The inclusion of fine floury particles is undesirable in for example tortilla making, as it causes stickiness of the masa dough (Chandrashekar and Mazhar 1999). A similar situation exists in South Africa where small particles are undesirable resulting into sticky stiff porridges.

As stiff porridges are eaten by hand, stickiness becomes a problem and can result in reduced sales for millers (personal communication, Broadhead, G., Chief Miller, Tiger Milling Inland Division, South Africa). Depending on the end use, mills optimize for the yield of a specific product of a specific quality. Examples are: clean semolina (stiff porridges in South Africa), flaking grits for breakfast cereals (Watson 1987a), clean grits for brewing (Gerstenkorn 1991) and flour with a specific fat content and particle size for flatbread production in India (Chandrashekar and Mazhar 1999). The general trend is to extract as much as possible clean vitreous endosperm within certain maximum allowed limits of fat and fibre in the end products. Mills can optimize for grain strength to a certain extent in order to maximize the yield of clean vitreous endosperm, by adjusting factors such as water addition during conditioning, adjusting the severity of the degermer system and adjusting the breaking power in the rolls. This can be done by adjusting speed differentials, flute sizes and amounts accompanied by adjustments in sieve sizes (personal communication, Broadhead, G., Chief Miller, Tiger Milling Inland Division, South Africa). A true estimation of the maximum potential yield of vitreous endosperm from a specific cultivar of maize will assist significantly during milling as a miller will then know how much of a certain product can possibly be extracted from the maize and the mill can be optimized accordingly. Although milling resistance and grain strength from a mechanical point of view also influence milling performance, modern mills can be adjusted to a large extent to accept a range of maize of varying strength, but with similar vitreous endosperm contents and still produce the same yield of vitreous endosperm derived products (personal communication, Broadhead, G., Chief Miller, Tiger Milling Inland Division, South Africa).

The technique would assist the miller to segregate grain at intake into bins of identified milling characteristics and then grist accordingly in order to minimize setting changes during milling.

The proportion of vitreous endosperm in maize has been shown to be related to other physical properties such as particle size index (Mestres, Louis-Alexandré, Matencio and Lahlou 1991; Yuan and Flores 1996), density

(Mestres, Louis-Alexandré, Matencio and Lahlou 1991), the Stenvert Hardness tester (Kirleis and Stroshine 1990; Li, Hardacre, Campanella and Kirkpatrick 1996) and percent floaters (Peplinsky, Paulsen and Bouzaher 1992). Percent floaters is a rapid test commonly used for rapid classification of maize into various strength classes. It is based on the principle of density, as vitreous endosperm is more dense and therefore heavier than opaque endosperm. By adding maize kernels to solutions of a certain specific gravity, maize kernels can be classified (usually gravimetrically) according to their densities by calculating the amount of kernels that float or sink (Gerstenkorn 1991). The measurements are prone to be influenced by other factors than only vitreous/floury ratios (Chandeshekar and Mazhar 1999). Watson (1987a) described some of these factors, for example physical changes induced in maize kernels during drying result into void spaces and air pockets within the kernels and these will influence the percent floaters reading. Kernels differ in the amount of void space within them even without the additional drying effects. The moisture contents of the kernels will also influence the measurements and usually moisture correction factors are developed for specific maize cultivars (Watson 1987a). In spite of the problems encountered with percent floaters, it is still widely used as a screening method to select floury maize for the wet milling industry (Fox, Johnson, Hurbugh, Dorsey-Redding and Bailey 1992; Zehr, Eckhoff, Singh and Keeling 1995) and for screening samples with different proportions of vitreous endosperm (Pratt, Paulis, Miller, Nelsen and Bietz 1995).

The use of NIR and NIT for estimating maize vitreousness was investigated by Pomeranz, Czuchajowska and Lai (1986b), Williams and Sobering (1993), Robutti (1995), Eyherabide, Robutti and Borras (1996) and Muluc (1997). Vitreousness can be estimated by NIR at 1680 nm, but requires grinding of the samples. The use of NIT at 860nm was found to be suitable for distinguishing between opaque and vitreous maize kernels and was found to be more sensitive in classifying kernels into different groups of vitreousness than NIR (Eyherabide, Robutti and Borras (1996). Although both methods (NIR and NIT) produce good correlations with selected tests for example percent floaters or, in the case of South African cultivars the hand dissection and milling test described by Vorwerck and Miecke (1973), results are not always reproducible over more than one season (personal communication, Randall, P.G., Director, P Cubed). The use of a laboratory roller milling performance test for the calibration of NIT equipment is currently being investigated using South African cultivars (personal communication, Randall, P.G., Director, P Cubed). These data are proprietary information as the method is being developed by a private company and it was not available for review by the author.

2.3.2 Specific tests for measuring the resistance to milling or crushing (grain strength)

Pomeranz, Czuchajowska and Lai (1986a) did a comparative study of maize strength measurements namely Stenvert Hardness, Particle Size Index (PSI) and Near-Infrared Reflectance (NIR). The Stenvert Hardness Tester measures the time taken to grind kernels through a fixed mesh size. Although it has been proven as a good indicator of maize strength (Li, Hardacre, Campanella and Kirkpatrick 1996), correlations between grain strength and yield of larger particle size fractions are not always correlated (Chandrashekar and Mazhar 1999).

The strong influence of maize kernel moisture content on grinding resistance was demonstrated by many authors (Shelef and Mohsenin 1969; Tran, deMan and Rasper 1981; Paulsen 1983; Pomeranz, Czuchajowska and Lai 1986a; Watson 1987a). These authors' findings help to explain the milling process used in a typical dry maize mill. The maize is tempered before milling in order to make the endosperm as well as the bran more resilient to breakage, in spite of the fact that it becomes "softer" according to mechanical pressure.

The use of compression tests such as the Instron Universal Testing Machine to measure kernel strength seem to be unsatisfactory because of high variability, according to Tran, deMan and Rasper (1981).

Jindal and Mohsenin (1978) developed a method for determining the dynamic hardness of maize involving impacting the kernels with a steel ball. Properties such as absorbed energy, coefficient of restitution, elastic properties and the yield pressure of the maize were determined. These measurements were used to show the effect of moisture content on aspects such as breakage susceptibility, but no correlations were made with milling performance.

Other strength measurements based on milling resistance include: the Tangential Abrasive Dehulling Device (TADD) used by Lawton and Faubion (1989), obtaining fractions using a micro hammer-cutter mill (Wu 1992) and a round wheel crusher (Bennet 1950). Lawton and Faubion (1989) adjusted the TADD method to accommodate sorghum, wheat and maize samples by changing and adjusting the type of sandpaper used for the abrasive dehulling step. Although their results indicated that the TADD could be used for maize kernels, they tested only three samples: popcorn, a floury white maize and a yellow dent maize. They did not test for small differences among samples of the same type of maize. Wu (1992) used a micro hammer-cutter mill to differentiate between fourteen maize samples in terms of Particle Size Index (PSI). His results were positively correlated with the true yields of clean flaking grits obtained from the same samples using a pilot-scale demerming and roller milling system. The round wheel crusher used by Bennet (1950) was initially developed for wheat milling resistance measurements, but was also tested on maize. Samples were crushed followed by sieving into fractions of a specific particle size. The author was able to differentiate between maize samples of different endosperm opacity (visual) by analyzing the PSI data.

2.3.3 Specific milling simulation tests

2.3.3.1 Industrial-scale milling

Full-scale milling trials under controlled conditions are seldom conducted because of the large quantity of maize required and the difficulty of controlling and measuring the variables involved in the processing. Paulsen and Hill (1985) conducted a industrial milling trial on a mill with two degermers at a capacity of 1780 tons in a 24 hour period. They found significant correlations between breakage susceptibility, stress cracks, floaters and the yield of flaking grits. Their aim was to find quality factors such as moisture, fat and protein contents as well as physical properties such as stress cracks in the whole kernels in order to predict the yield of flaking grits from artificially dried maize intended for the production of cornflakes. Although density-based methods such as test weight and percentage floaters were also used as screening methods to select maize, their study did not focus on the relationship between vitreousness and the yield of clean vitreous endosperm products. Maize in the USA is often dried artificially, giving rise to stress crack problems with a known effect on flaking grit yield. Predicting the yield of flaking grits in maize without stress cracks (such as found in the South African scenario) will require another in-depth milling study, as taking out the effect of stress cracks will change the behaviour and prediction models significantly.

Litchfield and Shove (1990) did a large scale industrial-milling trial in Japan using a minimum of 300 tons of maize for each trial. The maize came from two 7000 ton batches that were shipped from the USA to Japan and distributed among 8 mills for milling trials. Maize grits yield varied from 42.8% to 52.2% for the first milling trial between the eight mills and varied from 47.1 to 60.3% for the second milling trial between the eight mills. The quality of the maize was assessed using the Stenvert Hardness Tester with a value of 12 seconds milling time (Standard Deviation of 0.68) for the first batch and 12.9 seconds (Standard Deviation of 0.65) for the second batch. Percent Floaters differed considerably between the trials with an average value of 44% for the second trial, compared to 65% for the first trial. Stress cracks also differed significantly, with an average value of 6% cracked kernels for the second trial and 17.3% for the first trial. Unfortunately, no objective measurement was done to assess vitreousness of the samples. The second milling trial gave higher yields of grits for all eight mills than the first trial, but not enough data were available to make significant conclusions, apart from the significant difference in stress crack occurrence. Based on the findings of Paulsen and

Hill (1985), stress cracks would have had a significant effect on maize grit yield as was evident in the milling trial.

2.3.3.2 Small-scale milling simulation

Milling performance trials are usually done on smaller scale set-ups with equipment simulating the actual milling process. Although not ideal, parameters can be controlled better during the simulation trials than would otherwise be possible in large industrial mills. However, all the steps necessary to achieve good separation of products similar to those found in an industrial mill cannot be simulated fully on a small-scale system. Small-scale milling simulations tests can take many different forms, but can mainly be divided into three categories:

- Small-scale roller milling and sieving units without degerming
- Small-scale roller milling and sieving units with degerming prior to the first break
- Milling tests using other types of mills than roller mills.

Milling performance is usually measured in terms of yield of flaking grits of a defined quality (Paulsen and Hill 1985), semolina or small-size grits of a defined quality (Mestres, Louis-Alexandré, Matencio and Lahlou (1991), and regular or brewers grits (with very low specified fat and fibre contents) to assess the yield of vitreous endosperm products from the maize. These products are all derived mainly from the vitreous endosperm portion of the kernels, so as to keep the fat and fibre contents as low as possible. The total extraction of flour (Mestres, Louis-Alexandré, Matencio and Lahlou (1991), is also used in conjunction with the yield of offal (germ and bran products) in order to assess the total yield of maize products (Paulsen and Hill (1985). Many methods for small-scale roller milling and sieving exist. Mestres, Louis-Alexandré, Matencio and Lahlou (1991) used 4-5 kg batches in a SOCAM roller mill system using a semi-wet milling process followed by pin milling in an

Alpine pin mill. This method was developed by Mestres, Matencio and Faure (1990). An experimental milling system using a roller breaking system followed by sieving in a centrifugal sifter (an alternative process to degerming, but with a different action) and cleaning in a purifier (equipment supplied by Bühler Miag) was developed by Gerstenkorn (1991). It should be noted that these two procedures do not make use of a Beall or Bühler type maize degermer in the initial stages, but the degerming stage is replaced by alternative purification steps.

Methods making use of a degermer in the initial milling stage are more common. One of the most popular methods is the Milling Evaluation Factor (MEF) method developed by Stroshine, Kirleis, Tuite, Bauman and Emam (1986). In this method, maize is tempered, degermed, sieved into various fractions, aspirated and the final fatty germ residues removed by flotation. The yields of all these fractions are calculated as a formula expressing a MEF and it is used to indicate the yield of flaking grits. No roller milling is done in this method, as all milling is done by using two degermers in series. The batch size is 1.3 kg of whole maize.

Degermers are designed in various shapes, but all have a similar action, namely a rotating unit inside or outside a static unit (eg. round, conical or flat disk) applying a rubbing action to the maize kernels (Figure 4.2 Chapter 4). Stroshine, Kirleis, Tuite, Bauman and Emam (1986); Peplinski, Anderson and Mounts (1990); Peplinski, Paulsen and Bouzaher (1992), Pan, Eckhoff, Paulsen and Litchfield (1996) and Yuan and Flores (1996) used scaled-down versions of horizontal drum degermers (similar to the Bühler system) for milling tests using degerming as the only breaking mechanism. In contrast, Wu and Bergquist (1991) combined the use of a horizontal drum degermer with small-scale roller milling to produce a full range of products including flour using 4.5 kg samples of maize. Peplinski, Paulsen, Anderson and Kwolek (1989) combined horizontal drum degerming and roller milling to process 6 kg batches.

It appears as if small-scale milling methods are developed by each author depending on the specific needs of the research project. No standard method exists for comparison of the individual methods and therefore it is difficult to judge the effectiveness of each method. However, the primary objective of all the methods is to separate clean vitreous endosperm from the rest of the maize kernel, for example in the milling method described by Mestres, Matencio and Faure (1990). All small-scale milling methods are roughly based on four basic steps, namely addition of moisture to condition the kernels (values differ and are optimized for each method), initial crushing of kernels into large particles (with or without degermers of various shapes and sizes), reduction of the various kernels morphological parts (using roller and other mills of various specifications) and separation of the morphological parts by an array of sieve types, aspirators and gravitational methods.

As long as a specific method can differentiate between different classes of maize kernels in terms of aspects such as stress crack percentage, moisture contents, vitreous endosperm content and other properties, and do so repeatibly and statistically significantly, a method can be regarded as effective for the purpose. Therefore, due to a lack of a "milling standard" worldwide, results can only be compared with great caution. Examples of application-specific small-scale milling are (1) Yuan and Flores (1996) using a degerming step followed by sieving without further milling in order to compare the yields of large flaking grits for breakfast cereals from selections of white maize, and (2) the combination roller milling and sieving method designed by Mestres, Matencio and Faure (1990) for comparing the yields of fine maize grits similar to semolina obtained from the wheat milling process. Mestres, Matencio and Faure (1990) were investigating the possibility of including fine maize grits into durum wheat semolina for manufacturing pasta from a composite wheat/maize mixture. They were not concerned with the yield of large maize endosperm flakes and therefore did not include a degerming step in their small-scale milling test.

2.3.4 Estimation of the vitreous/opaque endosperm ratio in maize kernels by hand dissection

In spite of the time hand dissection takes, it is still regarded as the only fundamental method to use when evaluating methods for the determination of the ratios of vitreous and opaque endosperm in maize kernels. Maize kernels are generally soaked in water to make them softer before dissection or sectioning for microscopic and weighing purposes (Bennet 1950). Individual morphological parts are weighed to calculate the exact yield for an individual maize kernel (or a small group of kernels), The method is sometimes also referred to as "micromilling" (Peplinsky, Anderson and Alaksiewicz 1984; Louis-Alexandré, Mestres and Faure 1991; Yuan and Flores 1996; Dombrink-Kurtzman and Knutson 1997).

To interpret the data accurately, the history of the sample needs to be known. The history includes factors such as genetic variability and environmental factors. Differences in the vitreous/opaque ratio are caused by the environment on the development of the maize on the ear, within fields, between fields due to moisture, temperature and soil nitrogen supply and uptake (Hamilton, Hamilton, Johnson and Mitchell 1951). Hand dissection is of value especially when determining the effect of soil fertilization and environmental conditions on maize kernels (as these factors may influence the development and ratio of vitreous and floury endosperm) and references made to such experiments date back to 1903 (Hopkins, Smith and East 1903, according to Hamilton, Hamilton, Johnson and Mitchell 1951).

Several methods involving pre-soaking maize kernels followed by dissection and analyzing the individual components have been described. Kernels were soaked for a certain time period and dissected with a scalpel (Hamilton, Hamilton, Johnson and Mitchell 1951). A method whereby maize kernels were soaked for 28 hours in distilled water at 36°C before sectioning was also described by Bennett (1950). Dombrink-Kurtzman and Knutson (1997) soaked maize kernels in distilled water for five minutes and removed the pericarp and germ with a scalpel. After drying of the kernels overnight, the floury endosperm was drilled out with a Dremel Mototool (commonly used by dentists). Although the method is quicker than a method requiring excessive soaking, it caused some kernels to shatter due to the high shear of the drill.

Louis-Alexandré, Mestres and Faure (1991) equilibrated maize kernels to moisture contents of 11.5 and 15.5% respectively by subjecting the kernels to different controlled humidity atmospheres at room temperature for three weeks. Kernels were then sectioned and soaked in distilled water for 20 to 30 minutes, followed by separation into morphological parts. The different endosperm parts were dried at 130°C for 2 hours and weighed. Various vitreousness indexes were calculated, but the index used for further analysis was the percentage of vitreous relative to total endosperm on a dry weight basis. Kereliuk and Sosulski (1995) steeped maize kernels for three hours in 1 g/litre sodium metabisulphite solution before dissecting the kernel into bran, germ and endosperm using a razor blade. Apparently, the metabisulphite weakened the protein-starch adhesion and the different morphological parts could be separated more easily. This dissection technique would be more applicable in the field of measuring wet milling characteristics. In wet milling, kernels are soaked in a solution containing sulphur dioxide and lactic acid before further processing (Fox, Johnson, Hurburgh, Dorsey-Redding and Bailey 1992). Milling is done on the soaked kernels and differs fundamentally from dry milling. As this work focuses on dry milling properties, further discussions on wet milling are omitted.

2.3.5 Estimation of the vitreous/opaque ratio on cut kernel surfaces by visual or machine examination

As the hand dissection method is very tedious, the analysis of sectioned maize kernels for vitreous/opaque endosperm ratios by measuring the relative surface areas covered by the two types of endosperm is the most popular method to obtain a rapid estimation of the vitreous or opaque endosperm yield. Methods have been developed to correlate these ratios with the yield from hand dissection methods determined on a weight basis (Louis-Alexandré, Mestres and Faure 1991). Such methods involve: measuring different parts of cross-sectioned maize endosperm using a planimeter (Kirleis, Crosby and Lousy 1984), an Image Analyser (Kirleis, Crosby and Hously 1984; Watson 1987a; Louis-Alexandré, Mestres and Faure 1991) or vernier calipers (Li, Hardacre, Campanella and Kirkpatrick 1996). Although these methods are suitable for quantifying vitreousness in various applications, they are all destructive and do not allow for analysis of large sample sizes. Usually only 10 kernels per sample were analysed (Louis-Alexandré, Mestres and Faure 1991; Mestres, Louis-Alexandré, Matencio and Lahlou 1991). However, the cut surface method, as it gives high correlations with the hand dissection method, is a useful rapid method. Several studies used this method as a reference indicator of vitreousness when comparing the results of small-scale milling tests and other tests (Kirleis and Stroshine 1990; Mestres, Louis-Alexandré, Matencio and Lahlou 1991; Li, Hardacre, Campanella and Kirkpatrick 1996).

Some authors have reported studies using vitreousness measurements on only a very few kernels, for example Mestres, Louis-Alexandré, Matencio and Lahlou (1991) and Yuan and Flores (1996). Ten kernels in each case for each maize cultivar were tested. The reason for the small sample sizes appears to be the time consuming methods used for measurement of vitreous endosperm yield on single kernels.

Mestres, Louis-Alexandré, Matencio and Lahlou (1991) used a larger sample size (50 kernels per cultivar) when characterising West African maize cultivars according to their physico-chemical properties and their dry-milling behaviour. They evaluated vitreousness according to the method of Louis-Alexandré, Mestres and Faure (1991) by cross-sectioning the 50 maize kernels of each cultivar and measuring the ratio of vitreous endosperm expressed as a cut surface area index using a digitization tablet ("vitreousness index"). Although the authors were able to demonstrate a clear correlation between the hand dissection method and their calculated "vitreousness index", they could not obtain a significant correlation between "vitreousness index" and "semolina yield" (fine grit yield). They found a good correlation between vitreousness and kernel density (r = 0.92). However, the correlation between semolina (obtained from vitreous endosperm) and flour (obtained from opaque endosperm) obtained from the milling trial was only – 0.58. This was surprisingly poor, as the one is the inverse of the other. When vitreous endosperm content increases, the opaque endosperm content decreases and *vice versa*). The poor correlation underscored the problems that were encountered during the sieving of the flour and therefore, a relationship between vitreous endosperm yield and milling performance could not be demonstrated. According to the authors, the dry milling pilot method used was not successful because they had trouble obtaining efficient extraction of flour as it was observed that the sieving processes were not effective.

Results produced by Mestres, Louis-Alexandré, Matencio and Lahlou (1991) showed very high coefficients of variation for the hybrids tested. The hybrids originated from West Africa and the coefficients of variation were far higher than those found for other dent and flint hybrids in developed countries (Pomeranz, Czuchajowska, Martin and Lai 1985; Pomeranz and Czuchajowska 1987; Peplinsky, Paulsen, Anderson and Kwolek 1989). The coefficients of variation for sphericity and vitreousness within one cultivar were 10.6 and 34.1% respectively. The variability reflected the very high heterogeneity of individual kernels within the samples. Even 1000 kernel weights showed relatively high coefficients of variation.

The above shows how important it is to take into account all factors when evaluating vitreous or opaque endosperm yield and milling performance data, especially the variation of maize kernels within samples and problems encountered during the milling trials (for example sieving). Heterogeneity in maize within a cultivar and even on the same ear is well known (Wolf, Buzan, MacMasters and Rist 1952; Watson 1987a). This emphasizes the need for a clear understanding of the samples used for analysis – preferably the genetic history as well as the growing conditions (Wolf, Buzan, MacMasters and Rist 1952).

Li, Hardacre, Campanella and Kirkpatrick (1996) also used the measurement of vitreous/floury ratios by sectioning of the kernels followed by measuring the surface areas with a pair of vernier calipers, according to the method of Kirleis, Crosby and Hously (1984). They measured only 10 kernels each of 38 cultivars of New Zealand maize and found that the vitreous/opaque ratios correlated significantly and highly with maize kernel strength parameters determined by the Stenvert Hardness Tester. Great care was taken to ensure that the moisture contents of the milled samples were exactly the same, to exclude the potential of moisture effects on milling resistance. Li, Hardacre, Campanella and Kirkpatrick (1996), showed that even with only 10 kernels, vitreous to opaque ratio can be correlated with grain strength (electric power consumption during milling) in spite of high coefficients of variation for vitreous/opaque ratio.

Kirleis and Stroshine (1990) used the method of Kirleis, Crosby and Hously (1984) to classify maize into three classes of hardness, in order to measure the Milling Evaluation Factor (MEF). Rankings occurred within the expected order. Mestres and Matencio (1996) used the method described by Kirleis, Crosby and Hously (1984) as modified by Mestres, Louis-Alexandré, Matencio and Lahlou (1991) and found correlations between the vitreous/opaque ratio and the proportion (%) of the two γ-zein fractions on 18 maize samples collected from West Africa. In this research, it was also found that the vitreous or opaque endosperm ratios correlated significantly with the yield of coarse meal and fine flour. Mestres, Matencio and Louis-Alexandré (1995) developed a new milling performance test in the form of a laboratory friability test. In this test, coarse meal and fine flour is produced. The yields of coarse meal correlated well with vitreousness index even though West African cultivars with high variation coefficients of variation were again used.

2.3.6 Use of non-destructive machine vision technology

Until the development of IA (machine vision) technology, the only non-destructive technique available for classifying maize kernels was a visual examination (candling) on a light table. This is subjective and depends on the skill of the analyst. There are several references where the visual examination technique was used both for determining wet milling properties, for example Kereliuk and Sosulski (1995) and dry milling properties, for example Felker and Paulis (1993).

2.3.6.1 Need for machine vision technology

The importance of the development of equipment for rapid methods in food quality control is described by Torkler (1990), as a necessary tool to make an effective contribution to consumer protection, due to their ability to make a prompt statement regarding food quality. The increased costs and the increased control needs with high turnover rates makes the implementation of methods which require less personnel and less expensive operating budgets necessary.

For the food manufacturer, raw material cost is often the highest cost factor. Therefore, the manufacturer needs at the time of raw material delivery a rapid evaluation of the valuable constituents that will form the basis for the acceptance/ rejection decision of the product (Torkler 1990).

One type of rapid measuring system is known as machine vision technology or IA or Computer Vision and is used widely in a cultivar of industries including the food industry for material handling and sorting (Singh and Smith 1988).

2.3.6.2 Description of IA technology

IA is the science of making geometric and densitometric measurements in images from any source. Its main application is in quantitative microscopy, providing rapid, accurate and statistically significant data, replacing the traditional subjective methods (Leica QWin User Guide 1996).

IA first appeared as a technique in 1963 with the introduction of the “QTM”, a Quantitative Television Microscope designed by Metals Research Ltd, who later became part of Leica Imaging Systems Ltd. in Cambridge, England (Leica QWin User Guide 1996). The first instrument was used in metallurgical laboratories for quality control of steel cleanness and other microstructural measurements, at that stage for the space industry. Its usefulness in other fields soon became apparent and one of the earliest applications in the biological field was measuring the size of air spaces in the lung to quantify lung damage (Leica QWin User Guide 1996).

Computer Vision systems provide a means for obtaining a digital image of an object with a video camera. An array of picture elements known as pixels (the smallest part of the digital image that can be assigned a grayscale value), are digitized based on individual pixel (picture element) intensity or brightness. An eight bit A/D converter will provide 2^8 (256) gray levels of intensity for each pixel (Paulsen and McClure 1985). These gray values are defined in such a way that a value of zero equals black and a value of 255 equals white. Values in between give a linear transition from black to white. Image processing software is used to fashion the matrix of pixels and to measure parts of the image (Van Sonsbeek 1994). The pixel matrix can vary in size, but a matrix of 512 x 512 dots (pixels) is commonly used (Van Sonsbeek 1994). Other systems commonly used have a matrix (or special resolution) of 512 x 480 or 640 x 480 (Gunasekaran and Ding 1994). For best results, the spatial resolution of the camera is matched to that of the vision processor board (Gunasekaran and Ding 1994). The Leica 600 Image Analyser (as used in our laboratory at the CSIR) consists of a larger pixel matrix (spatial resolution) and usually a matrix of 764 x 575 dots is used (Leica QWin User Guide 1996). Digitized images may be stored temporarily or permanently on disk. Two terms commonly mentioned with computer vision systems are “image processing” and “pattern recognition”. Image processing involves steps taken to enhance an image and extract pertinent information. Pattern recognition involves procedures that allow the computer to identify whether an object is acceptable or if it should be rejected (Paulsen and McClure 1985).

The essential elements of computer vision systems include the following components:

- Image acquisition (using a high resolution Closed Circuit Digital (CCD) camera and a vision processor board (frame grabber))
- Image enhancement, preprocessing and storage
- Extraction of relevant features (using computer hardware and software)
- Measurement of relevant features
- Postprocessing such as statistical analysis, printing and storage of data (Gunasekaran and Ding 1994).

2.3.6.3 IA in cereal research

IA has been used for various types of cereal analysis such as: discrimination between wheat types, barley, rye and triticale kernels (Chen, Chiang and Pomeranz 1989), oat kernels (Sapirstein, Newman, Shwedyk and Bushuk 1986), the evaluation of starch types from wheat (Bechtel, Zayas, Dempster and Wilson 1993; Baldwin, Adler, Davies and Melia 1994), the quantification of kernel morphology variation in six-row barley (Gebhardt, Rasmusson and Fulcher 1993), detecting sprout damage in wheat (Thomson and Pomeranz 1991), quantifying wheat morphology (Symons and Fulcher 1986) and the prediction of milled rice fractions (Mathewson and Zayas 1986).

Paulsen and McClure (1985) emphasized the importance of proper illumination set-ups, especially in the analysis of cereal grains. Although a large amount of effort usually goes into optimizing pixel density, number of gray levels, speed of image acquisition and computer storage requirements, even a little effort into optimizing illumination of the samples can greatly improved images. Image processing cannot be correct for details that were never captured due to poor illumination. Paulsen and McClure (1985) published detailed information on the methodology of detecting maize kernels in order to obtain surface morphology data. They emphasized the use of diffuse light and highlighted other aspects such as the improvement in contrast if a feature is analysed against a dark background versus analyzing it against a light background. They found that a deep purple background gave the best contrast, but that a opaque black background was also suitable. They also highlighted the fact that the optimum detection levels of digital or video cameras tend to be at a different wavelength than the optimum detection level of the human eye. This can result in features being distinguished by the human eye which cannot be detected by the camera and *vice versa*. The use of filters and diffuse indirect light from fluorescent sources rather than incandescent light was mentioned, in order to reduce uneven illumination and shadow formation.

Sapirstein, Dexter and Bushuk (1986), used IA to determine the vitreousness of wheat. Images of transilluminated wheat samples were obtained with a monochrome Charged Coupled Device (CCD) camera connected to a computer with a frame grabber board. Histograms of pixel intensities were analysed. They were able to correlate the proportion of vitreous kernels as detected by the instrument to visually determined proportions.

Felker and Paulis (1993) were the first to attempt quantification of translucency on whole maize kernels by IA. As stated, the traditional evaluation method for translucency consists of visually scoring and assigning the maize kernels to arbitrary, discontinuous classes according to the ratio of vitreous to floury endosperm using a light box (candling) (Ortega and Bates 1983).

The methodology that Felker and Paulis (1993) used was to surround individual kernels with modeling clay to exclude excess light. The kernels were viewed on a light box with a monochrome video camera. They also only used 10 kernels per class but the samples were very homogenous, as kernels were pre-selected by hand for the analysis. They were able to classify a segregating F2 population of high-lysine maize (Quality Protein Maize) into 10 classes of translucency that correlated well with visually assigned translucency classes. They had a wide range of translucency classes to work with, ranging from 0% to 100% translucency as determined as a ratio of illuminated parts of the kernel to the total surface of the kernel visible on the light box. They also found that the grayscale value was inversely proportional to kernel thickness, although the correction factor they applied for kernel thickness did not have a significant influence on their rankings.

It is known that the intensity of light shining through slabs of glass decreases according to Lambert's law due to absorption of the light (Sears, Zemansky, and Young 1982) and the absorption rate are related to the material and the thickness. However, the translucency of maize kernels is also affected by many other factors such as light scattering and the heterogenous nature of the internal kernel morphology (two types of endosperm, germ and other parts). Felker and Paulis (1993) suggested that there is a linear relationship between grayscale (an indication of light intensity) and thickness, as opposed to Lambert's law which is logarithmic and does not take into account the effect of factors other than light absorption by the material. An average thickness of 4.5 mm was used as the standard. For each millimeter of departure from the mean thickness of 4.5 mm, the grayscale varied by 36.5%. This meant that if a maize kernel was 5.5 mm thick, the grayscale reading was 36.5% less than the standard. The thickness correlation factor published by Felker and Paulis (1993) is useful for further research.

Felker and Paulis (1993) also removed image background corresponding to the embryo area using the computer software in order to improve the correlation. However, some aspects which appear to require further investigation include:

- Correlation between grayscale values and translucency classes of normal maize (all their tests were done on Quality Protein Maize, which is genetically different from the standard types)
- The correlation between grayscale (resembling translucency) and true vitreous endosperm yield (either by milling or by dissection)
- Alternative methods of sample preparation, in order to replace mounting in modeling clay which is a time consuming method.

The only other published examples of the application of IA to maize involve the measurement of the particle size of maize starch (Jane, Shen, Wang and Maningat 1992; Campbell, Pollak and White 1994) and the detection of maize kernel size dimensions (lengths, widths and projected areas in a two-dimensional plane) as described by Paulsen, Wigger, Litchfield and Sinclair (1988).

IA has also been evaluated on other cereals. A method for the quantitative determination of vitreousness on whole grain samples of wheat was developed by Sapirstein, Dexter and Bushuk (1986). They correlated vitreousness results to the milling quality of durum wheat in terms of semolina yield. IA was also used for measuring wheat kernel morphological characteristics (Symons and Fulcher 1986; Sapirstein, Newman, Shwedyk and Bushuk 1986) and wheat starch granule size distributions (Bechtel, Zayas, Kaleikau and Pomeranz 1986; Zayas, Bechtel, Wilson and Dempster 1994).

Kirleis, Crosby and Hously (1984) measured vitreous endosperm areas of sectioned sorghum grains. Although sorghum also has vitreous and opaque endosperms, it does not have a transparent pericarp similar to maize and therefore, the grains had to be sectioned before IA could be done.

IA work on other cereals mainly for the determination of kernel dimensions has also been published. Examples are oat kernel morphology (Symons and Fulcher 1988), barley, rye and triticale classification (Chen, Chiang and Pomeranz 1989) and also some work on oilseed quality factors such as detection of discolouration due to fungal damage on soybeans (Paulsen, Wigger, Litchfield and Sinclair 1988).

2.3.6.4 Maize translucency measurements other than IA

Hall and Anderson (1991) used a light meter to measure the amount of light transmitted through a layer of close-packed maize kernels on a glass plate with a light source underneath. They correlated light transmittance (translucency) through maize kernels with percent floaters. Selected maize kernels were subjected to careful treatment during drying and handling in order not to induce additional variation in the floaters readings. A correlation of 0.98 was obtained. However, in this case the maize all came from the same location and was treated similarly. No attempt was made to correct for variations due to sphericity or kernel thickness. Unless the history of maize is known, the percent floaters can be an indication of various other properties and may not always be correlated to vitreousness. Although Hall and Anderson (1991) did not use an Image Analyser to quantify the intensity of the light, this experiment can be seen as the first attempt to quantify maize translucency measured on whole kernels. They correlated the measurements with another property, percent floaters, which has been correlated with vitreousness and yield of milled maize products in spite of its shortcomings.

2.3.7 Other indirect methods (physical methods and chemical methods) for measuring maize endosperm

Other methods have been explored to predict or explain endosperm vitreousness and opaqueness in maize kernels using various quality parameters such as: zein composition (Dombrink-Kurtzman and Bietz 1993), analysis of protein, starch, moisture, fat and fibre (proximate analysis), kernel density, floaters, starch and fatty acids (Kereliuk and Sosulski 1995), amylose content (Dombrink-Kurtzman and Knutson 1997), damaged starch and protein fractionation (Mestres and Matencio 1995), and other parameters such as breakage susceptibility (Kirleis and Stroshine 1990). These quality parameters were examined mainly to explain or quantify the differences between endosperm types. Except for stress cracks, they have not been widely used for predicting milling performance. One such report is by Mestres, Louis-Alexandré, Matencio and Lahlou (1991). They found correlations between ash content, kernel density and vitreousness, determined as surface area percentages on cross-sectioned kernels and also a correlation between ash content and semolina yield obtained by using a small-scale roller milling system without a degermer. The vitreousness of their samples, which were from central and east Africa, ranged from 6% to 80%.

They did not find significant correlations between vitreousness and semolina (fine grit) yield, but vitreousness correlated very well with density. Other workers in the field successfully used density, vitreousness and percent floaters to predict maize semolina yield (Manoharkumar, Gerstenkorn, Zwingelberg and Bolling 1978; Yuan and Flores 1996). Mestres, Louis-Alexandré, Matencio and Lahlou (1991) mentioned problems during the sieving stages of their experiment and it made their comparison between vitreousness and semolina yield inaccurate, explaining why they could not get significant correlations similar to other authors. Manoharkumar, Gerstenkorn, Swingelberg and Bolling (1978) also correlated maize semolina yield with kernel bulk density, percent floaters and kernel protein content. Their correlation coefficients varied from 0.49 to 0.9, depending on the particle size of the semolina fraction (n = 40).

Percent floaters is an indirect measure of kernel density and the percentage of floating kernels are determined in a sodium nitrate solution made up to a specific gravity of 1.275 (Wu and Bergquist 1991). These authors have shown that moisture content variations in maize kernels influences kernel density measurements. Some kernels also tend to suspend themselves in the middle of the solution and neither sink nor float, giving rise to variable results.

The range of percent floaters tested by Hall and Anderson (1991) was wide, from 7.7% to 97.7%. A smaller range may be more influenced by small differences in the density of individual kernels due to moisture content differences, stress cracks or other physical changes resulting in less accurate readings. In a study done by Manoharkumar, Gerstenkorn, Zwingelberg and Bolling (1978), reasonable correlations were obtained between percent floaters and the yield of fractions of maize semolina milled in a laboratory roller mill system. The correlations obtained indicated that vitreousness could possibly be used as an indication of semolina yield, as percent floaters was linked to the vitreousness of the kernels. Better correlations with semolina yield were obtained, however, using the bulk density (hectolitre mass) of the same kernels instead of the floaters test. This indicated that the floaters test results could have been influenced by other factors, in spite of the fact that all maize samples were of the same moisture content.

2.4 CONCLUSIONS

Apart from the research work of Hall and Anderson (1991), and Felker and Paulis (1993), no quantitative work has been done on using maize translucency as a non-destructive technique for predicting maize milling performance. Although candling has been around for a long time as a quality evaluation test, it has not been quantified and relies heavily on the experience of the analyst (and the ability of the human eye to differentiate). In general, although much work has been done on the development of laboratory assays suitable for predicting dry milling performance of maize, the general conclusion is that the individual parameters measured are not well defined and there is a lack of agreement amongst researchers. Terms such as "hardness", "vitreousness", "milling resistence" and "softness" and "opaqueness" are often used indiscriminately. Although many publications exist, the results are often confusing due to the undefined terms. It is also clear that few large-scale milling tests have been performed, mainly due to cost implications. It is, however, still necessary to ultimately test a developed analytical method for predicting maize milling performance in a large-scale experiment for final verification.

Process control is a necessity in the food industry due to quality and cost control requirements. None of the tests for predicting maize milling performance used to date with the possible exception of NIT appear to be suitable as potential on-line process control methods. Probably, the only method which show real potential is the candling method for assessing maize translucency. If this method can be quantified, it would have the potential for use as a process control standard. Machine Vision, replacing the human eye, is probably the best way of attempting to quantify the candling method. Apart from the potential of developing an on-line process control method for adjusting mills according to potential product yield depending on the percentage of vitreous or opaque endosperm in the kernels being milled, such a method would also have a wide application as a rapid non-destructive laboratory assay. As the development of an on-line machine vision process control unit is beyond the scope of this study, the focus will be on the development of a rapid non-destructive IA method for accurately predicting vitreous and opaque endosperm ratios in maize kernels on a single kernel level at a high degree of accuracy.