Maize kernel translucency measurement by Image Analysis and its relationship to vitreousness and dry milling performance

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I declare that the dissertation herewith submitted for the PhD Food Science degree at the University of Pretoria, has not previously been submitted by me for a degree at any other university.

ABSTRACT

MAIZE KERNEL TRANSLUCENCY MEASUREMENT BY IMAGE ANALYSIS AND ITS RELATIONSHIP TO VITREOUSNESS AND DRY MILLING PERFORMANCE

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A rapid non-destructive Image Analysis (IA) technique was developed for the determination of maize kernel endosperm vitreousness. Kernels were analysed using a Leica Q-Win Q500 IW-DX Image Analyser fitted with Leica Q-Win software and connected to a Sony XC-75 CCD camera. Kernel translucency measurements were optimised by using a light system that involved positioning whole kernels on top of a mask containing round illuminated areas (circles), smaller than the projected areas of the kernels, allowing light to shine through the kernels only. Correction factors allowing for constant illumination of kernels were developed to adjust for kernel size variation in relation to constant light area. Similarly, a correction factor for the effect of kernel thickness on detected translucency values were developed.

Significant correlations were found between corrected translucency values and vitreous and opaque endosperm yields as determined by hand dissection. These were: translucency as a percentage of the whole kernel and vitreous endosperm (mass %) (Translucency 1), $r = 0.77$, $p<0.00001$, and Translucency 1 and opaque endosperm (mass %), $r = -0.72$, $p<0.00001$ for white maize. Similar correlations were found for translucency as a percentage of endosperm (Translucency 2). Correlation coefficients increased significantly after kernel thickness corrections. Significant negative correlations were also found between corrected translucency values and Floating Number. For yellow maize, Translucency 1 correlation coefficients was $r = 0.78$,

$p<0.00001$ and $r = -0.71$, $p<0.00001$ respectively with similar correlations for Translucency 2. Correlations were obtained after applying both correction factors for exposure and thickness.

The IA technique was evaluated for predicting the yield of vitreous endosperm products during dry maize milling in laboratory and industrial-scale milling trials. Significant positive correlations were found between corrected translucency values and yields of milling products from vitreous endosperm. Experiments using a laboratory-scale experimental roller milling test without a degerming stage produced the following correlations: between Translucency 1 and semolina yield (mass %), 0.74, $p<0.001$ and Translucency 2 and semolina yield (mass %), 0.70, $p<0.001$. For industrial-scale milling, a Bühler industrial-scale maize mill (3 tons per hour) was used. The correlation between Translucency 1 and extraction at degermer (degermer overtail yield) was 0.93, $p<0.0001$. There was a similar correlation for Translucency 2. Yellow maize was degermed using a pilot-scale Beall-type degermer and the correlation between Translucency 1 and flaking grits > 3.9 mm was 0.67, $p< 0.001$.

The IA technique permits the non-destructive analysis of maize endosperm translucency on large samples of single kernels. It is suitable for rapid quantification of maize endosperm contents and predicting dry maize milling performance, as kernel translucency was significantly correlated with vitreousness in all instances. With further development of specific hardware and software, the technique has potential as an on-line maize kernel classification system in industrial mills. As the method is non-destructive, it is also suitable for classification of maize seed breeding material. It is also a potential method for the measurement of maize opacity as used by the wet milling industry, where opacity (the opposite of vitreousness) is related to maize starch yield.

UITTREKSEL

BEELDANALISE METING VAN MIELIEPIT LIGDEURLAATBAARHEID EN DIE VERWANTSKAP MET GLASIGHEID EN DROË VERMALINGSEIENSKAPPE

deur

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'n Vinnige nie-destruktiewe beeladanalise tegniek (IA) is ontwikkel vir die bepaling van mieliepit endosperm glasigheid. Pitte is met die Leica Q-Win Q500 IW-DX beeldanaliseerder toegerus met Leica Q-Win standard sagteware en 'n Sony XC-75 CCD kamera ontleed. Ligdeurlaatbaarheidsmetings van pitte is ge-optimiseer deur gebruikmaking van 'n ligsisteem waar heel pitte bo-op ronde verligte oppervlaktes (sirkels) geposisioneer is. Die verligte gebied se oppervlaktes was kleiner as die geprojekteerde oppervlaktes van die pitte en die beligting is regdeur die pitte verkry. Korreksiefaktore is aangebring om konstante beligting van pitte met veranderde groottes op 'n konstante beligtingsoppervlakte te verkry. Korreksiefaktore is ook vir die effek van pitdikte op waargenome ligdeurlaatbaarheidswaardes ontwikkel.

Met behulp van handdisseksie is betekenisvolle korrelasie tussen gekorrigeerde ligdeurlaatbaarheidswaardes en glasige sowel as ondeursigtige endospermopbrengste bevestig. Dit was: ligdeurlaatbaarheid as 'n persentasie van die heelpit (ligdeurlaatbaarheid 1) en glasige endosperm (massa persentasie), $r = 0.77$, $p<0.00001$ en ligdeurlaatbaarheid 1 en ondeursigtige endosperm (massapersentasie), $r = -0.72$, $p<0.00001$ vir witmielies. Soortgelyke korrelasies is vir ligdeurlaatbaarheid as 'n persentasie van endosperm (ligdeurlaatbaarheid 2) gevind. Korrelasies is bereken nadat beide korreksiefaktore ingereken is.

In geval van geelmielies was Ligdeurlaatbaarheid 1 korrelasiekoëffisiënte van r = 0.78, $p<0.00001$ en r = -0.71, $p<0.00001$, met ooreenstemmende korrelasies vir Ligdeurlaatbaarheid 2, gevind. Korrelasiekoëffisiënte het betekenisvol toegeneem nadat pitdikte korreksies aangebring is.

Die IA tegniek is geëvalueer vir die voorspelling van die opbrengs glasige endospermprodukte tydens droë vermalingstoetse in die laboratorium en tydens industriële vermaling. Betekenisvolle negatiewe korrelasies is aangetoon tussen gekorrigeerde ligdeurlaatbaarheidswaardes en flottasie-syfers van heelmielies.

Betekenisvolle positiewe korrelasies is tussen gekorrigeerde ligdeurlaatbaarheidswaardes en vermalingsprodukopbrengste van glasige endosperm aangedui. Eksperimente met 'n laboratoriumskaal eksperimentele rollermeuletoets, sonder 'n kiemverwyderingstap (ontkiemer), het die volgende korrelasies opgelewer: tussen Ligdeurlaatbaarheid 1 en semolina opbrengs (massapersentasie), r = 0.74, $p<0.001$ en Ligdeurlaatbaarheid 2 en semolina opbrengs (massapersentasie), r = 0.70, $p<0.001$. 'n Bühler industriële-grootte mieliemeule is vir industriële proewe (drie ton per uur) aangewend. Die korrelasie tussen Ligdeurlaatbaarheid 1 en ekstraksie tydens ontkieming (produkoorloop) was r = 0.93, $p<0.0001$. 'n Soortgelyke resultaat is vir Ligdeurlaatbaarheid 2 verkry. Geelmielies is m.b.v. 'n loodsaanleg Beall-tipe ontkiemer verwerk en die korrelasie tussen Ligdeurlaatbaarheid 1 en mieliegruis > 3.9 mm was r = 0.67, $p<0.001$.

Die IA tegniek is geskik vir die nie-destruktiewe analise van mielie endospermligdeurlaatbaarheid op 'n groot hoeveelheid enkelpit monsters. Dit is ook geskik vir vinnige kwantifisering van mielie endosperminhoud en droë vermalingspersentasie. Ligdeurlaatbaarheidsmetings is betekenisvol gekorreleer met glasigheid in alle gevalle. Die tegniek kan na verdere ontwikkeling van spesifieke harde- en sagteware vir 'n aan-lyn klassifiseringsisteem tydens industriële vermaling aangewend word. 'n Besondere potensiële aanwending van die nie-destruktiewe tegniek is die klassifikasie van mielietelingsmateriaal. Dit is ook moontlik om mielie ondeursigtigheid ("opacity") as teenoorgestelde van Ligdeurlaatbaarheid) tydens natvermaling te evalueer vir voorspelling van mieliestyselopbrengs.

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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

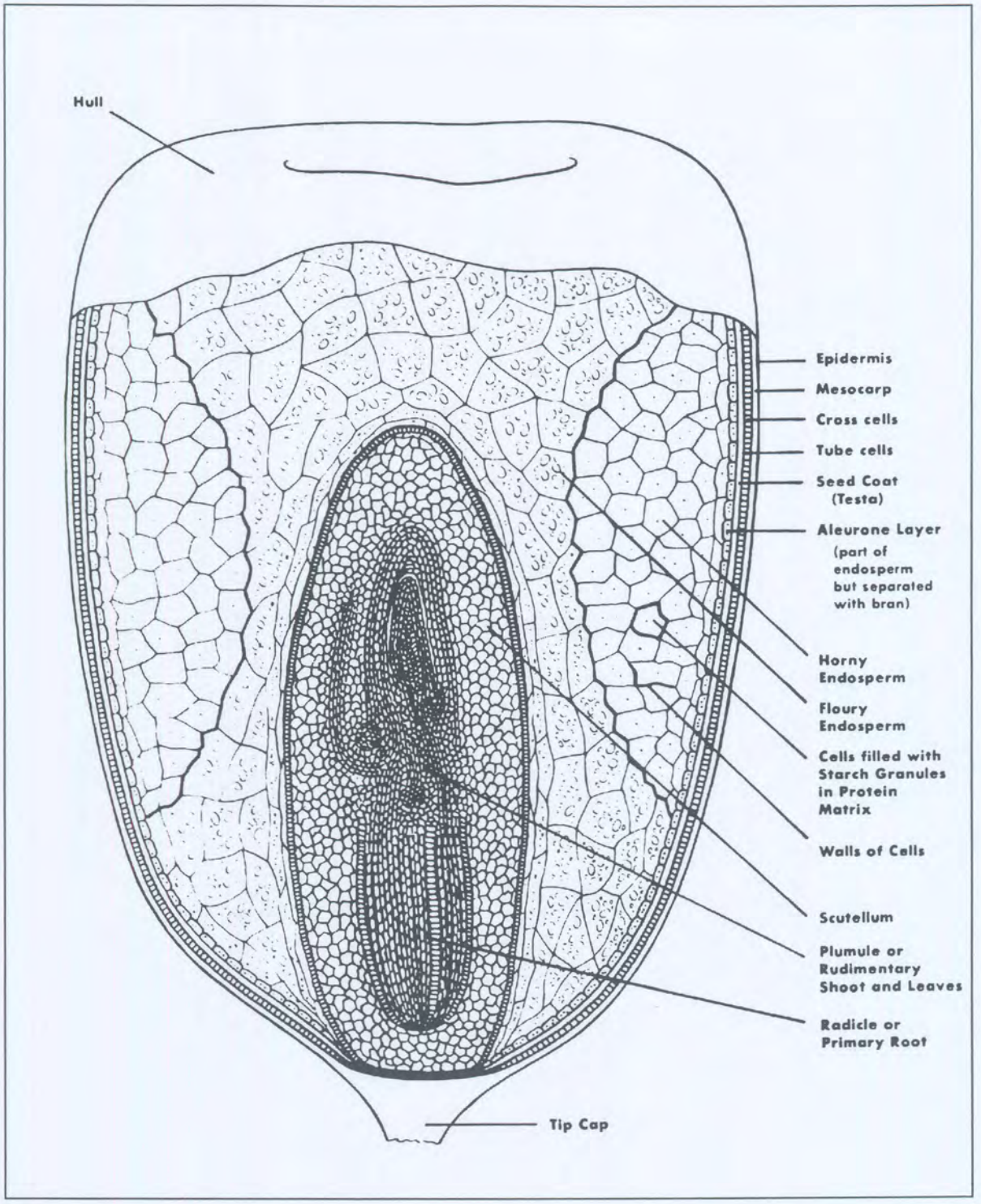
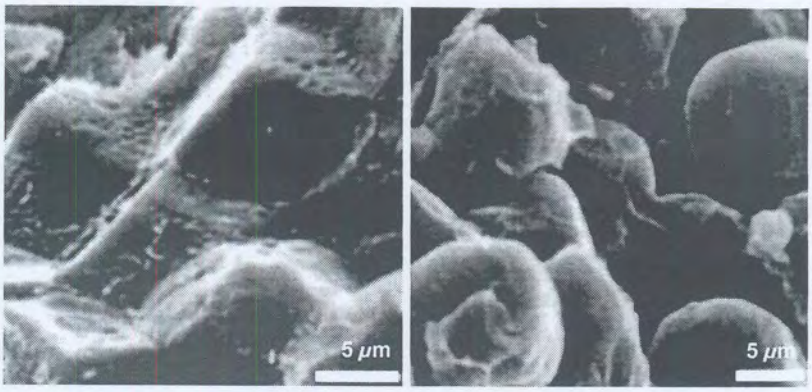


Figure 2.1 Longitudinal section of a dent maize kernel showing the morphology and the different endosperm types (Hoseney 1994)



A B

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).

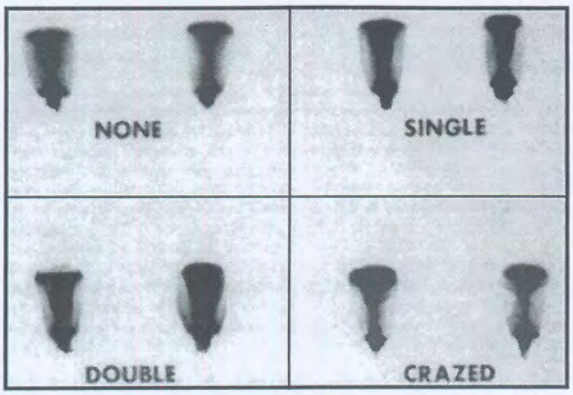


Figure 2.3 Maize kernels placed on top of a light box showing translucent and opaque parts. Maize kernels are also showing stress cracks which can be single, double or multiple (crazed) (Watson 1987a)

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 Hously 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.

CHAPTER 3: DEVELOPMENT OF A NON-DESTRUCTIVE IMAGE ANALYSIS (IA) TECHNIQUE FOR THE QUANTITATIVE MEASUREMENT OF MAIZE KERNEL TRANSLUCENCY

3.1 MATERIALS AND METHODS

3.1.1 Selection and preparation of kernels

Intact cobs of five white and three yellow dent maize industrial cultivars, (F2 segregating hybrids), supplied by Syngenta Seed Co (Pty) Ltd. were used for sampling of kernels. Samples were produced under irrigation at Delmas, Mpumalanga province, South Africa during the 2000 - 2001 growing season. The maize was allowed to dry naturally on the plant on the land before harvesting. Only sound intact maize kernels were used for IA and vitreousness measurements. Kernels considered damaged were: discoloured (including yellow kernels on a white cob due to accidental cross-pollination), poorly developed (obvious lack of proper endosperm development compared to the rest of the kernels on the cob), deformed (for example germ positioned on an abnormal place on the kernel), or abnormally small (relative to the other kernels on the cob, or less than 4.0 mm in length) kernels or those showing chips, fractures, stress cracks, or mechanical, insect or fungal damage. Kernels were removed from the cob by hand, then cleaned using a 4.0 mm opening sieve. Kernels were gently rubbed over the sieve by hand to remove excessive small kernels, pieces of bran coming from the cob and any other debris. After cleaning, kernels were stored in dry plastic containers with lids in the cool and dark. Where applicable for large samples, kernels were counted using a seed counter. Damaged kernels were removed from the samples before analysis. The number of kernels removed per 100 kernels varied from 5 to 15. A few of the cobs showed clear signs of fungal damage, leading to a higher proportion of damaged kernels. As the cultivars were dried naturally on the land, the percentage of stress cracks was low and most unsound kernels were removed due to size (too small) or fungal damage.

Table 3.1 Description of the South African maize cultivars used in the experimental work

Description	Allocated code number	Genetic history	Colour
SR 52 Bethal 00/01*	1	F2 cross	White
L390 I/K., 500/01: 434	2	F2 cross	White
CRN 3549 00/01: 429	3	F2 cross	White
R827/I, 500/01: 435	4	F2 cross	White
CRN 3549 500/01: 439	5	F2 cross	White
N282/FO, 500/01: 467	6	F2 cross	Yellow
N290, K, 500/01: 460	7	F2 cross	Yellow
N258 K/I, 500/01: 460	8	F2 cross	Yellow

* "01" referred to the year of harvest (August 2001)

3.1.2 Image Analysis

Kernels were analysed using a Leica Q-Win Q500 IW-DX Image Analyser (Leica Imaging Systems Ltd., Cambridge, United Kingdom) fitted with Leica Q-Win standard Microsoft Windows compatible software (Windows 95 software). The system was also fitted with a Sony XC-75 CCD Camera B/W (Sony, Tokyo, Japan) with 560 lines (fitted with a 35 mm zoom lens), a standard resolution image capture board (600 dpi) and a standard personal computer system and a 17 inch high resolution monitor. The spatial resolution of the images was 764 X 575 pixels and the calibration factor was 0.22 mm/pixel (both for x and y).

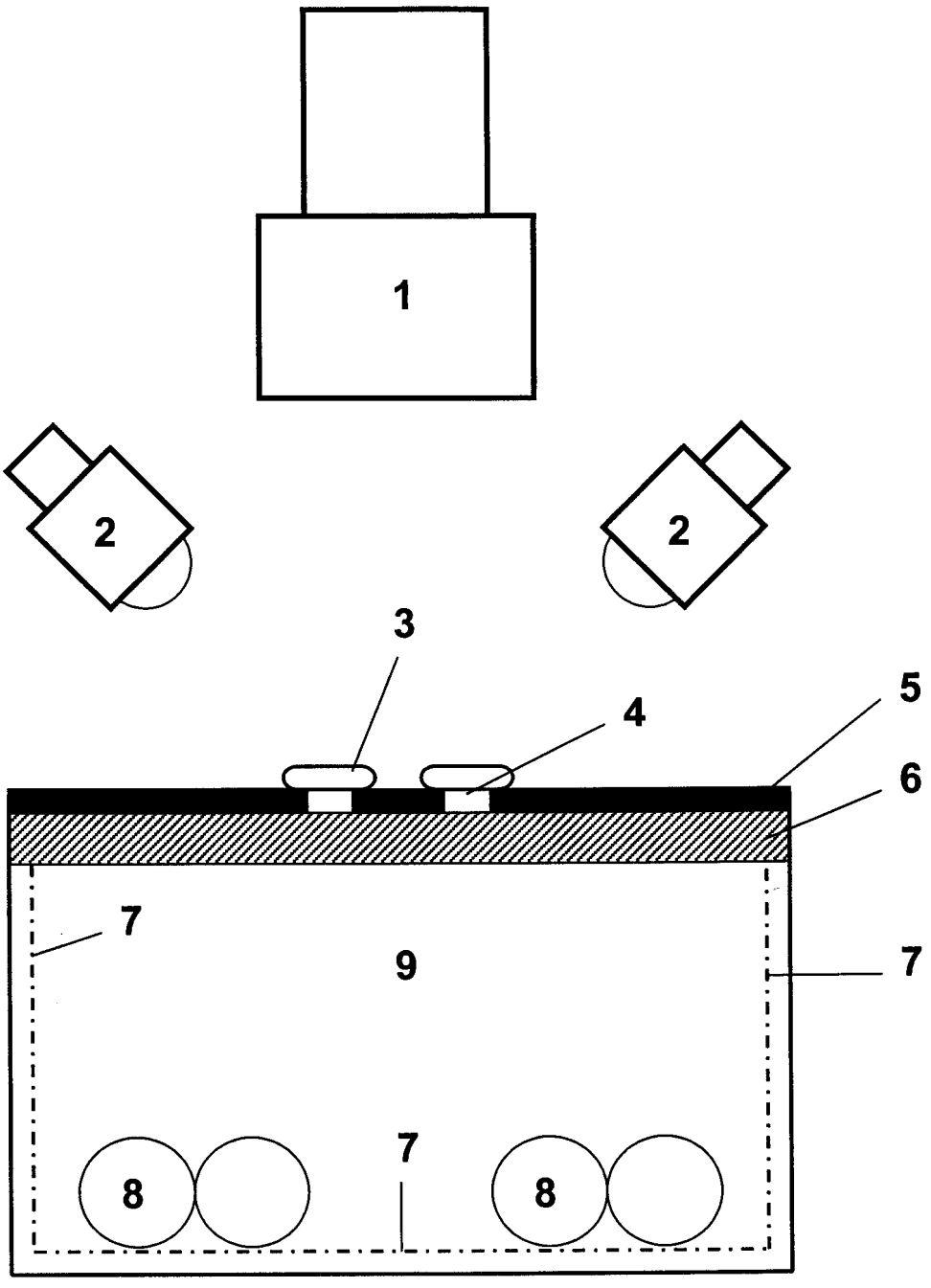


Figure 3.1 General design of maize translucency detection equipment: 1, digital camera with 35 mm lens; 2, incandescent lamps (40 W) for kernel illumination from above; 3, maize kernel positioned on top of circle; 4, round hole in black paper (circle); 5, black paper cover; 6, white Perspex layer; 7, mirrors covering inside walls; 8, double-tubed fluorescent lamps (11 W); 9, light box

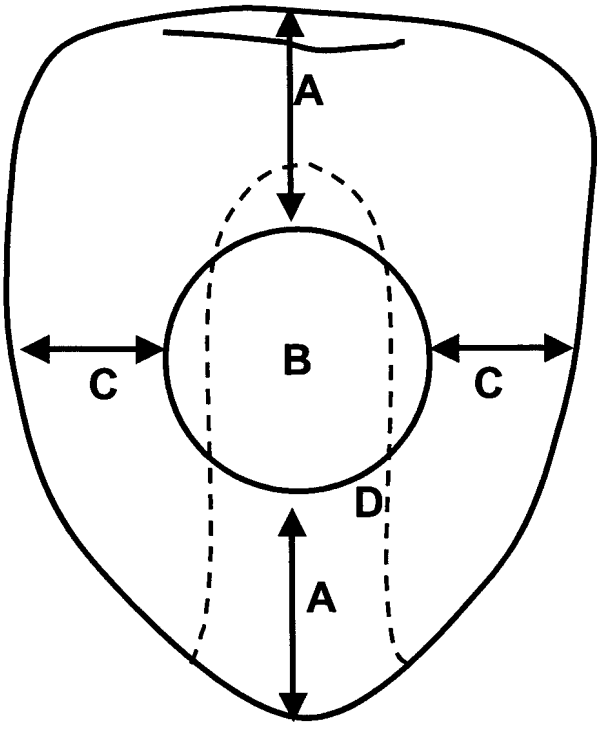


Figure 3.2 The positioning of an intact whole maize kernel on a circle to achieve translucent images. The distances marked "A" were the same and the two distances marked "C" were the same. Area B shows the position of the light circle beneath the maize kernel and line D shows the border of the germ on top of the kernel

Maize kernels were placed on a wooden light box (Fig 3.1), with a length and breadth of 50 cm and a depth of 30 cm. Two double-tubed fluorescent lamps (11 W, Osram Dulux G23 energy savers of 900 Lumen intensity, Lumilux, Italy) were placed next to each other inside the box. The box was fitted with mirrors totally covering all inside surfaces except the top surface. White Perspex (3 mm thick) was placed over the top and covered with black paper with round holes (circles) punched into it to resemble a mask. By placing kernels on top of the circles in the mask (Fig 3.2), partial illumination from below was achieved, instead of full illumination achieved by using modelling clay surrounding individual kernels on the light table (Felker and Paulis 1993). Light produced from uncovered areas was screened off as the circles were smaller than the kernels. Four additional incandescent lamps (40 W soft white, Osram, Italy) for illumination of the kernels from above were also placed

above the unit (Fig 3.1). All work was done in a dark room and a black cloth for additional covering of the camera and light box stand including all light fixtures was used. Three attributes per kernel were measured: 1, The projected kernel area (reflected light image); 2, Projected area of translucent endosperm (gray transmitted light image through the circle mask) and 3, Germ size measurement as determined by hand editing. The three measurements were combined for each kernel for analysis. Seven different sized circles were investigated (Table 3.2).

Table 3.2 Sizes of the circles in the paper mask used as a light source for detecting maize kernel translucency

Circle surface area (mm^2)[a]
17.2 (0.19)
29.6 (0.58)
43.8 (0.70)
48.3 (0.60)
66.2 (0.85)
88.4 (0.91)
113.4 (0.86)

a Means and standard deviation of circles

The IA system was set-up to accommodate a total of 49 (7 x 7) kernels simultaneously. Depending on the lens size, a large number of kernels can be measured simultaneously (100 or more circles are possible on the light box). The camera was set up at a fixed calibration and no changes were made during measurements. The camera was used with the shutter fully open and the Image Analyser video intensity gain/offset signals were adjusted for maximum picture contrast. For this system the gain/offset percentages were 50%/25%. At maximum contrast, the image background (black mask) had a gray value of 0 (black). Image brightness was optimised by ensuring that all gray levels were stretched as much as possible across the spectrum from

black to white (0 – 255). Transmitted light images of each kernel were captured after kernels were illuminated through the circle in the paper mask (Fig 3.2). The gray threshold level was set at a minimum of 54 for white kernels and at 44 for yellow kernels. All images captured were in monochrome (gray). The images were segmented for the gray value range 54 to 255 for white and 44 to 255 for yellow kernels, creating a binary mask image used for the measurement of the total translucent area of the kernels. The minimum threshold levels of 54 for white maize and 44 for yellow maize were chosen because they were sensitive enough to detect gray pixels from the samples with a small percentage of translucent endosperm, while still allowing sufficient scope for detecting pixels from samples with a large percentage of translucent endosperm, with a minimum of overexposure. By using the above levels, 201 levels of gray for white and 211 levels of gray for yellow maize per pixel can be detected allowing for a sufficient range of detection. Binary images were edited after detection using the features available in the Image Analyser software (Leica Imaging Systems Ltd., Cambridge, United Kingdom).

Translucency was measured with the germ facing up towards the camera. This was to allow sufficient light to enter the kernel via the circles without unnecessary scattering caused by the germ, as the germ covers most of the light exposure area. Although moisture content did not influence the readings significantly, excessive moisture ranges were avoided. A kernel moisture range of 10 – 14% (g moisture/100 g kernels) was used.

3.1.3 Effect of humidity exposure on the translucency of intact maize Kernels

As the effect of humidity was a concern with regard to its possible influence on translucency measurements, the translucency of kernels before and after exposure to a high humidity environment was measured. Three intact maize kernels of three cultivars of known moisture content determined by AACC method 44-18 (American Association of Cereal Chemists 2000) were

selected. The kernels were taken from samples stored at ambient conditions (Pretoria Relative Humidity fluctuating between 30 and 60% during the summer). Translucency was measured in triplicate as described in section 3.1.2. The kernels were then placed in a desiccator containing a saturated potassium dichromate solution (relative humidity 98%) (Stokes 1948). The container with the kernels was left at 25°C for 5 days. After exposure to the high humidity level, the translucency of the kernels was measured again.

3.1.4 Effects of translucency measurement methodology

3.1.4.1 Orientation of kernel position in relation to the direction of detection

Three white kernels were used for the assays. Measurements were made in triplicate on the same sized circle (29.6 mm^2). Measurements were done using two kernel orientations, namely vertical with kernel germ and tip cap direction facing 90° and horizontal with kernel germ direction facing 0°. The germ side faced the camera lens.

3.1.4.2 Binary amendment

Although some maize kernels gave a continuous translucent area with detection, most kernels gave two separate translucent areas. This was due to the distribution of opaque endosperm in the South African hybrids. Most of the opaque endosperm is in the middle section of the kernel. Therefore, most cultivars produced an image consisting of two vitreous sections on either side of the opaque middle section. This was not a problem if only one kernel was analysed, but when multiple kernels were analysed simultaneously, it was necessary to combine the area measurements of the two sides of each kernel by using the available "amendment" software.

Three types of amendments were tested to combine the area measurements for each kernel. A set of three kernels was used. Kernels, circle size, gray thresholds and orientation were kept the same.

Binary amendments tested were (Leica QWin User Guide 1996):

- Amendment one - the area measurements of each separate area combined manually.
- Amendment two - the combined area measurements using the vector element.
- Amendment three - the combined area measurement using the cross erosion and dilation element.

3.1.4.3 Repeat readings on the same kernels

As the video image was a live image, it could have been influenced by minute fluctuations in intensity caused by background noise such as electricity signals. Measurements were made in triplicate on the same kernels to determine the precision of the instrument. The standard deviation obtained gave an indication of the precision of the results. Pictures were taken of each step during the detection and IA procedure. This was followed by analysis of variance on the triplicate measurements on three kernels in order to determine the precision of the measurements. Tukey's honest significant difference (HSD) test was done using SAS PROC GLM procedures.

3.1.4.4 The effect of circle size on kernel illumination

Triplicate readings on three white kernels measured with four different circle sizes were done. Kernels were analysed as shown in Figure 3.2. Analysis of variance was done followed by paired comparison tests (Tukey's HSD). Tests were also done for interactions. The objective was to determine if a change in circle size would result in comparative changes in translucent area sizes for different kernels. If the circle size was increased at a fixed ratio, it was

expected that the detected area size would also increase at a similar ratio. It was expected that this ratio increase would be the same for all kernels. A significant interaction between individual measured kernels and circle size would have meant that additional variables such as the kernel size and thickness had a significant effect on the accurate detection of translucency in maize.

3.1.5 Optimisation of measurements

Measurements were optimised to allow for a fixed ratio between circle area and maize kernel area. Before a formula could be developed for calculating all maize kernel areas and adjusting detected translucent areas to produce values for fixed circle area/kernel area ratios, a series of calibration curves had to be developed. All maize kernels differ in size and shape and the size of a kernel would have an additional effect on the detected intensity of the translucency. In practise, it is not possible to adjust gray levels to allow for individual kernel size and shape and therefore, a fixed circle size was selected followed by the development of a calibration formula to adjust readings obtained for each kernel to a fixed circle area/kernel area ratio. The actual detected translucent area for a maize kernel was expected to be influenced by the following variables:

- Proportion of vitreous endosperm
- Size of the maize kernel
- Ratio of the circle area to the projected kernel area (bigger kernels will absorb more light to give lower readings)
- Thickness of the maize kernel
- Colour of the maize kernel.

3.1.5.1 Calibration of the fixed circle method of light exposure with the modeling clay method

Using modelling clay to surround each maize kernel on a light box, effectively produces a "circle" area/kernel area ratio of one, as the "circle" area is the same as the kernel area. The "circle" in this case was not round, but the same shape as the kernel area. This is the most correct method, but it is impractical to implement on a large scale, as it would require each kernel to be embedded individually with clay. By using a fixed sized circle slightly smaller than the kernels, with kernels placed on the circle all light was shone through the kernels. However, as the area of the circle was fixed, only a fixed amount of light shone through and kernels with larger areas gave lower readings than they should. Kernels with smaller areas gave higher readings than they should have. A calibration curve was therefore developed in order to allow all readings on all kernels to be adjusted to a fixed circle area/kernel area ratio to produce comparable results to those possible from the modeling clay method.

Three white kernels of one F2 hybrid of a South African cultivar were used for this experiment. The areas of translucent endosperm in the three maize kernels were measured using modeling clay on the IA. Background light was excluded using the modeling clay. Each kernel was then measured again in triplicate using a range of 7 different sized light circles, as described in Table 3.2. Gray value ranges for image segmentation for the kernels illuminated through the circles in the paper mask were adjusted until the same sized (area) binary image masks were created as those produced by using modelling clay. These data were analysed statistically and used to develop a relationship between the intensity of the gray pixel levels and the ratio of the light circle area to the area of the kernel. The relationship was then used later to correct for the readings on each individual kernel based on the kernel size and area.

Exposure percentage (EX) was calculated as follows:

$$EX = \frac{\text{Circle area } (mm^2)}{\text{Projected maize kernel area } (mm^2)} \times \frac{100}{1}$$

Analysis of variance was performed (LSD paired comparison test) to determine if the measured translucent areas were the same at every exposure percentage (EX). Eight exposure percentages (seven using fixed circles of different sizes and one using the modeling clay or kernel area) were tested and the hypotheses tested were:

Ho: T1=T2=T3=T4=T5=T6=T7=T8
Ha: T1≠T2≠T3≠T4≠T5≠T6≠T7≠T8.

T = Mean area of translucent endosperm at eight levels of EX.

The correlation coefficient (Pearson) between the EX and the determined minimum gray threshold detection level was determined following fitting a linear regression line to the data.

3.1.6 Translucency correction factors

3.1.6.1 Corrections for exposure

Three intact kernels were taken from the middle section of one cob from each of the cultivars for light intensity tests. In order to have the same number of white and yellow kernels for the analysis, three additional yellow kernels were taken from cultivars 7 and 8 (Table 3.1). The maize kernels were analysed in groups of three per cultivar. Each set of three kernels were subjected to light exposure using four different sets of circles, according to the method described in section 3.1.2.

The areas of the detected translucent parts were used for the calculation of the increase in translucency at a constant gray threshold level, but with increased exposure. A constant gray threshold level was used for all measurements and the level selected was all gray pixels on levels 54–255 for white maize and a level of 44–255 for yellow maize.

Projected maize kernel area was measured after measuring the translucency and an algorithm for the two detections was programmed into the software (Leica QWin User Guide 1996). The increase in translucency as a function of increased circle size in relation to projected kernel area size was calculated as follows for each exposure area bigger than the reference (for the same maize kernel):

The translucent area increase (TI) was expressed as a percentage as follows:

$$TI = \frac{(T_l - T_r)}{T_r} \times \frac{100}{1}$$

T_l = Translucent area at larger circle area

T_r = Translucent area at reference circle area

With the above formula, the translucent area increase (TI) will have a value of 0 for the reference exposure area. Calibration curves were plotted for all five cultivars with TI (Dependent variable) and EX (Independent variable). Linear regression lines were fitted for each cultivar followed by an adjustment in order to have a fixed EX at a zero point TI of 0%. With surrounding the kernels with modeling clay, 100% of the projected kernel area was exposed to light and these measurements were used as reference points at 100% EX. After the adjustment, the data were combined to produce a calibration curve. The resulting regression lines were used for future correction of translucent area measurements on maize kernels placed on fixed-sized light circles in the paper mask. Corrections were achieved by measuring the projected areas of individual maize kernels produced from a reflected light image followed by adjusting the size (area) of the detected binary translucent area mask images accordingly using the regression line. These calculations therefore took into account the size effect of maize kernels on the intensity of the transmitted light

images. Based on the optimum circle size as shown in Fig 3.2, a fixed circle of size 29.55 mm^2 was selected for further calculations.

Corrections for exposure were made to measure all kernels at a fixed ratio of circle area vs. projected kernel area using the following formulas:

$$\text{True translucent area } (mm^2) = \frac{\text{Thickness adjusted translucent area } (mm^2)}{\text{Correction factor for exposure}}$$

Correction factor for exposure = 1 + (TI/100), where TI = (4.02 x EX) – 55 (white maize), and EX = (circle area (mm^2)/Total kernel area (mm^2)) X 100.

Thickness adjustment is discussed in section 3.1.6.2.

The relationship between TI and EX for white maize was determined by linear regression of the calibration curve where TI = 4.02 EX – 55.
($r = 0.91$, $R^2 = 0.83$, $n = 60$, $p < 0.001$).

The relationship for yellow maize was: TI = 3.58 EX – 47, $r = 0.90$, $R^2 = 0.81$, $n = 60$, $p < 0.001$.

The translucency percentages using the corrected translucency values were calculated as follows:

$$\text{Translucency 1} = \frac{\text{True translucent area } (mm^2)}{\text{Projected kernel area } (mm^2)} \times \frac{100}{1}$$

$$\text{Translucency 2} = \frac{\text{True translucent area } (mm^2)}{\text{Endosperm area } (mm^2)} \times \frac{100}{1}$$

Endosperm area (mm^2) = Projected kernel area (mm^2) – germ area (mm^2)

3.1.6.2 Corrections for kernel thickness

A thickness correction factor was calculated by selecting ten kernels from each cultivar, measuring the translucency and the corresponding kernel thickness. The same kernels were then sanded on the flat side opposite from the germ with fine sandpaper to reduce the thickness by 0.5 mm increments. Translucency was measured at each individual thickness. Sandpaper with a grit size of 1000 was used in order to produce a smooth polished kernel surface. The seed coat layers of the kernels were transparent as only maize cultivars with a clear pericarp were used. Kernels with a coloured pericarp due to fungal or other damage were not measured. Measurements on the sanded kernels were discontinued when the germ was exposed after removing successive layers of tissue. The percentage increase in translucency was calculated for each kernel using the non-sanded measurement as a basis. Data were combined and a linear regression line fitted to the data. The resulting thickness correction factor was then used to adjust translucency readings together with the Exposure percentage (EX). Separate values were determined for white and yellow maize. The thickness of the kernels was measured by standing kernels on their sides and the distance between the top and bottom edges measured using Image Analysis. The average of five thickness measurements spaced evenly along the longitudinal axis of each kernel was calculated. Forty nine kernels were measured per cultivar and the mean value of each cultivar used for the thickness correction calculations.

3.1.7 Vitreousness determinations on single kernels (mass fraction)

3.1.7.1 Hand dissection of maize kernels

As the maize kernels were too hard for any of the dissection methods described in the literature (Louis-Alexandré, Mestres and Faure 1991; Yuan and Flores 1996; Dombrink-Kurtzman and Knutson 1997), a modified method was developed. Intact maize kernels (49) from each of the eight cultivars

were used. Each individual maize kernel was weighed (four decimal places precision). After weighing, the kernels were subjected to IA to measure the translucency and thickness and the values were adjusted according to the calibration curves. Each kernel was numbered after IA using a water resistant black marker pen from no 1 – 49 with the number corresponding to the number allocated by the Image Analyser. The numbered kernels were placed in plastic containers and filled with distilled water. The containers were sealed and the kernels soaked at 4°C for five days. After soaking, each kernel was weighed after excess water had been removed from the kernels using soft tissue paper. After weighing, each kernel was sectioned open longitudinally using a scalpel to obtain two flat halves. The two halves were then brushed with a Jordan V toothbrush (Junior size) under running water to remove all visible opaque (mealy) endosperm. Any opaque endosperm that could not be brushed out, was carefully scraped out using a scalpel.

After removal of opaque endosperm, the kernels were again weighed (the two halves together) after excess water had been removed using soft tissue paper. After weighing, the vitreous endosperm, germ and bran were separated using a scalpel. The vitreous endosperm was weighed after the separation. Vitreous endosperm was determined by weighing the actual dissected vitreous portions of each kernel after cleaning, while opaque endosperm was determined as the mass difference after brushing. The moisture content of each cultivar was determined before soaking on maize kernels from the same cob of which the dissected kernels were obtained using AACC method 44-18 two-stage drying (American Association of Cereal Chemists 2000). Moisture contents varied from 10.1 to 12.9%.

3.1.7.2 Calculation of the yield of vitreous and opaque endosperm

The dry masses of all weighed fractions of each maize kernel were calculated using the moisture content data obtained from the determination on the cultivar before soaking. The moisture content after soaking was calculated for

each individual kernel using the weight increase of the kernel after soaking. It was assumed that the moisture contents of the kernels after brushing and the vitreous endosperm after dissecting was the same as that of the whole kernel after soaking. To reduce the possibility of moisture loss during dissection, each kernel was dissected and weighed before the next kernel was taken out of the soaking water. The brushing of the kernels under running water also helped to reduce moisture loss during dissection. The yield of vitreous and opaque endosperm for each individual kernel was calculated as:

$$\text{Vitreous endosperm (\%)} = \frac{\text{Dry mass of vitreous endosperm}}{\text{Dry mass of whole kernel}} \times \frac{100}{1}$$

$$\text{Opaque endosperm (\%)} = \frac{\text{Dry mass of whole kernel} - \text{dry mass after brushing}}{\text{Dry mass of whole kernel}} \times \frac{100}{1}$$

The above calculations were also done without corrections for moisture.
Significant differences between different cultivars were tested using the Kruskal-Wallis (Keller and Warrack 2000) test for non-parametric data where sample residuals did not show normality, otherwise analysis of variance was done.

3.1.8 Statistical calculations and the development of regression models between translucency and endosperm yields

Analysis of variance and Tukey's HSD were performed on the results where applicable. The Kruskal Wallis test was performed on data where residuals did not show normality. Pearson correlation coefficients were determined followed by linear regression where applicable. The analysis were done in SAS (SAS 1989) using PROC REG, PROC GLM, PROC UNIVARIATE ((test for normality) and PROC CORR. All data were considered significant at $p < 0.05$. Data with smaller p-values (higher significance) were labeled accordingly. Z-transformations were done for the Pearson correlation

coefficients in order to test whether changes in the correlation coefficients were significant. Data were interpreted according to the tables provided by Diem and Seldrup (1982).

Correlations and regression calculations were done and levels of significance were calculated. Slopes for the regression models fitted were also tested for significance. Residuals of IA data were normally distributed and therefore no significant differences existed between mean and median values of the data hence only the mean and standard deviation values were calculated for all data. The correlation coefficient r was determined as a measure of the stochastic dependence of the dependent and independent variables used for determining relationships between IA measurements and milling yield data. A standard error of the regression slope was calculated along with a t-test to determine if the slope differed significantly from the horizontal or not. With this specific test, conclusions can be made on the data independent of the units of the graphs on the axes. A relationship may have a significant slope, but may seem flat on a line diagram because of the units of measurements or *vice versa*. Single tailed correlations and t-tests were used throughout the experimental work as the relationships tested were predicted for example higher translucency values were expected to predict a higher yield of vitreous endosperm. The exposure only corrections were not shown in the results, only the thickness corrections and the combined effects of thickness and exposure.

3.2 RESULTS

3.2.1 Image set-up

Pictures of the various stages in the procedure used for capturing images of vitreous endosperm in maize are shown in Figure 3.3.

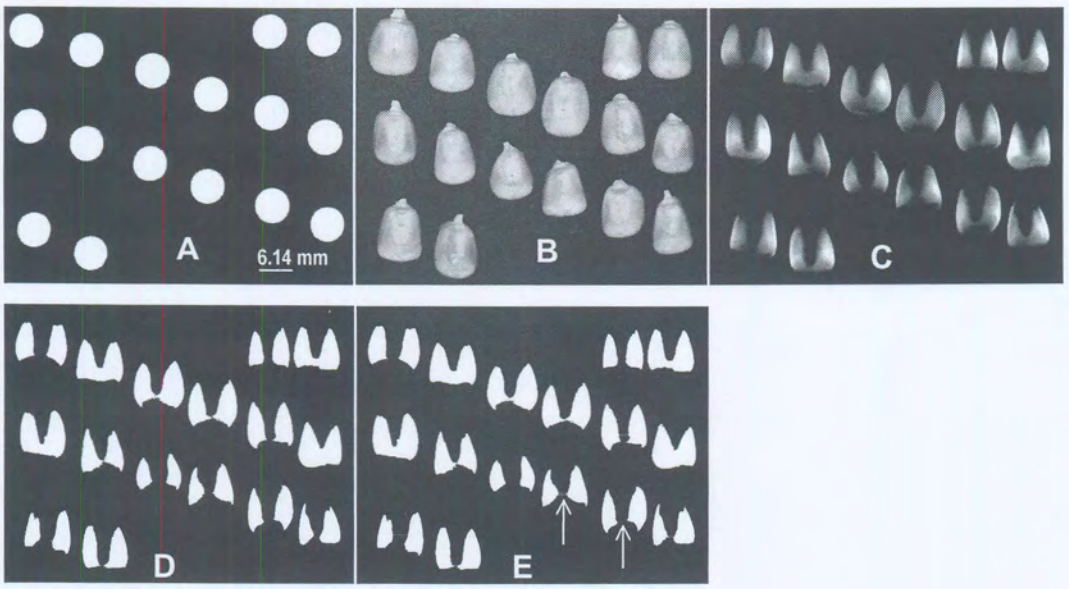


Figure 3.3 The appearance and detection of maize kernels on the light box. A, appearance of circles with light source below before maize kernels were placed on top; B, intact kernels with illumination from underneath and above; C, translucent endosperm visible with illumination from underneath through the paper mask; D, computer generated detected areas covering the translucent parts generated in C; E, paired sections of the areas connected using the vector element (thin connecting lines – see arrows) in order for the computer to calculate the areas as a combined single area for a kernel. All pictures are at the same magnification

3.2.2 Effect of humidity exposure on the detected translucent area of intact maize kernels

The results of the translucency measurements done on three kernels of three white maize cultivars before and after exposure to a relative humidity of 98% are shown in Table 3.3.

Table 3.3 The effect of air relative humidity on the detected translucent area (mm^2) measured in three white maize cutivars

Treatment	Cultivar 1	Cultivar 2	Cultivar 3
Before humidity exposure	79.22 (4.76)[a]**	81.34 (6.43)[b]*	49.82 (6.96)[c]
After humidity exposure	75.62 (3.17)[a]	82.62 (2.71)[b]	48.46 (8.89)[c]

* Mean and standard deviation

** Different superscripts in columns indicate statistically significant differences ($P<0.05$)

Results show that no significant differences existed between detected translucent areas of kernels before and after humidity exposure (Table 3.3).

3.2.3 Effects of translucency measurement methodology

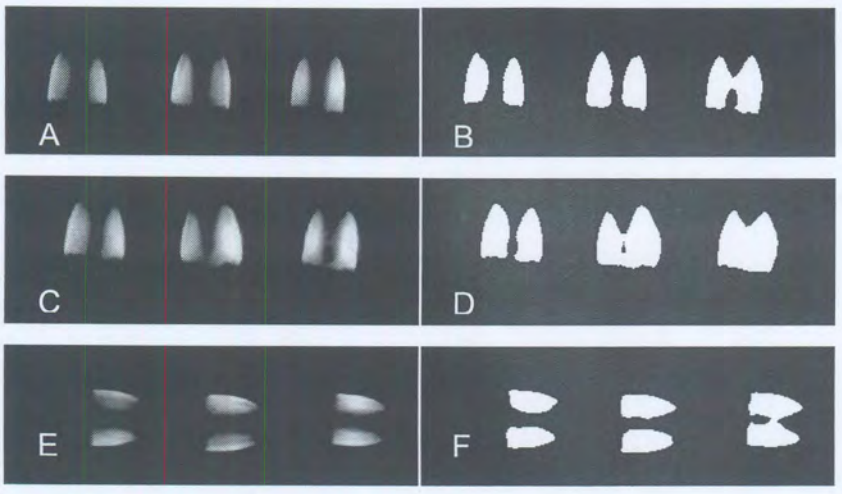


Figure 3.4 The effect of changing circle size on the appearance and size of the translucent area of the same maize kernels. A, captured image for three maize kernels, circle size at 29.5 mm^2; B, detected pixel area at circle size of 29.5 mm^2 (gray threshold between 54 and 255); C, captured image for the same three maize kernels, circle size at 43.77 mm^2; D, detected pixel area at circle size of 43.77 mm^2 (gray threshold between 54 and 255); E, similar to A, but at a different orientation; F, similar to B, but at a different orientation

The effects of changing circle size on the translucent area detected and the effect of kernel orientation are illustrated in Figure 3.4. The larger circle size (C and D) caused larger binary images if pixels were detected in the same intensity range as compared to smaller circle size (A and B). Kernel orientation had no effect on the shape and size of the binary images.

Measurements of the detected translucent areas at constant gray levels at four different circles are given in Table 3.4. The effect of binary amendment is shown graphically in Figure 3.5.

Table 3.4 The effect of circle size on the detected translucent area (mm^2) at constant gray level and constant amendment for three white maize kernels measured at each of four circle sizes

Circle size (mm^2)	**Kernel 1**	**Kernel 2**	**Kernel 3**
17.2	34.72 (1.49) [a*]	33.75 (1.15) [a**]	38.52 (0.19) [e]
29.6	57.04 (0.36) [b]	52.46 (0.37) [f]	62.11 (0.27) [g]
43.8	78.12 (1.25) [c]	71.22 (1.02) [h]	78.79 (0.93) [c]
48.3	88.86 (0.92) [d]	83.33 (0.37) [j]	84.05 (0.85) [i]

* Mean and standard deviation of triplicate measurement on each kernel

** Different superscripts in rows as well as columns refer to statistically significant differences (Tukey HSD test, paired comparisons, $p < 0.001$).

The results in Table 3.4 show that with increased circle size, the detected translucent area also increased for each kernel due to the increased amount of light entering the kernels. There was also small variation in the detected size of the same kernel with the same circle size.

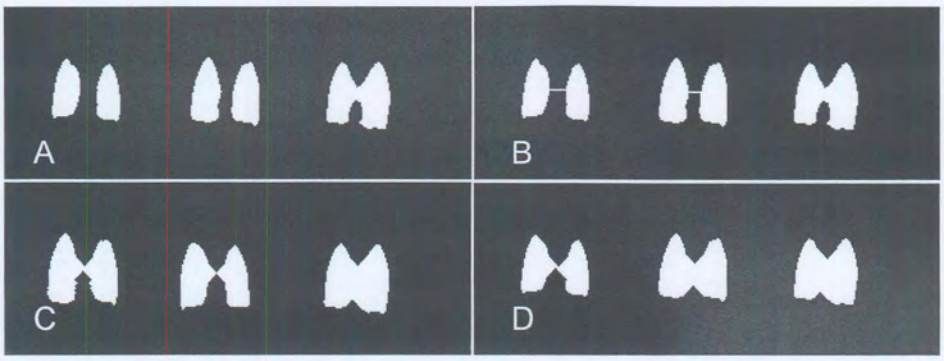


Figure 3.5 Comparisons between different techniques of combining detected surface areas earmarked for measurement. A, detected binary; B, correcting areas using the vector function; C, connecting areas using the amend (dilation and erosion function – cross element); D, connecting areas using the amend function (dilation and erosion function – octagon element)

The effect of kernel orientation and amendment method as illustrated in Figures 3.4 and 3.5 on the measured detected translucent area is shown in Table 3.5. Figure 3.5 clearly shows an effect of amendment method on the distortion of the binary image covering the detected translucent areas of the kernels. Amendment B (linking areas with a thin line) had the least amount of visible distortion to the areas.

Table 3.5 The effect of kernel orientation, binary amendment method and repeat analysis on the detected translucent area (mm^2) of maize kernels

Description	Orientation (detected area, mm^2)	Description	Amendment (detected area, mm^2)	Description	Repeat Analysis (detected area, mm^2)
Vertical (90°)	58.9 (4.9) [a]*	No amendment***	58.6 (4.0) [a]	First analysis****	58.3 (4.8) [a]
Horizontal (0°)	58.1 (4.9) [a]***	Vector amendment	58.6 (3.9) [a]	Second analysis	58.9 (5.2) [a]
		Dilation and erosion amendment	62.5 (4.3) [b]	Third analysis	58.3 (4.9) [a]

* Mean and standard deviation

** Different superscripts in columns refer to statistically significant differences ($P< 0.01$)

*** No amendment (manual adding of detected areas)

**** Repeat analysis was done on the same set of kernels

Kernel orientation had no effect on the detected translucent areas (Table 3.5). Amendment method did have a significant effect on the detected translucent areas. The vector amendment did not increase the measurements significantly, but the dilation and erosion amendment did increase the measurements significantly (as was also visible in Figure 3.5). Repeat analysis produced results which were not significantly different from each other, indicating that the set-up of the measurement system as described was stable and precise after the initial settings were completed.

3.2.4 Optimisation of measurements

Table 3.6 Comparison between the mean detected translucent areas (mm^2) of maize kernels of different sizes (three kernels per size) measured using a constant circle (29.6 mm^2) and modeling clay for light exposure

Size of kernels**	**Size 1 (mm^2) 96.9 (3.1) ***	**Size 2 (mm^2) 154.7 (9.9)**	**Size 3 (mm^2) 104.7 (2.6)**	**Size 4 (mm^2) 77.9 (2.3)**
Circle (29.6 mm^2)	29.8 (1.7) [a**]	72.9 (6.4) [b]	85.2 (0.1) [c]	0.32 (0.17) [d***]
Modeling clay	29.7 (3.0) [a]	86.7 (5.5) [c]	85.4 (2.7) [c]	0.16 (0.10) [d***]

* Mean and standard deviations

** Different superscripts in rows as well as columns indicate statistically significant differences (LSD analysis of variance, $p<0.05$).

*** These kernels had very low levels of translucency (they were almost completely opaque).

**** The size of the kernels was the total projected area of a kernel laying flat as detected by the camera, three kernels of similar size were selected for each size group and standard deviations show size variation between three kernels in the group

The results in Table 3.6 show that different area sizes were detected for size 2, but the same areas were detected for the other sizes when comparing the two methods (modeling clay vs. fixed circle). This indicated that the areas detected using modeling clay cannot be repeated using a fixed circle for illumination and other unidentified factors were present that influenced the results.

The effect of the exposure percentage (EX) on the gray threshold detection level to produce the same translucency area on the same maize kernels at eight different exposure ratios is shown in Table 3.7. A plot of the measurements is shown in Figure 3.6 with a linear regression line fitted. The Pearson correlation coefficient was determined for the data followed by a fitted linear regression line.

Table 3.7 The effect of exposure percentage (EX) on the gray threshold detection level necessary to produce the same translucent area (mm^2) on the same maize kernels

Description	Setting 1*	Setting 2	Setting 3	Setting 4	Setting 5	Setting 6	Setting 7	Setting 8***
Circle area (mm^2)	17.2 (0.2) [a]**	29.6 (0.6) [b]	43.8 (0.7) [c]	48.3 (0.6) [c]	66.2 (0.9) [d]	88.4 (0.9) [e]	113.4 (0.9) [f]	154.6 (1.0) [g]
Exposure percent EX (%)***	11.3 (0.9) [a] ******	19.2 (1.5) [b]	28.4 (2.2) [c]	31.3 (2.1) [c]	42.9 (3.2) [d]	57.3 (3.5) [e]	73.5 (4.3) [f]	100 (0.0) [g]
Gray threshold for detecting the same translucent area at each circle size	9****	32	60	69	97	144	168	244
Actual detected translucent area (mm^2) at each circle	86.5 (2.1) [a]	86.6 (5.7) [a]	86.7 (2.5) [a]	86.7 (2.7) [a]	86.5 (6.0) [a]	86.4 (5.1) [a]	86.6 (6.5) [a]	86.7 (5.5) [a]

* Setting number using the same three maize kernels (projected kernel area was 154.6 (1.0) mm^2 in all instances as the same kernels were used)

** Different superscripts within rows indicate statistically significant differences (LSD Analysis of variance, $p < 0.05$)

*** Setting 8 = modeling clay with an exposure percentage of 100 (kernel area/kernel area x 100)

**** The same threshold level was used for the three kernels within each group (threshold levels were not adjusted for individual kernels as well)

***** EX = (Circle area/Total kernel area) x 100

****** Mean and standard deviation

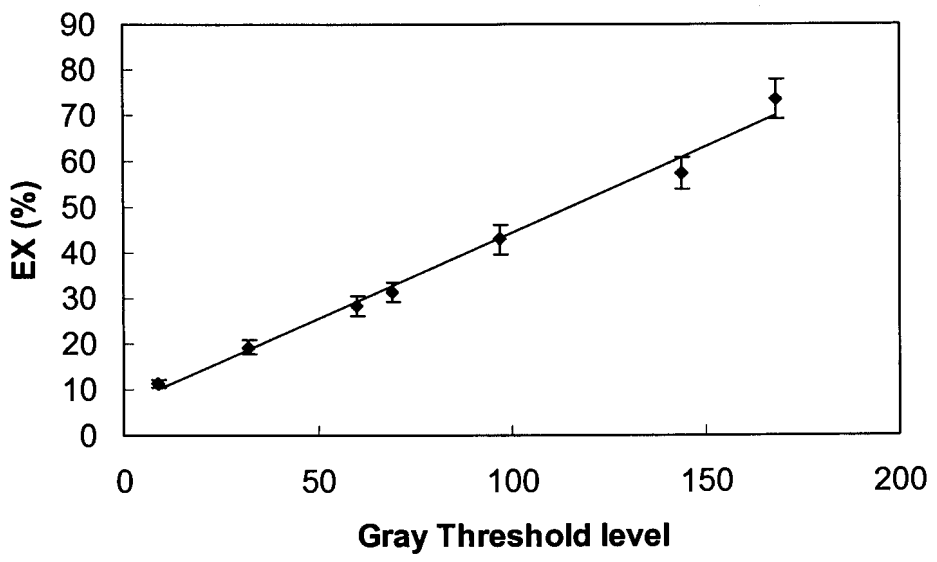


Figure 3.6 The relationship between gray threshold detection level and exposure percentage (EX) when three intact white maize kernels were measured at seven different circle sizes. The gray threshold levels were set for each EX at such a level that the same translucent area on the kernels was detected (see Table 3.7). $r = 0.99$, $y = 0.37x + 6.53$, $R^2 = 0.98$

3.2.5 Optimisation of translucency correction factors

3.2.5.1 Corrections for exposure

The effect of EX on TI using three maize kernels of one cultivar is shown in Figure 3.7. After adjusting the data to refit the regression line at a TI of 0% and an EX of 15%, the new regression line is shown in Figure 3.8. The EX of 15% was chosen as the smallest circle size relative to the kernel size. Below this value, kernels were unevenly illuminated.

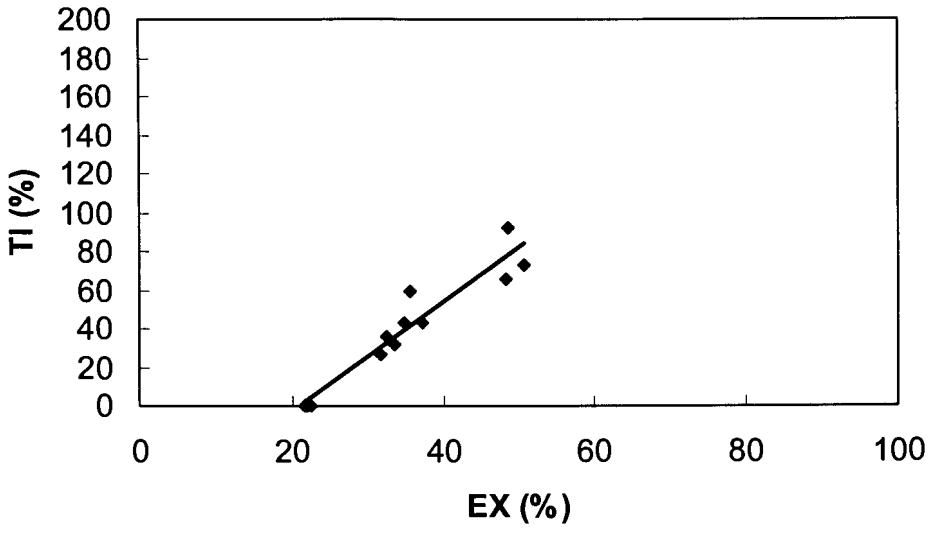


Figure 3.7 The effect of exposure percentage (EX) on the translucency increase percentage (TI) of maize kernels of cultivar 1, without a fixed zero TI correction, $y = 2.8x - 59.0$, $R^2 = 0.92$

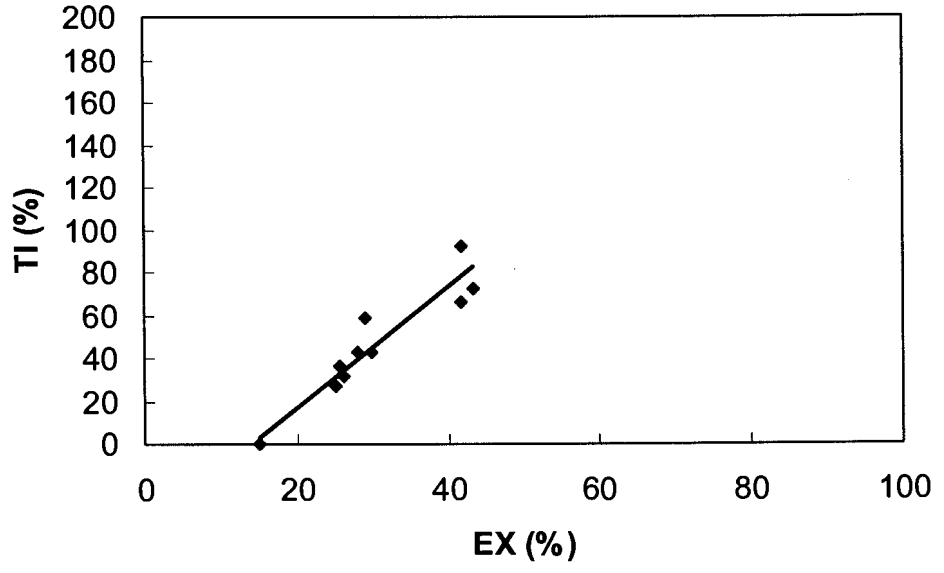


Figure 3.8 The effect of exposure percentage (EX) on the translucency increase percentage (TI) of maize kernels of cultivar 1, with a fixed zero TI correction at an EX of 15%, $y = 2.8x - 39.8$, $R^2 = 0.92$

The slope and R^2 of each fitted line for each cultivar at an EX of 15% and TI of 0% is summarized in Table 3.8.

Table 3.8 Regression data for fitted linear regression lines for EX and TI of eight maize cultivars after adjustment to an EX of 15%

Cultivar number	Slope	Pearson r	R^2
1 (SR 52)	2.83	0.96*	0.92
2 (L290)	4.19	0.98*	0.97
3 (CRN 429)	4.95	0.97*	0.91
4 (R 827)	3.36	0.96*	0.94
5 (CRN 439)	4.63	0.95*	0.92
6 (N282)	2.56	0.99*	0.99
7 (N290)	4.41	0.94*	0.92
8 (N258)	3.82	0.88*	0.85

* $p<0.0001$ for all relationships

The correlation between EX and TI was highly significant for each individual cultivar (Table 3.8). Small differences existed in the slope of the lines of the cultivars (Table 3.8), but after combining all the results, the relationships were still highly significant with high correlation coefficients (Figures 3.9 and 3.10). The values for white and yellow maize kernels were not combined because white and yellow maize translucent areas were detected at different gray thresholds. The reason for the difference was the slightly darker image obtained when the yellow kernels were converted to a monochrome image than the white kernels. Similar correlation and regression analyses were done for the remaining 7 cultivars and in all cases adjustments were made to allow for a fixed zero point EX of 15% at a TI of 0%. After adjusting the data to refit the regression line at a TI of 0% and an EX of 15%, the regression line is shown in Figure 3.8. The EX of 15% was chosen as the smallest circle size for a maize kernel relative to the kernel size. Below this value, maize kernels

tended to become unevenly illuminated which could influence the results of the translucency measurement.

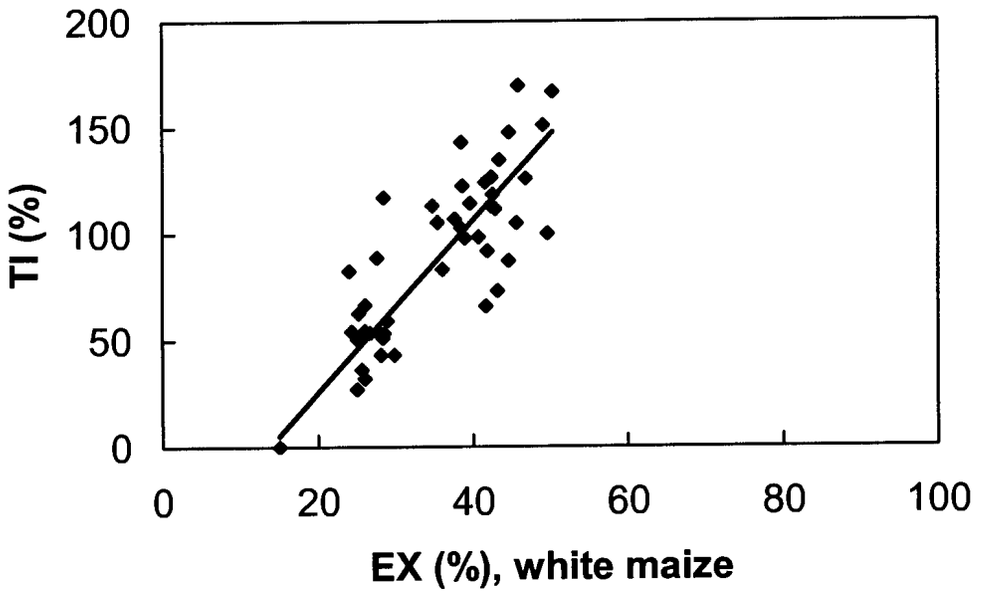


Figure 3.9 The effect of exposure percentage (EX) on the translucency increase percentage (TI) of five combined white maize cultivars. $y = 4.02x - 55$, $r = 0.91$, $R^2 = 0.83$

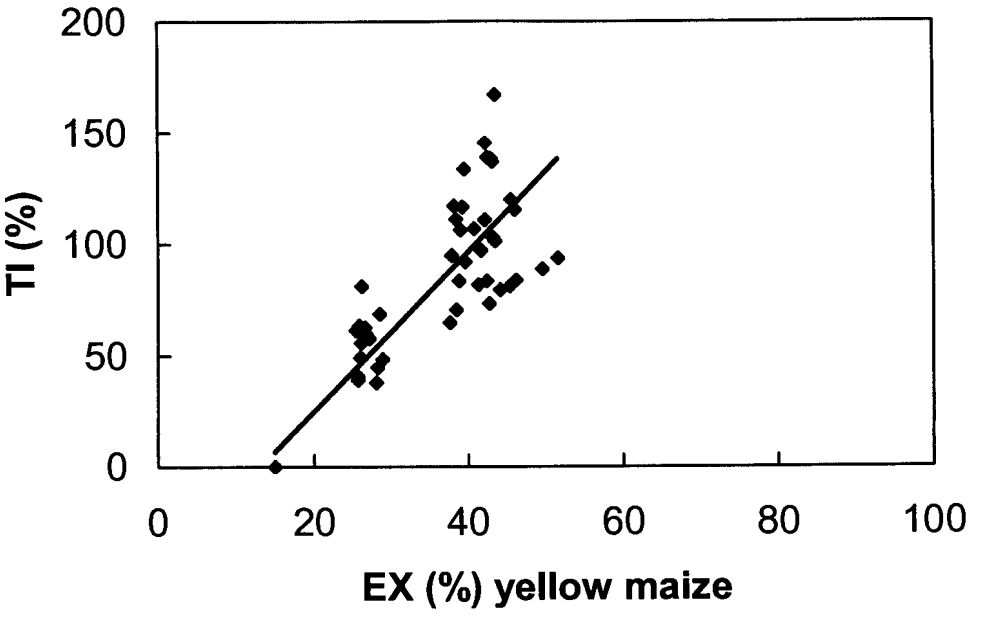


Figure 3.10 The effect of exposure percentage (EX) on the translucency increase percentage (TI) of three combined yellow maize cultivars. $y = 3.58x - 47$, $r = 0.90$, $R^2 = 0.81$

3.2.5.2 Corrections for kernel thickness

The relationships between the thickness of maize kernels and the change (%) in the detected translucent area (mm^2) at constant gray threshold and EX levels were linear (Figures 3.11 and 3.12). Both linear regressions were highly significant ($p < 0.001$). The thickness factors for white and yellow maize were the same.

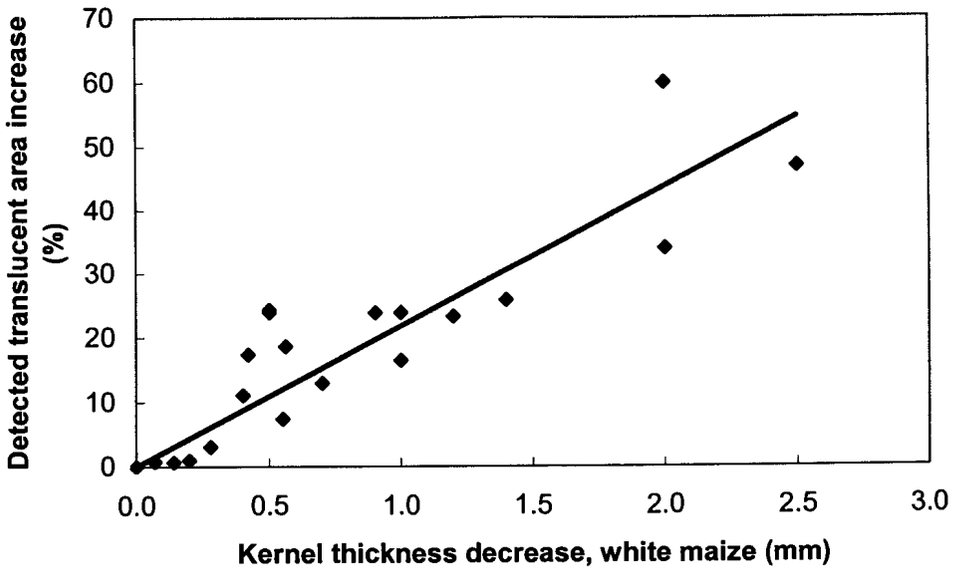


Figure 3.11 The effect of white maize kernel thickness decrease (mm) on the detected translucent area increase (%). y = 21.86x, r = 0.89, R^2 = 0.78

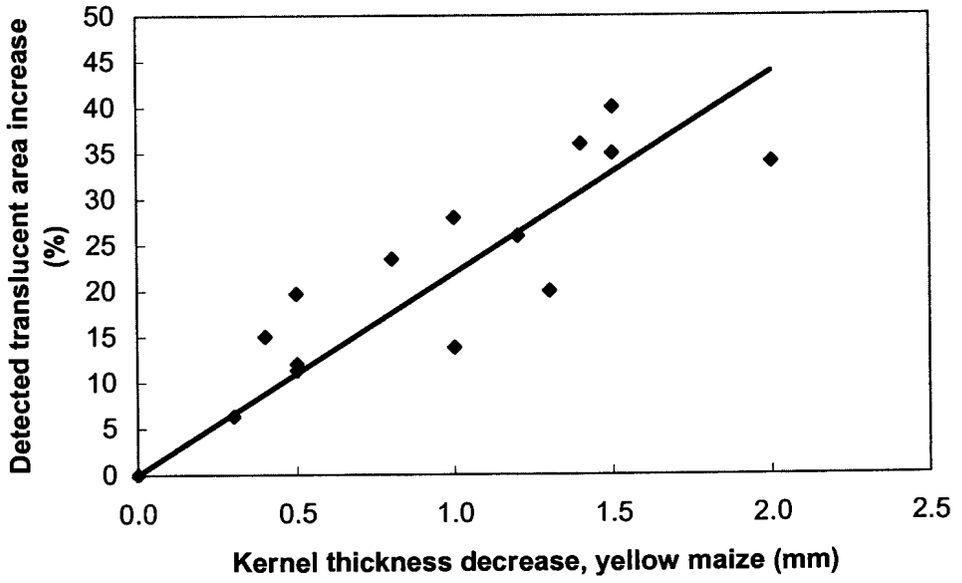


Figure 3.12 The effect of yellow maize kernel thickness decrease (mm) on the detected translucent area increase (mm). y = 21.94x, r = 0.88, R^2 = 0.74

3.2.6 Vitreousness measurements on single kernels (mass fraction)

3.2.6.1 Hand dissection of maize kernels

Images showing the dissection procedure are given in Figure 3.13.

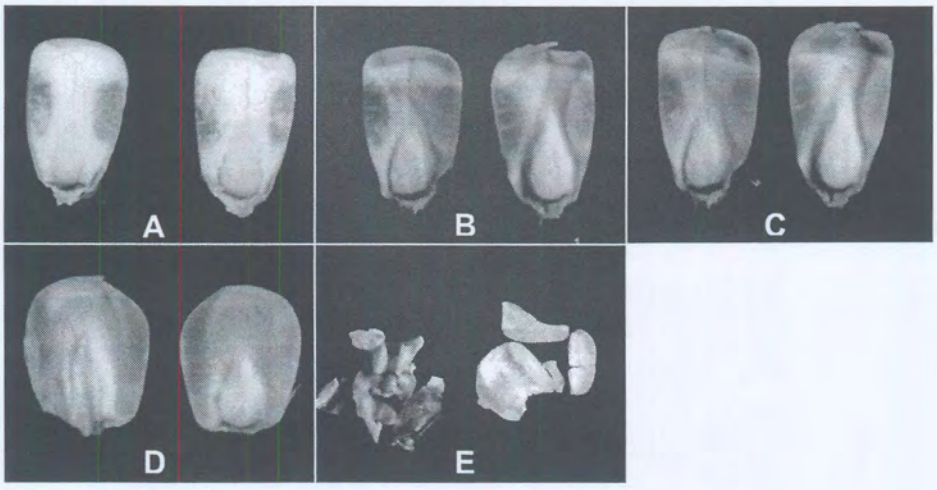


Figure 3.13 Longitudinal sections cut using a scalpel of yellow and white dent maize kernels after soaking in water for five days at 4°C. A, vitreous and opaque endosperm, yellow kernels; B, partial removal of the opaque endosperm with a toothbrush, yellow kernels; C, removal of all opaque endosperm after scraping with a scalpel followed by brushing, yellow kernels; D, similarly cleaned white kernel (all opaque endosperm removed by brushing and scraping); E, the dissected morphological parts of the white kernels

3.2.6.2 Calculating the yield of vitreous and opaque endosperm

Table 3.9 Hand dissection measurements on 49 kernels of five F2 white maize hybrids

Cultivar		Kernel mass (g)	Vitreous endosperm (g)	Opaque endosperm (g)	Vitreous (%)	Opaque (%)
1 (SR52)	Mean	0.46	0.21	0.14	46.3 [a*]	31.3 [a**]
	St. Dev	0.03	0.02	0.02	3.2	3.3
	Median	0.46	0.21	0.14	46.2	31.2
2 (L390)	Mean	0.39	0.25	0.06	63.1 [b*]	15.7 [b]
	St. Dev	0.04	0.02	0.02	3.0	3.4
	Median	0.39	0.25	0.06	63.7	15.2
3(CRN 429)	Mean	0.45	0.25	0.10	55.8 [c*]	22.0 [c]
	St. Dev	0.03	0.02	0.02	2.8	2.9
	Median	0.44	0.25	0.10	55.5	21.6
4 (R827)	Mean	0.34	0.18	0.09	53.2 [d*]	26.6 [d]
	St. Dev	0.05	0.03	0.02	3.2	2.8
	Median	0.32	0.17	0.09	52.9	26.2
5(CRN 439)	Mean	0.39	0.21	0.09	54.0 [d*]	23.8 [e]
	St. Dev	0.04	0.02	0.02	3.5	4.1
	Median	0.39	0.21	0.09	54.1	23.4

Vitreous (%) – vitreous endosperm % of total kernel mass (moisture free basis)
Opaque (%) – opaque endosperm % of total kernel mass (moisture free basis)

* Kruskal-Wallis test
** Different superscripts in columns refer to statistically significant differences ($P < 0.05$)

Table 3.10 Hand dissection measurements on 49 kernels of three F2 yellow maize hybrids

Cultivar		Kernel mass (g)	Vitreous endosperm (g)	Opaque endosperm (g)	Vitreous (%)	Opaque (%)
6 (N282)	Mean	0.32	0.16	0.08	49.9 [a*]	26.3 [a**]
	St. Dev	0.03	0.05	0.03	13.4	10.2
	Median	0.32	0.17	0.08	55.3	23.4
7 (N290)	Mean	0.42	0.22	0.10	52.7 [b*]	23.1 [b]
	St. Dev	0.05	0.03	0.02	3.5	3.1
	Median	0.42	0.22	0.09	52.6	23.1
8(N258)	Mean	0.42	0.24	0.09	56.4 [b*]	22.4 [b]
	St. Dev	0.03	0.02	0.01	3.1	2.4
	Median	0.42	0.23	0.09	56.0	22.1

Vitreous (%) – vitreous endosperm % of total kernel mass (Moisture free basis)
Opaque (%) – opaque endosperm % of total kernel mass (Moisture free basis)

* Kruskal-Wallis test
** Different superscripts in columns refer to statistically significant differences ($P < 0.05$)

3.2.7 Statistical calculations and the development of correlations

3.2.7.1 IA measurements

Table 3.11 Image Analysis measurements on 49 kernels of five white F2 maize hybrids

Cultivar		Tra (mm^2)	Trb (mm^2)	Trc (mm^2)	Tr1 (%)	Tr2 (%)	Length (mm)	Width (mm)	Thickness (mm)	Total kernel area (mm^2)	Germ area (mm^2)
1 (SR52)	Mean	45.3	45.3	32.0	26.5[a*]	40.7[a*]	13.7	11.7	4.7[a**]	120.0	41.9
	St. Dev	9.3	9.3	6.6	5.5	8.3	0.5	0.3	0.6	6.3	3.6
	Median	43.5	43.5	31.2	25.2	38.9	13.6	11.7	4.6	120.0	41.9
2 (L390)	Mean	82.6	91.7	58.2	56.0[b*]	87.2[b*]	12.8	10.5	5.2[b]	104.0	36.2
	St. Dev	6.9	7.6	5.4	4.9	8.9	0.5	0.5	0.4	6.8	3.8
	Median	83.5	92.7	58.9	56.3	87.0	12.8	10.6	5.3	105.0	36.1
3 (CRN 429)	Mean	92.9	84.5	61.8	48.8[c*]	61.5[c*]	13.9	11.6	3.9[c]	127.0	26.0
	St. Dev	9.0	8.2	7.6	4.4	5.4	0.5	0.5	0.3	7.7	4.4
	Median	94.5	86.2	61.5	49.4	62.2	14.1	11.7	3.9	127.0	35.4
4 (R827)	Mean	83.5	76.2	45.5	47.6[d*]	59.4[d*]	12.6	9.9	3.9[c]	96.0	18.9
	St. Dev	6.4	5.8	2.3	4.5	6.7	0.5	0.6	0.5	8.8	3.8
	Median	82.8	75.5	45.3	47.0	58.3	12.8	9.9	3.7	95.0	18.2
5 (CRN 439)	Mean	84.6	77.2	50.4	46.7[d*]	57.4[d*]	12.6	11.2	3.9[c]	108.0	20.2
	St. Dev	8.3	7.6	6.6	4.1	4.7	0.4	0.3	0.4	6.0	2.9
	Median	84.1	76.7	50.0	46.4	57.7	12.5	11.2	3.9	108.0	20.6

Tra – translucent area without corrections
Trb – translucent area with thickness corrections
Trc – translucent area with thickness and exposure ratio corrections
Tr1% - Translucent area % formula 1 (section 3.4.2)
Tr2% - Translucent area % formula 2 (section 3.4.2)
* Kruskal-Wallis test
** Different superscripts in columns refer to statistically significant differences ($p<0.05$)

Table 3.12 Image Analysis measurements on 49 kernels of three yellow F2 maize hybrids

Cultivar		Tra (mm^2)	Trb (mm^2)	Trc (mm^2)	Tr1 (%)	Tr2 (%)	Length (mm)	Width (mm)	Thickness (mm)	Total kernel area (mm^2)	Germ area (mm^2)
6 (N282)	Mean	53.5	51.5	28.3	34.5[a*]	56.5[a*]	12.3	8.6	4.4[a**]	82.0	31.8
	St. Dev	18.7	18.0	10.1	12.0	19.8	0.5	0.35	0.5	4.1	3.1
	Median	60.0	57.7	31.7	38.4	63.2	12.4	8.5	4.4	82.3	32.1
7 (N290)	Mean	69.5	69.5	42.4	44.5[b*]	74.6[b*]	13.4	10.1	4.6[a]	95.4	37.9
	St. Dev	10.0	10.0	6.4	6.3	11.4	0.4	0.5	0.6	4.9	4.7
	Median	69.8	69.8	42.5	44.6	74.7	13.4	10.1	4.6	96.6	37.1
8 (N258)	Mean	80.4	80.4	49.3	51.4[b*]	79.4[b*]	12.6	10.8	4.6[a]	95.9	33.7
	St. Dev	7.2	7.2	5.0	4.4	7.6	0.3	0.3	0.4	4.1	3.4
	Median	80.6	80.6	48.8	51.4	79.2	12.5	10.8	4.5	95.2	33.6

Tra – translucent area without corrections
Trb – translucent area with thickness corrections
Trc – translucent area with thickness and exposure ratio corrections
Tr1% - Translucent area % formula 1 (section 3.4.2)
Tr2% - Translucent area % formula 2 (section 3.4.2)

* Kruskal-Wallis test

** Different superscripts in columns refer to statistically significant differences ($p<0.05$)

3.2.7.2 Correlations and optimisation of relationships

Table 3.13 Product moment correlation coefficient (r) and coefficient of determination (R^2) matrixes for white maize. N = 245 for each data set

	Transl.1[a] (no corrections)	Transl. 2[b] (no corrections)	Transl. 1 (thickness correction)	Transl. 2 (thickness correction)	Transl.1 (thickness and exposure corrections)	Transl. 2 (thickness and exposure corrections)
Vitreous[c] (mass %) r	0.60**	0.74***	0.75***	0.79***	0.77***	0.81***
Vitreous (mass %) R^2	0.36	0.55	0.55	0.63	0.59	0.65
Opaque[d] (mass %) r	-0.53*	-0.67**	-0.67**	-0.74***	-0.72***	-0.77***
Opaque (mass %) R^2	0.28	0.45	0.45	0.54	0.51	0.60

a Translucent area (% whole kernel)
b Translucent area (% of endosperm)
c vitreous endosperm mass % of whole kernel
d opaque endosperm mass % of whole kernel
* $p < 0.001$; ** $p < 0.0001$;*** $p < 0.00001$ for level of significance of the correlation coefficient (significantly different from 0).

Table 3.14 Product moment correlation coefficient (r) and coefficient of determination (R^2) matrixes for yellow maize. N = 146 for each data set

	Transl.1[a] (no corrections)	**Transl. 2[b] (no corrections)**	**Transl. 1 (thickness correction)**	**Transl. 2 (thickness correction)**	**Transl.1 (thickness and exposure corrections)**	**Transl. 2 (thickness and exposure corrections)**
Vitreous[c] (mass %) r	0.84***	0.80***	0.83***	0.79***	0.78***	0.76***
Vitreous (mass %) R^2	0.71	0.64	0.69	0.62	0.62	0.59
Opaque[d] (mass %) r	-0.76***	-0.76***	-0.75***	-0.75***	-0.71***	-0.72***
Opaque (mass %) R^2	0.58	0.58	0.57	0.57	0.51	0.52

a Translucent area (% whole kernel)
b Translucent area (% of endosperm)
c vitreous endosperm mass % of whole kernel
d opaque endosperm mass % of whole kernel
* $p < 0.001$; ** $p < 0.0001$; *** $p < 0.00001$ for level of significance of the correlation coefficient (significantly different from 0).

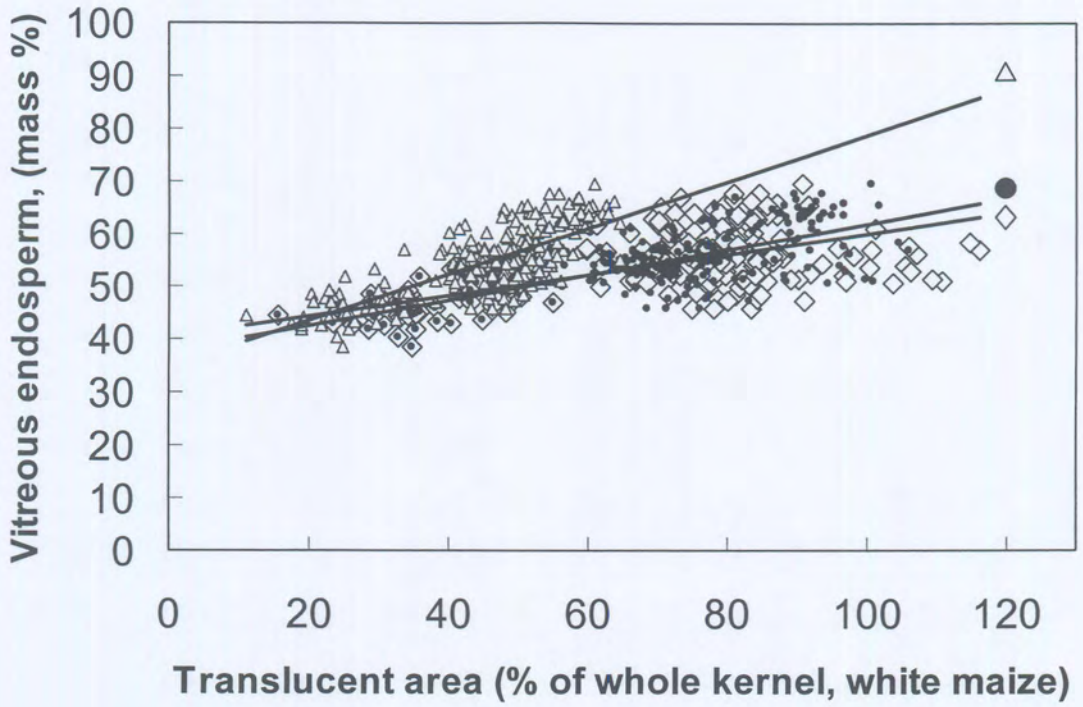


Figure 3.14 The effect of applying translucency correction factors (CFs) on the relationship between vitreous endosperm (mass %) as determined by hand dissection and the translucent area (% of whole kernel) of white maize as determined using IA. "◊", before any CFs (y = 0.19x + 41, r = 0.60); "●", after thickness CF (y = 0.24x + 38, r = 0.75) and "△", after thickness and exposure CFs (y = 0.44x + 35, r = 0.77), n = 245. r = 0.60 differed significantly (Fisher test) from r = 0.75 and r = 0.77 ($P<0.01$). r = 0.75 and r = 0.77 did not differ significantly from each other (Fisher test). Slopes were significantly different from the horizontal

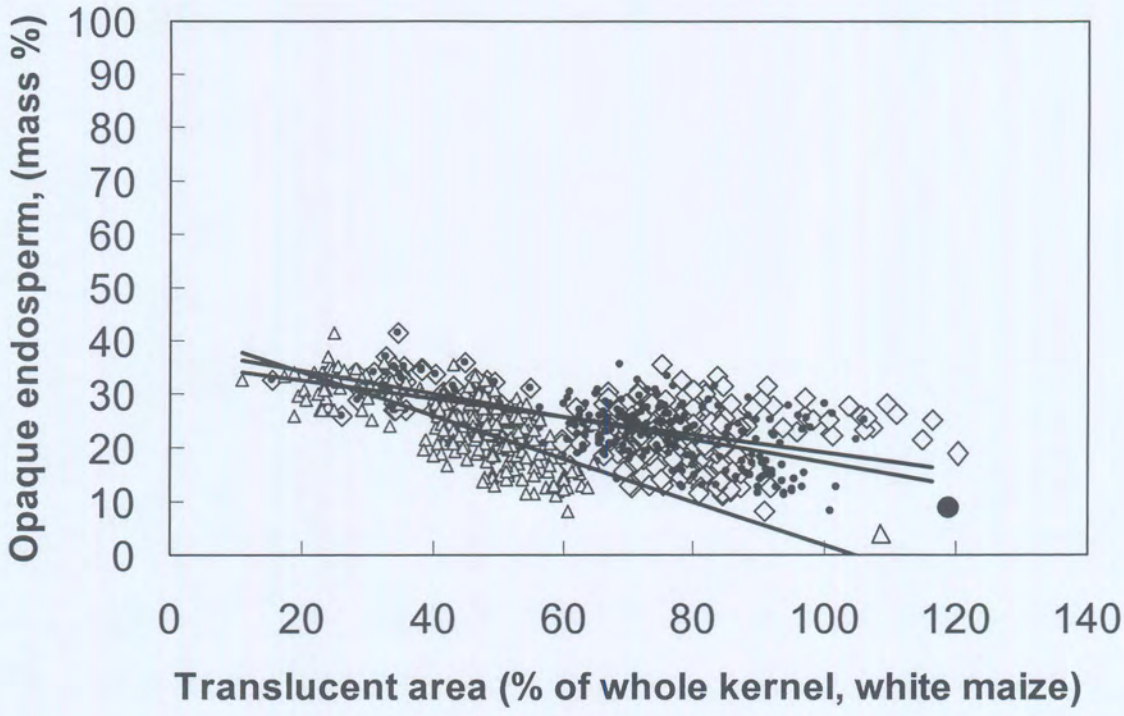


Figure 3.15 The effect of applying translucency correction factors (CFs) on the relationship between opaque endosperm (mass %) as determined by hand dissection and the translucent area (% of whole kernel) of white maize as determined using IA. "◊", before any CFs ($y = -0.17x + 36$, $r = -0.53$); "●" after thickness CF ($y = -0.22x + 39$, $r = -0.67$) and "△", after thickness and exposure CFs ($y = -0.40x + 42$, $r = -0.72$), $n = 245$. $r = -0.53$ differed significantly from $r = -0.67$ ($P<0.05$); $r = -0.53$ differed significantly from $r = -0.72$ ($p < 0.001$); $R = -0.67$ and $r = -0.72$ did not differ significantly from each other (Fisher test). Slopes differed significantly from the horizontal

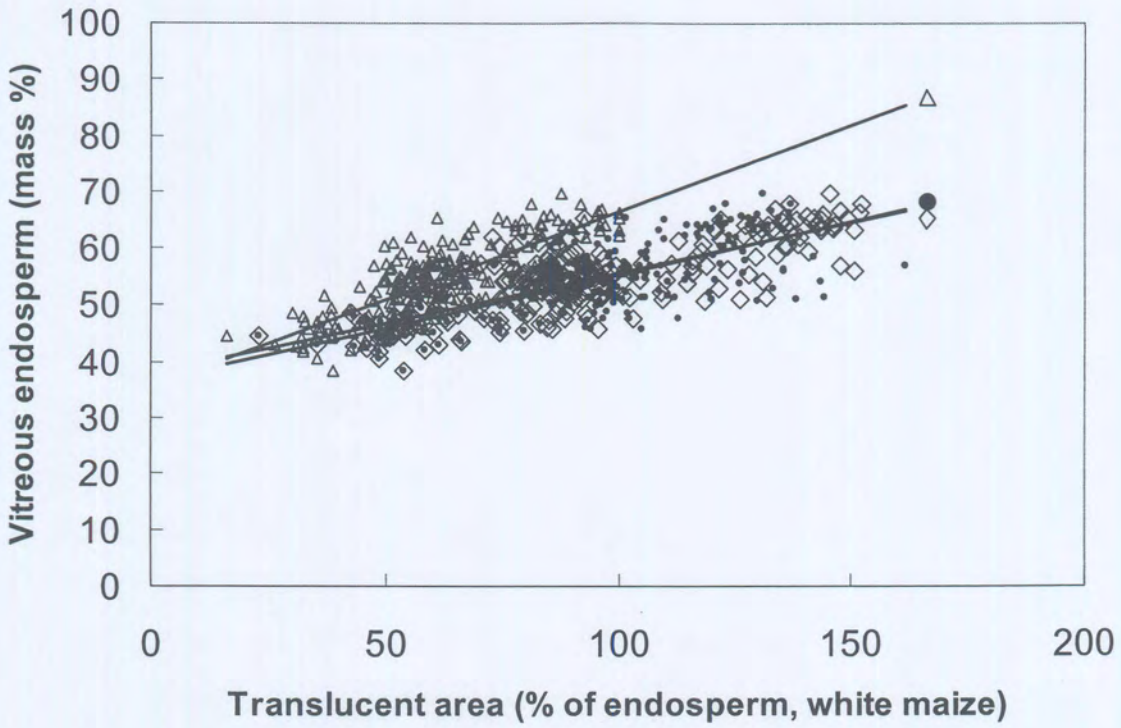


Figure 3.16 The effect of applying translucency correction factors (CFs) on the relationship between vitreous endosperm (mass %) as determined by hand dissection and the translucent area (% of endosperm) of white maize as determined using IA. "◊", before any CFs (y = 0.19x + 37, r = 0.74); "●", after thickness CF (y = 0.18x + 38, r = 0.79) and "△", after thickness and exposure CFs (y = 0.31x + 35, r = 0.81), n = 245. r-values did not differ significantly from each other (Fisher test). Slopes differed significantly from the horizontal

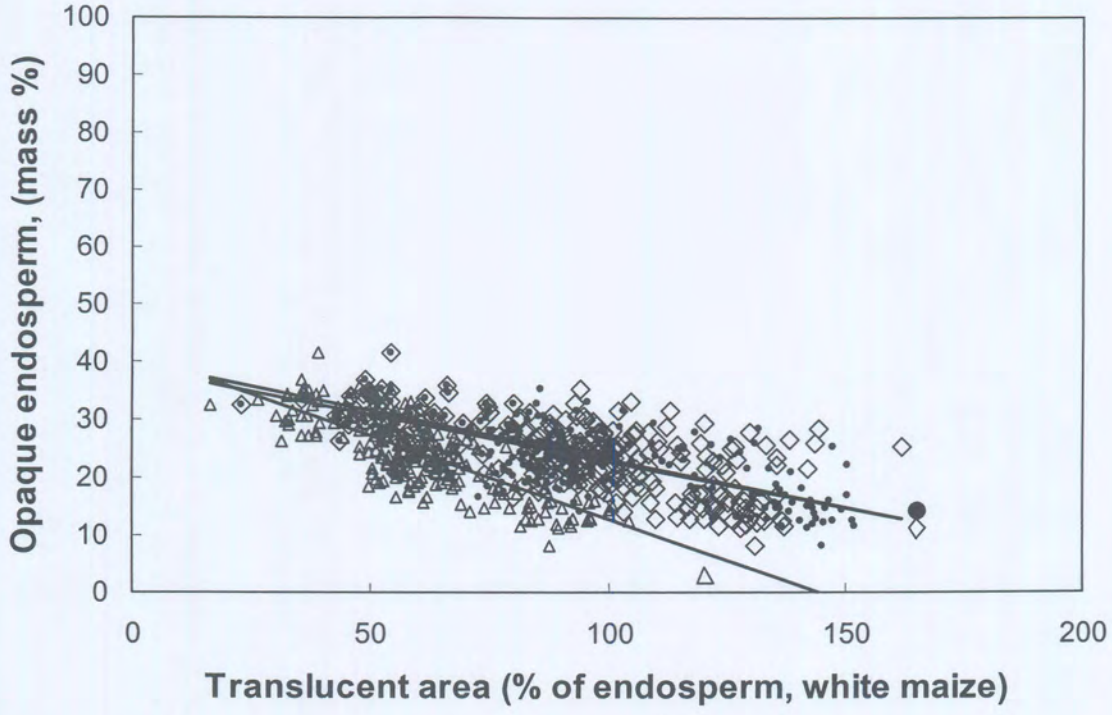


Figure 3.17 The effect of applying translucency correction factors (CFs) on the relationship between opaque endosperm (mass %) as determined by hand dissection and the translucent area (% of endosperm) of white maize as determined using IA. "◊", before CFs (y = -0.17x + 41, r = -0.67); "●", after thickness CF (y = -0.16x + 39, r = -0.74) and "△", after thickness and exposure CFs, (y = -0.29x + 42, r = -0.77), n = 245. r = -0.67 differed significantly from r = -0.77, but other r-values did not differ significantly from each other (Fisher test). Slopes differed significantly from the horizontal

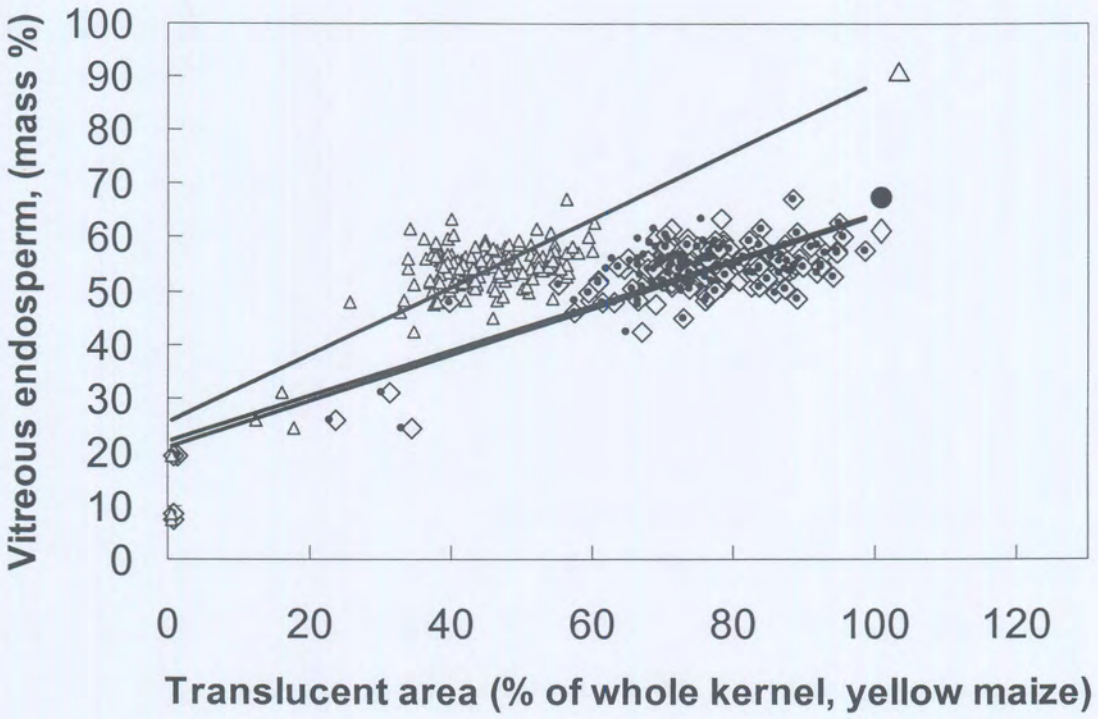


Figure 3.18 The effect of applying translucency correction factors (CFs) on the relationship between vitreous endosperm (mass %) as determined by hand dissection and the translucent area (% of whole kernel) of yellow maize as determined using IA. "◊", before CFs (y = 0.43x + 21, r = 0.84); "●", after thickness CF (y = 0.42x + 22, r = 0.83) and "△", after thickness and exposure CFs (y = 0.63x + 26, r = 0.79), n = 146. r-values did not differ significantly from each other (Fisher test). Slopes differed significantly from the horizontal

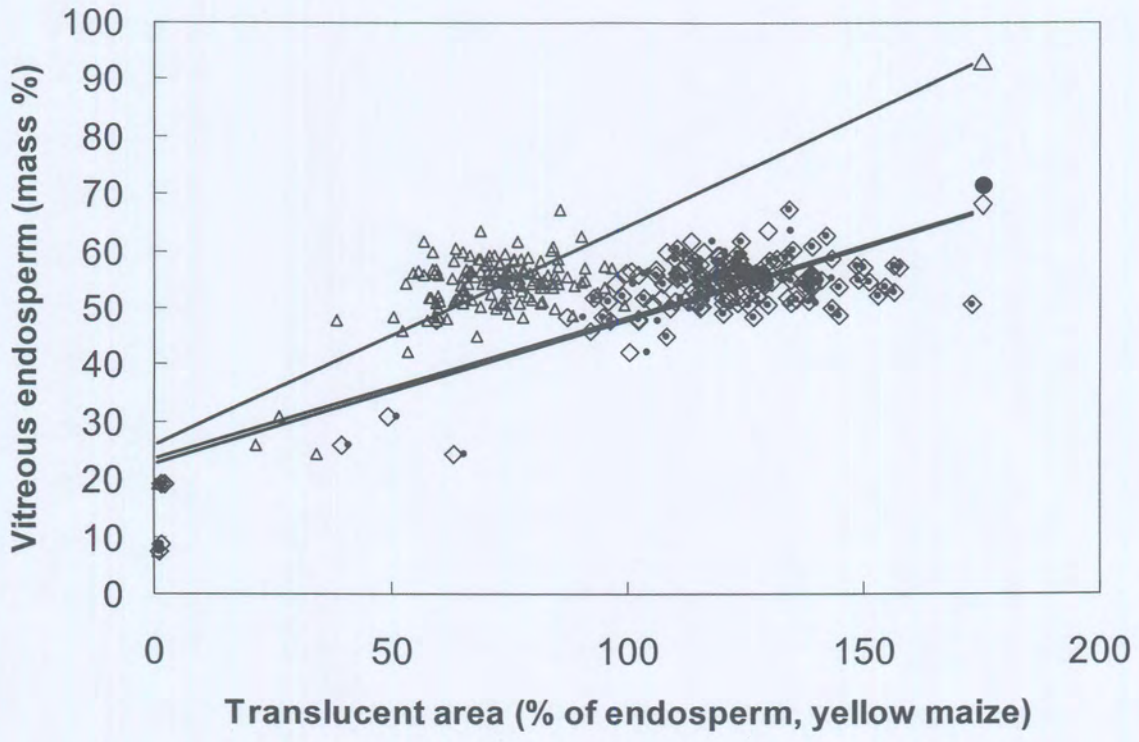


Figure 3.19 The effect of applying translucency correction factors (CFs) on the relationship between vitreous endosperm (mass %) as determined by hand dissection and the translucent area (% of endosperm) of yellow maize as determined using IA. "◊", before any CFs (y = 0.25x + 23, r = 0.80); "●", after thickness CF (y = 0.25x + 24, r = 0.79) and "△", after thickness and exposure CFs (y = 0.38x + 26, r = 0.76), n = 146. r-values did not differ significantly from each other (Fisher test). Slopes differed significantly from the horizontal

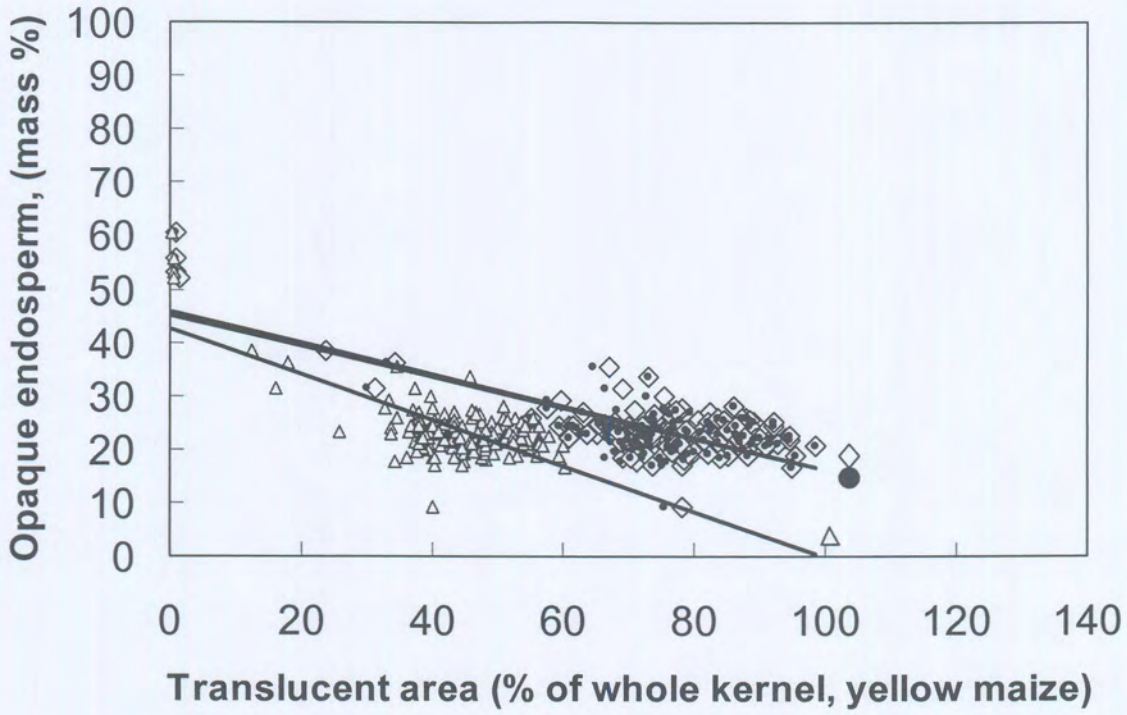


Figure 3.20 The effect of applying translucency correction factors (CFs) on the relationship between opaque endosperm (mass %) as determined by hand dissection and the translucent area (% of whole kernel) of yellow maize as determined using IA. "◊", before CFs (y = -0.30x + 46, r = -0.76); "●" after thickness CF (y = -0.29x + 45, r = -0.75) and "△", after thickness and exposure CFs (y = -0.43x + 43, r = -0.71), n = 146. r-values did not differ significantly from each other (Fisher test). Slopes differed significantly from the horizontal

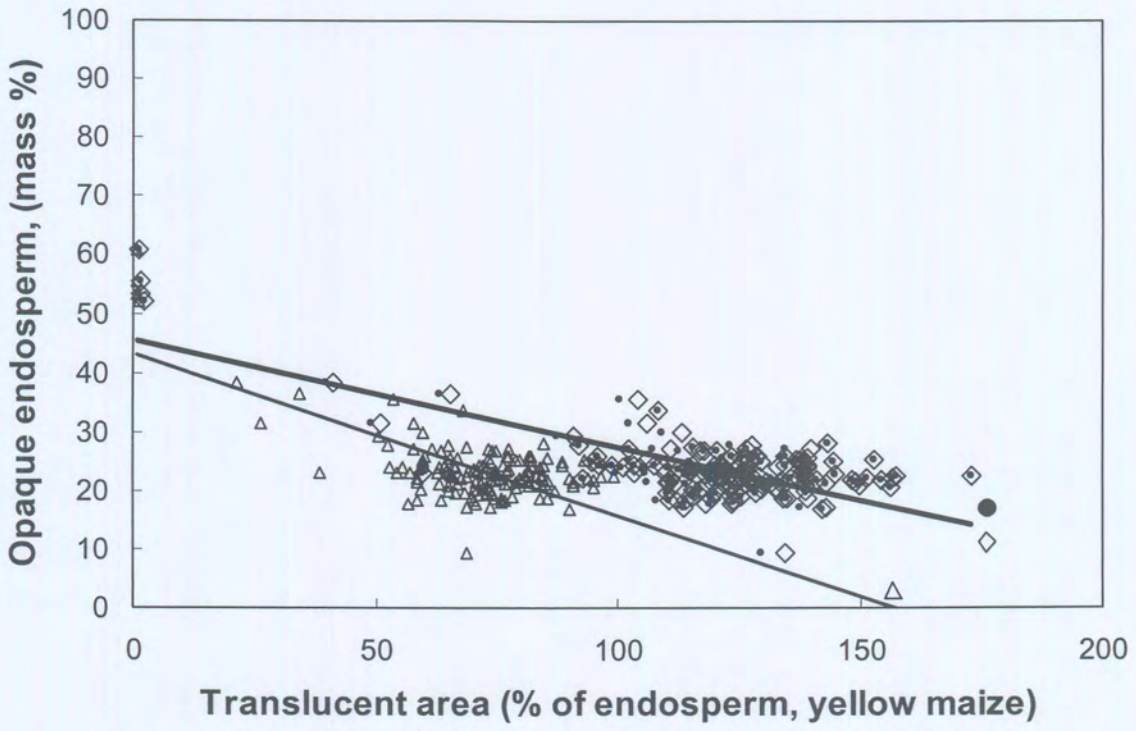


Figure 3.21 The effect of applying translucency correction factors (CFs) on the relationship between opaque endosperm (mass %) as determined by hand dissection and the translucent area (% of endosperm) of yellow maize as determined using IA. "◊", before CFs (y = -0.18x + 46, r = -0.76); "●", after thickness CF (y = -0.18x + 45, r = -0.75) and "△", after thickness and exposure CFs, (y = -0.28x + 43, r = -0.72), n = 146. r-values did not differ significantly from each other (Fisher test). Slopes differed significantly from the horizontal

Results of hand dissection measurements for white and yellow maize (Tables 3.9 and 3.10) as well as the results of the translucency measurements for white and yellow maize (Tables 3.11 and 3.12) showed statistically significant differences between cultivars. This indicated that the results were suitable for further analysis by linear regression. The three yellow cultivars (Table 3.12) had the same thickness.

Correlation coefficients for white maize (Table 3.13) increased after the application of the correction factors. Thickness correction had a bigger effect than exposure correction. For yellow maize (Table 3.14), thickness correction had no effect on the correlation coefficients, but corrections for exposure reduced the correlation coefficients. These trends occurred both for the vitreous and opaque endosperm results. For white maize, correlation coefficients improved significantly after thickness corrections. For white maize, these improvements were significant in most cases. As the yellow maize cultivars did not differ significantly in thickness (Table 3.12), the effect of thickness correction could not be demonstrated. Small differences in the actual thickness values did change the graphs very slightly, but not significantly. However, the yellow maize already had a highly significant and strong correlation before any corrections were made, as opposed to the white maize, which had a poor correlation before corrections. White maize cultivars also differed significantly in thickness.

Corrections for exposure had a bigger effect on the slopes of the linear regression lines than corrections for thickness (Figures 3.14 – 3.21). Slopes increased after corrections for exposure in all cases, both for white and yellow maize, while thickness corrections had only a limited effect with no changes in all cases except for the results in Figures 3.14 and 3.15 (relationship between translucent area as a percentage of the whole kernel and vitreous and opaque endosperm yields for white maize).

In all cases (Figures 3.14 – 3.21), the ranges of the translucency measurements were adjusted to realistic values after corrections for exposure.

Slopes differed significantly from the horizontal in all cases (t-test) which can be ascribed to the high number of observations.

3.3 DISCUSSION

It was clear from the beginning of the study that damaged kernels had a significant effect on the obtained image. As the amount of damaged kernels would vary among samples, they were excluded from the analysis.

As long as the selected circle size was smaller than the kernels, very few problems occurred with light escaping past the sides of the kernels. The method was significantly more convenient than having to embed each kernel in modeling clay and high-resolution images (Figure 3.3) could be obtained.

The camera used in the study was a T.V. standard high resolution digital camera using a 3CCD device for transforming the analogue light signals into digital form. The resolution total of 439300 pixels (764x575) is in the normal range for cameras used for machine vision imaging, although cameras with up to 1 gigabyte of pixels are available. Cameras used for machine vision imaging make use of a 3CCD device where the three primary colours are detected separately for more contrast (Leutron, 2004). The detection of kernels using a light table with a mask covering excess light was necessary because of the tendency of the light to reflect causing a significant loss of image contrast. The very bright background when viewing kernels without a mask caused a shadowing effect on the kernels that reduced the visibility of the internal structures of the kernels. Reflection of the light also caused features on the surface of the kernels to become visible which further reduced image clarity. Although "blooming" of image sensors, where very bright light contaminates adjacent darker pixels can be dealt with using the imaging and digital camera software, the corrections were not sufficient to eliminate the effect of shadowing and reflection, thereby making the use of the mask necessary.

Although only a preliminary test was done, humidity did not have any effect on the detected translucency. A humidity of 98% is high enough for mould growth on the kernels and was chosen as an extreme value (differing from the ambient relative

humidity) for detecting a potential effect. As no effect was observed, further analysis was discontinued.

The comparison of the triplicate readings using three maize kernels measured at four circle sizes is given in Table 3.4. The standard deviations show the detection differences between triplicate readings. These differences were caused by small changes in kernel position as kernels were placed by hand with the circles as closely as possible in the middle of each kernel as well as fluctuations in electricity and other machine-related "noise". These differences did not have a significant influence on differences between measurements at different circle sizes.

The results in Table 3.4 show an increase in detected translucent area for each increase in circle size. It was expected that the detected area would increase similarly for each kernel (a similar ratio). However, a statistically significant interaction was found between circle size and the individual kernels. This interaction is clear when comparing the increase in area sizes for each kernel. At a circle size of 17.2 mm^2, kernel 3 had the largest translucent area, but at a circle of 48.3 mm^2, kernel 3 had a translucent area similar in size to kernel 2 and both were smaller than kernel number 1. This indicates that there was more unexplained variability influencing the results and the two potential variables that were identified for further evaluation, were the ratio of the circle area to the surface area of the kernels and the thickness of the kernels. Highly significant differences occurred between the measurements taken at the four different circle settings (Table 3.4). The interaction was shown by the lower sensitivity of the measurements at the circles of 17.2 and 48.3 mm^2. At these two settings, two kernel translucent areas could not be distinguished from each other, while at settings number two and three (Table 3.4), all three kernel translucent areas could be distinguished from each other. The reason was that the ratio of the light area versus the kernel area had to be at an optimum. If the circle area was too small (circle of 17.2 mm^2), the kernel area was illuminated unevenly. If the circle area was too large, overexposure of the camera detector occurred with excessive light blurring the image. Kernels with small differences in translucency were detected without a significant difference. The circle of 29.6 mm^2

was sensitive enough to distinguish between the three different kernels and was chosen for future work.

Some maize kernels produced a single translucent area after detection, but most kernels produced two separate areas, due to the distribution of opaque endosperm in the hybrids. As the opaque endosperm tended to be concentrated around the germ in the maize kernels, it divided the translucent endosperm into two parts on either side of the germ. There are different combining methods for the two areas: connecting with a vector (thin straight line) or using the dilation and erosion feature, where pixels were added and subtracted according to set patterns (Leica QWin User Guide 1996). These methods were tested in order to select the best method with the least amount of changes to the measured area as illustrated in Figure 3.5. The different procedures had significant effects on the measured area. The vector method produced data that did not differ significantly from the unaltered measurement, but using the customary dilation and erosion method, produced significant differences in results. It was therefore decided to use the vector method of combining areas for future readings on multiple kernels. In the method of Felker and Paulis (1993) only single kernels were measured and the total area of gray was measured for the whole field. However, in this study, when multiple kernels were measured, the areas belonging to each kernel had to be combined in order to obtain the correct results. The combination method of choice will depend on the software available for the test. Software could also be developed to accommodate the unique measurements required in this work, for example allowing automatic combination of the two detected areas by the computer for each kernel using a programmed algorithm.

No significant differences occurred between repeat readings for measurements (Table 3.5). This indicated a negligible level of background noise from the images during detecting and that the measurements were highly repeatable. Kernel orientation (Table 3.5) had no significant effect on the detected translucent areas. This indicated that the circular shaped circles allowed for kernel areas to be detected

repeatably regardless of the kernel orientation. This will simplify a potential on-line detection process considerably.

As the maize kernels differed in size (variability which is characteristic of all biological material), it was suspected that using a fixed circle size for each measurement could have lead to additional variation in the translucent areas detected. Therefore, it was decided to compare measurements of the same kernels using a fixed circle versus modeling clay for excluding excess light.

A comparison of the two illumination methods is shown in Table 3.6. In the case of cultivars 1,3 and 4, the translucent areas detected at the fixed size circle and the modeling clay were identical, but for cultivar 2, the areas differed significantly. Cultivar 4 had virtually no translucent endosperm and was used as an extreme case reference point. The fact that the same area sizes could not be detected in all instances led to the conclusion that the ratio of the circle area to the kernel projected area had to be fixed mathematically in order to allow for similar amounts of light entering each kernel. The effect of the minimum gray detection threshold level on EX when the same three maize kernels were exposed to seven different circles is shown in Table 3.7. The gray detection levels were set at such a level that the same average detected translucent area was obtained for each set of three kernels. Analysis of variance showed that no differences existed between the translucencies of each set. LSD Paired comparison tests also confirmed that no significant differences existed between translucencies and the null-hypothesis as defined in section 3.1.5.1 was accepted (the alternative hypothesis that significant differences do exist was rejected). Following this result, a plot was made of gray threshold level versus EX to determine whether a significant relationship existed. A very strong highly significant linear relationship was found (Figure 3.6). This showed that it was possible to adjust for the effect of EX on gray level based on kernel size if the circle is fixed. However, a fitted line would have had to be developed with more maize kernels of various sizes and also two separate lines would have to be created for yellow and white maize.

From the results shown in Figure 3.6, it was concluded that using a constant circle size, the measured translucency of each kernel could be standardized, although maize kernels differ in size. This is an improvement to the Felker and Paulis (1993) IA method for quantifying maize translucency, as the relationship can be programmed into the computer software. In addition, any maize kernel of any size can be measured by simply placing it on a circle without the need for sample preparation such as modeling clay. In an on-line system, kernels could be moved over a fixed sized circle for quick detection. As the projected kernel area is also detected for every kernel during the assay, the size of the kernel can be taken into account if the size of the circle is known.

The effect of decreasing the thickness of kernels on the increase of the detected translucent area is shown in Figures 3.11 and 3.12. The relationships found were both linear and did not follow the curve of Lambert's law for the absorption of light (Sears, Zemansky and Young 1982). The probable main explanation for this is that maize kernels have a heterogeneous morphological structure (Watson 1987a). Additional scattering of light resulting from the different relative sizes and positions of the opaque endosperm and germ would have influenced the measurements. One of the biggest problems was to develop an effective method for measuring kernel thickness. In the developed method, kernels were sanded with abrasive paper to various thicknesses, then the translucent area was measured. It was not practical to remove the vitreous endosperm alone and sand it into disks of different thickness as it would not have resembled the effect of an intact kernel. Removal of the vitreous endosperm for the determination of absorption coefficients for comparison among cultivars, or endosperm colours in order to develop a better understanding of the factors influencing the absorbance of light by maize kernels could be considered. The scope of this work did not allow for further in-depth studies such as these.

The thickness measurement method developed in this work therefore only gave an indication of the thickness effect and will require some refining to confirm or adjust the thickness correction factor in future work. The effects of cultivar, relative size of germ, kernel shape and colour (except between white and yellow) were not

measured due to time constraints. It is strongly recommended to investigate these effects further in future as this might explain more variation in the translucency results. The slopes of the white and yellow maize kernel thickness effects were similar. This was not expected as the yellow colour had an affect on the general settings required to detect the total area of translucent endosperm. More levels of gray (from 44 to 255) for yellow kernels were measured than for white kernels (from 54 to 255). In spite of these differences, the reduction in detected translucent area will be the same with each mm increase in kernel thickness for both white maize and yellow maize. It is not known, however, how accurate the measurements were and the effect will have to be tested using the percentage translucency for the prediction of the yield of vitreous and opaque endosperm fraction in maize products (dry milled or hand dissected).

In order to accurately measure the mass percentage of opaque and vitreous endosperm, it was required to remove the opaque endosperm completely without damage to the kernels. To do this, it was necessary to soak the kernels for five days in water in order to have them soft enough to be cut open with a scalpel. Opaque endosperm was brushed out easily, except for some in the top end of the kernels. This could be scraped out easily using a sharp object such as a scalpel. Care had to be taken not to damage the germ during brushing, but apart from that, no other damage occurred to the kernels. As kernels would have fermented if kept in water at ambient temperature for five days, they were kept at refrigerated conditions. Vitreous and opaque endosperm could be easily distinguished in both white and yellow kernels. The strength of this improved maize dissection method is that it allows easy separation of the opaque endosperm from the rest of the maize kernel. It was found, however, that some opaque endosperm tended to stick to the vitreous endosperm in a thin layer which could not be brushed out without producing some damage to the germ. The germ becomes very soft during the soaking process and a few kernels had to be discarded after the whole germ came loose during the brushing. The biggest drawback of the procedure is that kernels had to be dried again afterwards which could lead to experimental errors if the drying is too severe.

Kernels must be dried slowly, preferably at temperatures below 50°C for the best results.

The summarized hand dissection data on the kernels are given in Tables 3.9 and 3.10. Vitreous endosperm percentage for white maize based on the whole kernel mass varied between 46.3% for the lowest amount and 63.7% for the highest amount. This range of 17.4% is narrower than ranges reported for other African maize cultivars (44.6%) (Louis-Alexandré, Mestres and Faure 1991) where weighed endosperm vitreousness was measured using hand dissection. The range for the yellow cultivars (Table 3.10) was narrow, between 49.9 and 56.4%. Both white and yellow cultivars have been bred and optimized in South Africa for various traits during the past few decades such as milling performance (personal communication, Randall, P.G., Director, P Cubed) and fungal resistance (personal communication, Esterhuyzen, A., Researcher, Monsanto). Therefore, the maize have become quite homogenous which would explain smaller variation in results.

The correlation coefficient r between % vitreous endosperm and % opaque endosperm as determined on the total of 245 kernels of the white maize was -0.946 ($P < 0.00001$), with $R^2 = 0.895$ (linear regression) showing that the dissection procedure was precise. For yellow maize $r = -0.935$ ($P < 0.00001$) and $R^2 = 0.874$. Vitreous and opaque endosperm was expected to be inversely proportional to each other as the amount of opaque endosperm lost during brushing will be reflected as a lower amount of vitreous endosperm retained. In order to reduce the effect of only subtracting the lost opaque endosperm mass from the total mass of the maize kernels, the vitreous endosperm was also dissected out and weighed after the pericarp and germ was removed. Unfortunately, the opaque endosperm cannot be dissected out as a stand-alone fraction as it disintegrates too easily. Opaque endosperm could only be measured as a mass difference before and after brushing. The results for the two sets for white and yellow maize were similar, showing that there was very small experimental error.

The same percentages of endosperm were obtained for fractions calculated on a dry basis and calculated on an "as is" moisture content. This is advantageous because no drying of fractions is necessary afterwards, provided that samples are weighed and dissected immediately after opaque endosperm removal and not allowed to dry out partially.

Mean values for IA on each maize hybrid are given in Tables 3.11 and 3.12. Variability of the translucent area measurements depended on the calculation method and the addition of the correction factors. The standard deviations decreased slightly or stayed the same after correcting for thickness (Trb), but decreased further in all instances after correcting for exposure percentage as well (Trc). This was observed both for the white and the yellow cultivars. This indicated that a portion of the variability in translucent area detected occurred as a result of faulty readings due to either over or under-exposure and in some cases due to thickness differences. The correction factors for exposure percentage can be programmed into the image analyser software for automatic correction at the detection stage. The calculation of the translucency as a percentage of the projected kernel area had the lowest standard deviation when calculated as a percentage of the total kernel area (Tr1, Tables 3.11 and 3.12). When the germ area was subtracted from the total kernel area before calculating the translucency percentage, the standard deviations increased slightly (Tr2). This increase is ascribed to the fact that the area of the germ had to be detected by indicating the area using a computer mouse marking the boundary between the germ and the endosperm. It could not be detected automatically because the contrast between the germ and the endosperm was not high enough for the computer to distinguish between the gray levels. This hand-detection method of the germ was prone to errors such as an inability to distinguish clearly where the germ boundary began and in many cases the germ area detected was too large. Pieces of pedicel (coming from the maize cob when the kernels were removed) often adhered to the tip cap and as the tip cap and germ areas were measured together, the pedicel pieces also increased the total area measured. The experimental error resulted in increased variability of the calculated translucent endosperm areas.

The individual samples showed similar standard deviations for vitreousness as those reported for vitreousness indexes calculated on sectioned kernels from other African maize cultivars (not South African cultivars) (Louis-Alexandré, Mestres and Faure 1991). However, as standard deviations for translucency indexes of individual samples have not been calculated previously for normal maize, the amount of variation cannot be compared. But it would also have been influenced by factors such as sample homogeneity and sample size. Translucency varied between 26.5% and 56% for Tr1 (a difference of 29.5%) and between 40.7% and 57.4% for Tr2 (a difference of 16.7%) for white maize. Translucency varied between 34.5 and 51.4% (Tr1) and 56.5 and 79.4% (Tr2) for yellow maize. The range of translucency was small when compared to translucency levels tested for high lysine maize (100% difference between lowest and highest levels) (Felker and Paulis 1993). In order to detect significant differences between samples within such a narrow range of translucency, sample size per cultivar was large (49 kernels) as compared to the 10 kernels used for the high lysine maize samples subjected to IA (Felker and Paulis 1993).

All correlation coefficients were significant at the 99.9% level ($P<0.001$) for white maize. For yellow maize, p was significant at < 0.00001. The high significance can be largely attributed to the high number of observations ($n = 245$ for white maize and $n = 146$ for yellow maize) and hence the resulting high number of degrees of freedom (Murdoch and Barnes 1973). There were significant negative relationships between opaque endosperm and translucency for both white and yellow maize. Before corrections, the difference between correlation coefficients of Translucency 1 and Translucency 2 for white maize was statistically significant (Table 3.13), but for yellow maize, the difference between Translucency 1 and Translucency 2 was not significant (Tables 3.14).

Larger standard deviations occurred in the measurements of Translucency 2 (Tables 3.11 and 3.12). This was possibly due to a small experimental error caused by adding the germ area by hand using the "draw" function on the image analyser. As the differences between the correlation coefficients for translucent area 1 and

translucent area 2 and vitreous endosperm was not statistically significant in any instance after all corrections, it can be concluded that both methods would be suitable for predicting percentage endosperm yield. To calculate the translucency 2 value involves excluding of the germ area by a manual step using the "draw" function. The manual step cannot be implemented during a quick in-line application. Translucency 1, however, can be calculated using the computer programme only.

Thickness correction increased the correlation coefficients of white maize significantly, indicating that the thickness of the kernels did account for a significant portion of the variation (Table 3.13). The thickness correction factor of 21.9 found in this work was smaller than the thickness correction factor of 36.5 for high lysine maize as calculated by Felker and Paulis, 1993. However, as the method for determination of the high lysine factor was not described in full by these authors, it is not possible to do a direct comparison. Thickness correction did not have a significant influence on gray measurements in high lysine maize (Felker and Paulis 1993) but it must be emphasized that translucency classes tested for high lysine maize varied between 0 and 100% in contrast to the variation of 29.5% in translucency of the results reported here for white maize. Although the thickness effect could not be demonstrated on yellow maize because the thicknesses of the three cultivars were the same, the thickness correction factor developed was exactly the same as the one for white maize and the factor will be used for further research.

After thickness correction (Tables 3.11 and 3.12), there were still many values indicating area percentages of more than 100%. This was the result of the effect of variable exposure percentages on the translucent area measurements due to variations in kernel sizes, but constant circle sizes (Figures 3.14 – 3.21). After corrections were made for exposure percentage in order to have a constant value for all detections, the slopes of the curves increased significantly, suggesting stronger relationships which accounted for more variation. The corrections for exposure also increased correlation coefficients slightly, but not significantly. Slope increase after corrections for exposure was consistently observed in all instances, both for white and yellow maize.

With the method developed in this study, 49 kernels could be analysed in five minutes when determining both Translucency 1 and Translucency 2 values. The sample size limitations were linked to the size of the camera lens as the speed of detection were the same for any number of kernels placed on the mask. However, as the kernels were placed manually on the mask and it was another time limiting factor. Larger sample sizes took longer to be placed on the mask, but a trained analyst could pack 49 kernels within one minute. Editing the germ area was more time consuming and it lengthened the total time for the analysis to 5 minutes for 49 kernels. The results of this study have shown that editing of the germ area is not necessary and it can be eliminated. Therefore, the time for analysing one sample using only Translucency 1 was reduced to 2 minutes. However, further research is needed in order to develop an automated system for placing the kernels on the mask. For in-line analyses, an automated sampling system will be needed to analyse representative samples taken from kernels moving past on a conveyor belt. With further research, a continuous system could be developed based on these observations.

3.4 CONCLUSIONS

A rapid non-destructive test has been developed for quantitatively measuring the translucency of large samples of maize kernels on a single kernel level using Image Analysis. No sample preparation is necessary (thereby for example removing the need to mount individual kernels in modeling clay) for these measurements and the sample size can be adjusted depending on the properties of the camera lens.

A correction factor to allow for constant illumination of kernels has been developed allowing the use of a single size light circle for illuminating maize kernels of varying size. As the projected area of kernels is measured during the assay, the correction factor can be programmed into the computer software to adjust illumination. The correction factor for exposure consistently increases the slope of linear regression lines fitted for correlating translucency and endosperm yield (vitreous as well as opaque) data for both white and yellow maize. It also eliminates translucency values of more than 100%, which are the result of overexposure in the case of small kernels.

A thickness correction factor, which has a significant effect on the strength of the relationships between translucency and vitreousness, has been developed for white dent maize. The thickness correction is significant within the narrow range of translucency (less than 30% difference between lowest and highest level) of the samples tested. A thickness correction factor was also developed for yellow maize, but its effect could not be demonstrated as the yellow cultivars measured did not differ in thickness. The yellow cultivars did, however, already give highly significant and strong correlation between translucency and vitreousness before any corrections as opposed to the white maize, which gave poor correlations before corrections.

The translucency of maize kernels is significantly correlated with vitreousness and opaqueness indexes determined by hand dissection. Relationships were developed for potentially predicting the yield of vitreous or opaque endosperm dry milling

products by using a translucency index allowing for possible future use as an analytical or quality control tool by maize millers.

In this work, kernels were placed over the illumination circle for transmitted light by hand. However, as kernel orientation had no effect on the detected translucent areas and good correlations were achieved without the need to measure the area of germ, the only requirements for accurate measurements are that the circles must be in the middle of the kernel and that the germ side must face the camera. At this stage in the development of the method, a trained analyst can measure up to 49 kernels in two minutes including packing the kernels on the mask if orientation is ignored.

During the research, it was found that if the light circles in the masks were not exactly in the middle of the kernels, results were not significantly influenced as long as no light escaped around the edges of the kernels. It was found that when kernels were placed ensuring that the light circles were covered completely, the circles tended to be in the middle of the kernels automatically possibly due to the selected size of the circles in relation to the average projected area size of the kernels.

The new method will allow for new insights into biological variation to be found within individual maize lines in terms of translucency as large numbers of kernels can be analysed in a quick and non-destructive manner.

CHAPTER 4: APPLICATION OF THE DEVELOPED IMAGE ANALYSIS MAIZE TRANSLUCENCY METHOD TO ESTIMATE THE YIELD OF DRY MILLED MAIZE PRODUCTS IN LABORATORY AND INDUSTRIAL SYSTEMS

4.1 OBJECTIVES

The objectives of the experiments described in this chapter were as follows:

- To test the validity of the developed image analysis maize translucency method (Chapter 3) as a tool for predicting the yield of vitreous endosperm products produced by laboratory and industrial milling tests
- To compare the formulas developed in three independent and different laboratory and industrial milling tests
- To compare the IA translucency measurement methods for predicting the yield of white and yellow milled maize products.

4.2 DEFINITIONS

Due to the fact that various terms are used in the literature to describe similar products or processes during maize milling, the following definitions of terminology were used in this study in order to allow for consistency:

Yield of milled products (or "milling yield) - weight of a specified product within a certain particle size and composition range, calculated as a percentage of the weight of the whole kernels, either cleaned or uncleaned depending on the mill's specifications (fully defined in section 1.1).

"Super" products – referring to a group of products consisting of reducing the particle size of the primary product, namely clean flaking grits. These products include the sum of the yields of the samp, rice, grits and super maize meal, or, in other words, the yields of flaking grits, coarse grits, medium grits, fine grits or "semolina" and maize meal or "cornmeal". These products all have fat contents of less than 1%,

unless otherwise specified (in some cases, fat contents of up to 1.5% are permissible).

Extraction at degermer – during degerming, the germ and bran are stripped from the endosperm and two fractions are obtained. The first fraction, large pieces of endosperm, also known as the "tail hominy", proceeds through the end of the degermer. This fraction is sifted and part of it is isolated as large flaking grits. The remainder is sent to the roller mills for reduction and cleaning into smaller fractions such as coarse, medium and fine grits. The "thru stock" stream, which is the second fraction obtained during degerming, contains mostly germ, bran, break flour and some small pieces of endosperm. It passes through a screen on the underside of the degermer (Alexander 1987). As it is more difficult to separate the small pieces of endosperm from this fraction, it is better to minimise this fraction and maize kernels with a lower tendency to break up into small particles will produce more "tail hominy" and less "thru stock" which ultimately leads to a higher yield of "super products". Extraction at degermer is therefore defined as the weight percentage of "tail hominy" as a percentage of the total weight of the two combined fractions.

Clean endosperm (super) products – endosperm products containing a low amount of fat, crude fibre and other contaminants, produced by the milling process.

Semolina – a convenient term to describe clean fine maize grits derived from vitreous endosperm with a particle size of 250 to 1000 microns and a low fat content. It is a term "borrowed" from the wheat milling process because of the resemblance in appearance of the fine vitreous endosperm maize grits to wheat semolina.

Dunst – a term to describe maize flour or "break flour", as described in Table 2.1, which is obtained from the opaque endosperm fraction after breaking open of the maize kernels.

4.3 MATERIALS AND METHODS

Three independent milling and different experiments were undertaken. In each experiment, image analysis translucency measurements of the maize were made

before milling as described in section 3.1. A range of products was produced in each of the three experiments. These products resembled ranges of vitreous endosperm products produced by the industrial dry milling process. Product yields were calculated on a weight percentage basis and the results were correlated with the translucency measurements. Accompanying analyses such as percentage floaters, fat content and other measurements were also made where applicable.

4.3.1 Experiment 1

Laboratory scale roller milling of 20 industrial white maize samples

4.3.1.1 Materials

Twenty samples of industrial white maize (mixed cultivars) obtained from various mills (100 kg each) were tested. The mills obtained the samples from different production areas in South Africa and samples were supplied in bulk by commercial farmers.

4.3.1.2 Methods

4.3.1.2.1 Image Analysis

Image analysis was done on 50 kernels (using an adapted mask to allow for 50 kernels instead of 49 as described in Chapter 3) selected from each of the 20 industrial samples, as described (section 3.1). Thickness was measured on each individual kernel instead of an average using the method described in section 3.1.6.2. Translucency values (%) before and after corrections for thickness and exposure were calculated for each sample. Measurements done on damaged kernels were discarded for statistical reasons. Measurements were done at the CSIR, Pretoria, South Africa on samples taken from each batch of maize before it was sent to the Federal Research Centre for Cereal and Potato Processing in Detmold, Germany for experimental milling tests.

4.3.1.2.2 Floating number (floaters, mass%)

Whole maize kernels were used and the floating number (similar to the percentage floaters test but at a different solution density of 1.25 instead of 1.275) was determined using the method described by Gerstenkorn (1991). Analysis was done at the Federal Research Centre for Cereal and Potato Processing. From a representative sample, 100 intact kernels were selected and placed into a sodium nitrite solution with a density of 1.25. The kernels were adjusted before the test to a moisture content of 12.0%. The solution was stirred for 30 seconds, and the floating and sunken kernels were taken out with a spoon. The kernels were then placed on filter paper, dried and weighed. The weight of the floating kernels was given as a percentage of the total weight of 100 kernels on a dry basis and the test was done in triplicate. The test was repeated if the deviation of two tests exceeded 10%.

4.3.1.2.3 *Fat and moisture contents*

Fat contents of products obtained during the experimental milling were measured using the Soxhlet method (AACC 30-20) (American Association of Cereal Chemists 2000) and moisture contents by using the oven drying method (AACC 44-18) (American Association of Cereal Chemists 2000). Analyses were done at the Federal Research Centre for Cereal and Potato Processing.

4.3.1.2.4 *Experimental milling of maize*

Experimental milling of the 20 industrial white maize samples was done at the Federal Research Centre for Cereal and Potato Processing. The method was as described by Gerstenkorn (1991). The milling procedure is given in detail in Figure 4.1. Maize was dampened to 20% moisture and allowed to stand (condition) for 18 hours. After conditioning, each sample was ground between two coarse-fluted rolls (maize laboratory mill roll specifications given in Figure 4.1), and the germ parts and "hulls" were separated using a centrifugal sifter. The grind (<3.3 mm) was put into a purifier in which the first grits fraction with a fat content lower than 1% was obtained. The separated material was then milled again in a Bühler experimental mill with fine fluted rolls. The milled product was then separated in a purifier into second and third grits fractions and also to first and second dunst fractions.

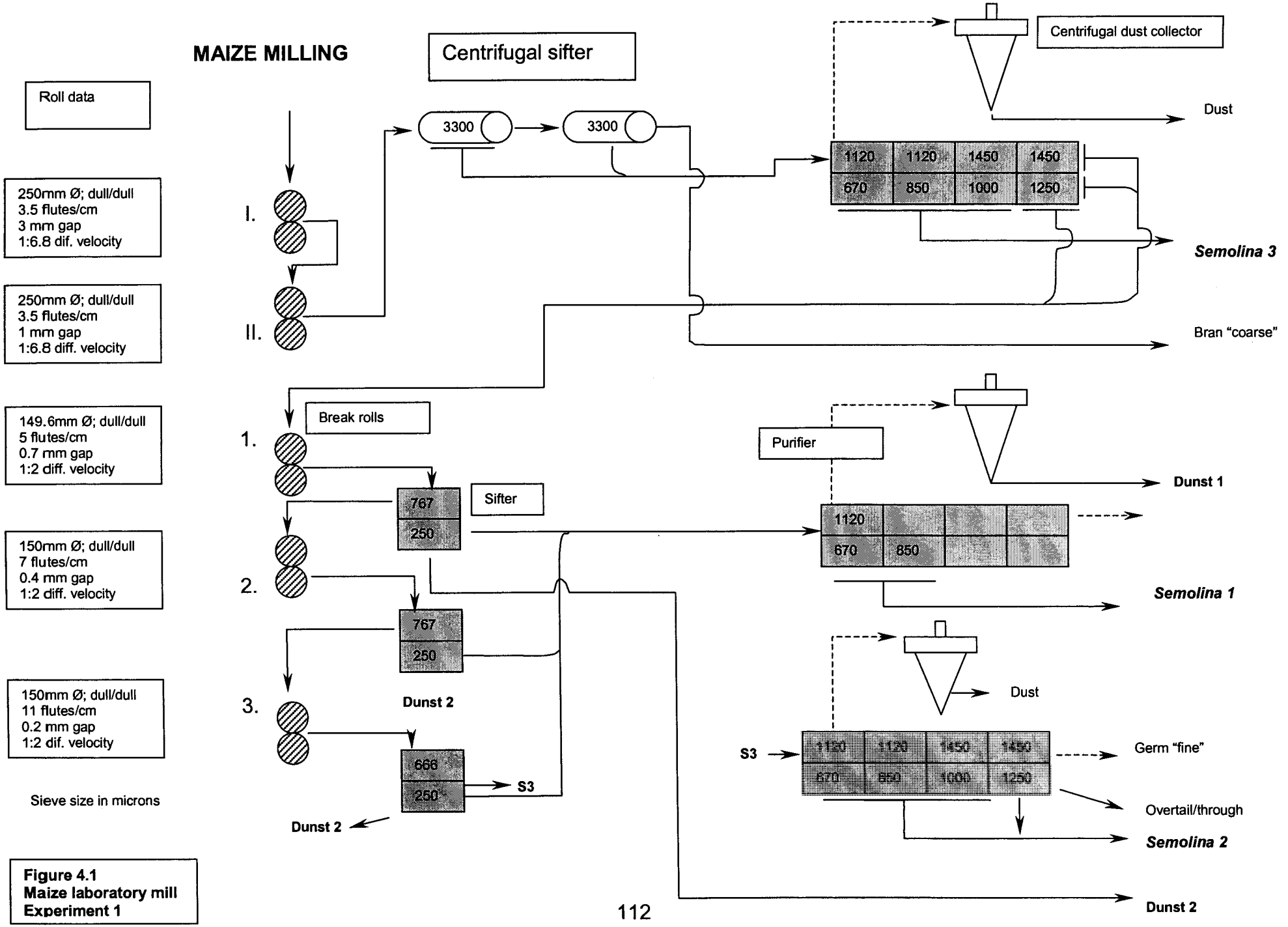


Figure 4.1
Maize laboratory mill
Experiment 1

4.3.1.2.5 Calculations and correlations

The yields of the milling process were calculated as follows:

- Total semolina yield (total yield of clean grits < 1000 microns) – the combined yield (%) of the first, second and third grits (semolina 1, semolina 2 and semolina 3) fractions with a particle size of more than 250 microns, but less than 1000 microns (Figure 4.1). The fat contents of the three combined grits fractions were in all cases less than 1.35%. The yield was calculated as a weight percentage of the combined three grits fractions calculated on a whole kernel weight basis.
- "Milling resistance" – this is an indication of the resistance of the vitreous endosperm to breakage. It was the weight percentage of the first semolina fraction (Figure 4.1) as calculated on a whole kernel weight basis.
- In both cases, yields were calculated on a moisture-free basis based on the weight of cleaned whole maize before milling. Moisture-free basis calculations were used to exclude the effects of moisture contents on the weight of the end products.

Translucency measurements were correlated with the following data obtained from the experimental milling:

- Total yield of clean grits (semolina), 250 - 1000 microns, including the fat content on an "as is" basis
- Total yield of clean grits (semolina), 250 - 1000 microns, on a fat free basis
- Milling resistance – as the yield of the first clean grit fraction (semolina 1), 250 - 850 microns.
- Floating number – correlations between translucency and floating number, and between floating number and total yield and milling resistance were determined.

4.4.2 Experiment 2

Industrial milling of eight white and two yellow maize samples

4.4.2.1 Materials

- Eight samples of industrial white maize (mixed cultivars) obtained from seven production areas in South Africa and one pure cultivar (SR 52), twelve tons of each
- Two samples of industrial yellow maize, one from South Africa and one imported from the USA (twelve tons of each)

4.4.2.2 Methods

4.4.2.2.1 Image Analysis

Image analysis was done on 50 kernels selected from each of the 10 samples as described in section 4.3.1.2.1. Thickness was measured on each individual kernel instead of an average using the method described in section 3.1.6.2. Samples were taken at the industrial milling test site. Measurements on damaged kernels were discarded for statistical reasons.

4.4.2.2.2 Moisture contents

Moisture contents of the whole maize before conditioning and of the conditioned maize before degerming were measured using the oven drying method AACC 44-18, (American Association of Cereal Chemists 2000). Measurements were done at the CSIR, Pretoria, South Africa.

4.4.2.2.3 Experimental milling of maize

A Bühler industrial maize mill was used for the trials (Tiger Milling Training Mill, Bloemfontein, South Africa). It had a capacity of 3 tons per hour with one degermer, one turbo sieve, two sets of break rolls, one set of break grinding rolls, three sets of reduction rolls and full sieving, aspiration and cleaning accessories capable of

manufacturing the whole range of industrially produced dry milling products (Tiger Milling Training mill, Bloemfontein, South Africa).

Approximately twelve tons of maize of each selected batch were transported to the milling site. The mill had two storage silos and the maize was divided into two batches. The batches of maize (approximately six tons) were drawn into the first conditioning bin and conditioned to 14% moisture for four hours. After the first conditioning, the maize pericarp surface was wetted to 18% moisture in a conveyor just prior to degerming (approximately five minutes between addition of water and milling).

Maize batches took two hours to be milled. To allow the mill to reach steady-state conditions, samples were taken and yields were calculated during the second hour of milling. Samples and calculations were done as follows:

- Moisture samples were taken after the second conditioning before the degermer stage
- The overtails and thrus at the degermer were collected for one minute at the beginning, middle and end of a 510 kg "batch" during milling. The two fractions were combined and weighed and the extraction at the degermer percentage calculated as the weight of the overtails divided by the combined weight of overtails and thrus.
- When 510 kg of maize had been milled, a warning signal was sounded indicating that the hominy chop (offal) collected during the 510 kg batch milling was to be put aside and weighed. Immediately after the signal the collection of the next 510 kg milling cycle's offal was commenced. Values for the weight of offal were taken during the second hour of milling.
- The mill was set to produce only one product, namely a maize meal with a specific set of specifications (see below). Fractions obtained from the vitreous endosperm were ground until the specified particle size distribution was reached and therefore no separate fractions of grits (coarse, medium or fine) were produced, as all fractions were expressed as maize meal. The yield was calculated in terms of maize meal (mass) as a percentage of

whole maize (mass) before conditioning and in this case before cleaning as well.

- A sieving test using a 500 µm opening sieve was done on the thru fraction of the degermer. The flour collected was weighed. The weight of the flour collected was calculated as a weight percentage on the weight of the thrus and represents the amount of break flour from the opaque endosperm produced by each sample of maize during degerming.

The maize meal produced had to conform to the following specifications:

Moisture – maximum of 14%, AACC 44-18, (American Association of Cereal Chemists 2000)
Fat, AACC 30-20, (American Association of Cereal Chemists 2000) – maximum of 2.3% (moisture-free base)
Ash, AACC 08-01, (American Association of Cereal Chemists 2000) – maximum of 1.36 (moisture-free base)
Particle size – minimum of 95% must go through a 500 µm opening sieve.

Due to an unforeseen problem in the mill during the milling of the SR52 white cultivar, not enough maize was available to allow the mill to reach steady-state, in order to calculate the yield of maize meal. Therefore, only nine samples of maize (instead of ten) were used for the measurement of the yield of maize meal. Two or three 510 kg test runs were measured for each batch depending on the amount of maize available after steady-state was reached.

Due to the fact that the mill belonged to a private company, a detailed flow chart of the design of the mill cannot be given here, but a summary of the process used is given below:

Step 1 – weighing before conditioning and cleaning
Step 2 – first conditioning bin (4 hours, 14% moisture)
Step 3 – second conditioning bin (5 minutes, 18% moisture)
Step 4 – conditioned maize through a magnet before milling
Step 5 – degerming to produce thru and overtails fractions (5 mm screen)

Step 6 – thrus to turbo sieve to produce offal fraction (coarse) and mixed fraction for aspirator

Step 7 – overtails to sieve for division into two fractions for aspiration and for the first and second break rolls

Step 8 – after the first and second breaks, products sieved, aspirated and gradually reduced using the remaining sets of one break grinding roll set and three reduction roll sets. The mill also had a purifier and aspiration system suitable for separating smaller grit particles from the similar sized germ and bran particles.

The whole mill was designed and built by Bühler (Uzwil, Switzerland) and all units in the mill consisted of Bühler manufactured units designed for the dry milling of maize. Systems were similar to the industrial systems in most industrial mills in South Africa.

4.4.2.2.4 Calculations and correlations

Translucency measurements were correlated with the following milling data:

- Extraction at degermer (weight of overtails/(weight of thrus plus overtails))
- Total extraction of maize meal (all endosperm extracted was used for producing maize meal). Two products were produced during milling namely maize meal and offal. Two calculations were done, namely yield of maize meal without moisture correction (as is basis) and yield of special maize meal on a dry basis.
- Percentage of break flour in the thrus fraction (weight percentage) after degerming

4.4.3 Experiment 3
Laboratory scale milling of 12 yellow maize samples

4.4.3.1 Materials

Twelve samples of industrial yellow maize cultivars planted and produced by a seed company (10 kg of each) (Monsanto, South Africa)

4.4.3.2 Methods

4.4.3.2.1 Image Analysis

Image analysis was done on 50 kernels selected from each of the 10 samples as described in section 4.3.1.2.1. Thickness was measured on each individual kernel instead of an average as described in section 3.1.6.2. Samples were taken at the industrial milling test site. Measurements on damaged kernels were discarded for statistical reasons.

4.4.3.2.2 Moisture contents

Moisture contents of the whole maize before conditioning and of the conditioned maize before degerming were measured by using an oven drying method, AACC 44-18, (American Association of Cereal Chemists 2000).

4.4.3.2.3 Degerming of maize

A small pilot-scale Beall-type maize degerminator designed and built by the CSIR (Pretoria, South Africa) capable of degerming 60 kg of maize per hour (see Figure 4.2 for a line diagram) was used.

Yellow maize (10 kg of each sample) was conditioned before degerming. Conditioning was done in two stages, namely a first stage (14% moisture for 16 hours) and a second stage (18% moisture for 30 minutes). After conditioning, maize was fed into the degermer using a vibratory feeder at a feeding rate of 1 kg per minute. The degermer's rotating cone rotated at a speed of 900 rpm. The gap was set at 2 cm between the cone and plate and a resistance weight of 1 kg was used on the exit plate. The exit plate covered the overtails end of the degermer and depending on the weight attached to it, could only be lifted by the pressure of the outgoing degermed maize. The heavy resistance weight allowed maize to be broken

up into smaller pieces and also allowed maize to be degermed more thoroughly. The weight was kept constant for all the samples. In this experiment, the screen normally used to separate thrus from overtails was replaced by a solid plate with knobs. This was done because of the difficulty of quantitatively cleaning the degermer with a screen fitted, especially with small samples. As the samples were derived from cultivar tests at a seed producing company, larger samples could not be provided. The plate arrangement allowed the production of a single mixed fraction consisting of the thrus and overtails and they were separated further by sieving and aspiration as a combined fraction.

After degerming, samples were sieved using the following sieve opening sizes: 3.9 mm, 3.6 mm, 3.3 mm, 2.9 mm, 2.4 mm, 1.01 mm and fines (thrus from the 1.01 mm sieve). Grits obtained from each fraction were then aspirated using a laboratory aspirator. This was done to separate the grits from the bran and germ particles. The yield of grits was calculated as a weight percentage of grits based on the weight of the whole maize which had a moisture content of 12 - 14%) before degerming and conditioning. Moisture contents of the grits were between 13 and 14% after sieving and aspiration, as drying occurred during the operations. Germ and bran obtained from the aspiration steps were combined with the fines fraction (thru 1.01 mm) to obtain an offal fraction. However, the fines fraction itself consisted of break flour only, as it was formed from the opaque endosperm of the maize during the degerming process. Milling was not done after degerming, as correlating translucency against the yield and size of flaking grits obtained was the primary aim of this experiment.

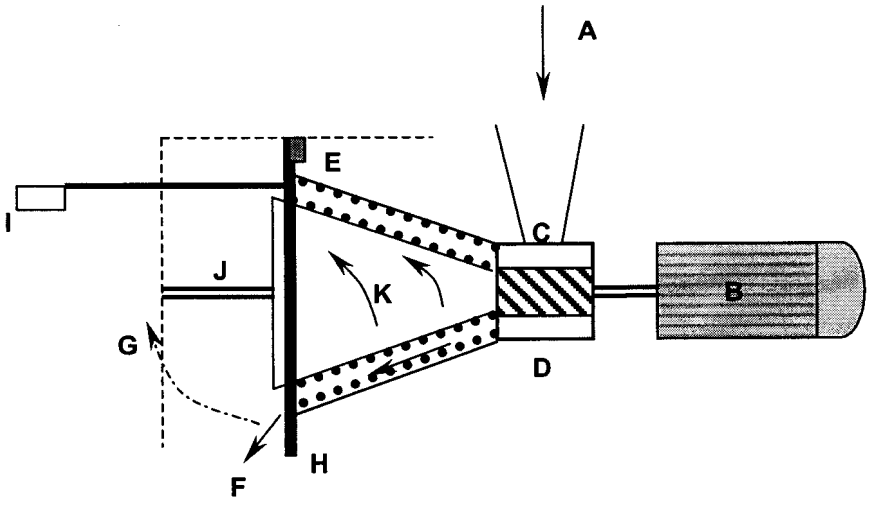


Figure 4.2 Line diagram of pilot scale Beall-type degermer: A is the flow of incoming conditioned whole maize; B is the motor turning the axis; C is the feed section with a screw feeder; D is the degerming section with maize being degermed in the area between the cone and the plate (knobbed area); E is a hinge for the resistance plate; F is the outgoing degermed maize (thru and overtail fractions combined); G is the direction of the resistance plate H being pushed away by the outgoing maize; I is the weight container for adding a specific weight onto the resistance plate and J is the axis of the unit rotating with the inner cone, with K the inner cone

In Figure 4.2, solid arrows show the direction of the maize moving between the rotating cone and static housing while being degermed. The cone surface and inner surface of the housing around the cone are both fitted with steel knobs to allow for a running and shearing action. Note that the cone K rotates freely and independently from the housing and protrudes through an opening inside the resistance plate.

4.4.3.2.4 Calculations and correlations

Translucencies were correlated with the following data from the degermer:

- Yield of large grits (>3.9 mm) as a weight percentage of whole maize before conditioning
- Yield (weight percentages) of combined grits fractions > 3.6 mm and >3.3 mm (typical ranges for breakfast cereal flaking grits) (Alexander 1987)
- Total endosperm (grits > 1.01 mm)

4.4.4 Statistical analysis

Correlations and linear regression calculations were done and tests for significance were calculated. Slopes of the regression models fitted were also tested for significance. Residuals of image analysis data were normally distributed and therefore no significant differences existed between mean and median values of the data. Hence, only the mean and standard deviation values were calculated for all data. The correlation coefficient r was determined as a measure of the stochastic dependence of the dependent and independent variables used for determining relationships between image analysis measurements and milling yield data. The z-transformation calculation was used for testing whether two correlation coefficients differed significantly from each other where applicable (Diem and Seldrup, 1982). A standard error of the regression slope was calculated along with a t-test to determine if the slope differed significantly from the horizontal or not. With this specific test, conclusions can be drawn from the data independent of the units of the measurements. A relationship may have a significant slope, but may seem flat on a line diagram because of the units of measurements or vice versa. Analysis of variance was done where applicable and Duncan groupings were calculated ($p<0.05$) to indicate significant differences between measurements. Correlations and t-tests were single-tailed since the relationships were predicted. The exposure only corrections were not shown in the results, only the effect of thickness and the combined effect of thickness and exposure.

4.5 RESULTS

4.5.1 Experiment 1

4.5.1.1 Image Analysis

Image analysis data are summarised in Table 4.1. Statistically significant differences were found for both Translucency 1 and 2 measurements (Table 4.1), indicating that the data were suitable for further analysis by linear regression. Morphology data

(thickness, total area and germ area) also showed statistically significant differences among the 20 samples.

Table 4.1 IA translucency and morphology data on twenty industrial white maize samples (45–50* kernels per sample)

Sample no		Tra (mm^2)	Trb (mm^2)	Trc (mm^2)	Tr1 (%)	Tr2 (%)	Thickness (mm)	Total area (mm^2)	Germ area (mm^2)
		**							
1	Mean	49.5^{abc}	59.0^{abc}	37.5^{ab}	36.0^{bc}	53.7^{ab}	4.9^{abcdef}	102.5^{abcd}	33.5^{abc}
	Std Dev	12.4	14.9	10.9	8.7	12.4	0.7	11.4	6.1
2	Mean	43.1^{defg}	50.8^{efghi}	30.7^{defg}	31.6^{def}	46.6^{efgh}	4.8^{abcdef}	95.9^{efg}	30.5^{defg}
	Std Dev	11.3	13.9	10.1	8.2	12.0	0.6	11.3	5.5
3	Mean	43.0^{defg}	53.1^{cdef}	33.4^{bcde}	32.6^{cde}	47.6^{cdefg}	5.1^{ab}	102.6^{abcd}	31.3^{bcdef}
	Std dev	9.8	14.1	9.4	8.6	13.8	0.5	10.9	5.1
4	Mean	36.7^{hi}	43.7^{ijk}	27.1^{fgh}	26.9^{hi}	40.5^{ij}	4.9^{abcd}	98.3^{bcde}	31.2^{bcde}
	Std Dev	7.2	8.5	6.2	5.1	8.5	0.8	17.9	7.8
5	Mean	38.4^{ghi}	47.6^{fghij}	29.2^{efg}	29.4^{efgh}	42.1^{ghji}	5.1^{a}	98.8^{def}	29.3^{efghi}
	Std Dev	5.8	10.2	7.2	6.1	9.3	0.8	8.4	5.4
6	Mean	47.8^{bcd}	57.6^{bcd}	37.0^{ab}	35.0^{bcd}	52.7^{abcd}	4.9^{abcde}	105.1^{abc}	34.4^{a}
	Std Dev	9.3	13.6	10.7	7.8	12.0	0.6	13.1	5.2
7	Mean	47.2^{bcde}	55.5^{bcde}	35.7^{abc}	33.8^{bcd}	50.3^{bcde}	4.8^{abcdef}	105.2^{ab}	33.7^{ab}
	Std Dev	7.4	11.5	8.9	6.7	10.4	0.7	11.5	5.7
8	Mean	44.8^{cdef}	50.8^{efghi}	30.7^{defg}	31.6^{def}	47.2^{defg}	4.7^{def}	96.7^{efg}	31.1^{cdef}
	Std Dev	11.0	12.2	8.0	7.5	12.7	0.6	8.7	5.4
9	Mean	35.0^{i}	39.8^{k}	23.6^{h}	24.9^{i}	37.0^{j}	4.6^{ef}	94.9^{efg}	30.4^{defg}
	Std Dev	8.4	10.6	6.9	6.6	10.5	0.5	13.1	6.2
10	Mean	37.8^{hi}	45.2^{hijk}	27.5^{fgh}	28.0^{fghi}	40.8^{ij}	4.8^{abcdef}	97.0^{efg}	30.0^{defgh}
	Std Dev	12.4	17.7	12.4	10.5	14.7	0.8	11.2	5.2
11	Mean	53.0^{a}	64.5^{a}	39.2^{a}	40.1^{a}	57.7^{a}	5.0^{abc}	97.2^{efg}	29.6^{efghi}
	Std Dev	10.5	13.1	10.2	7.6	11.0	0.5	12.2	5.3
12	Mean	50.7^{ab}	60.4^{ab}	37.2^{ab}	37.3^{ab}	53.1^{abc}	4.9^{abcdef}	99.7^{def}	29.1^{fghi}
	Std Dev	11.1	13.8	9.4	8.5	13.3	0.6	9.9	5.1
13	Mean	45.4^{cde}	55.8^{bcde}	36.0^{abc}	33.9^{bcd}	49.3^{bcdef}	5.1^{ab}	105.9^{a}	32.4^{abcd}
	Std Dev	11.5	15.1	10.3	9.1	14.3	0.6	9.7	4.6
14	Mean	43.1^{defg}	48.7^{efghij}	28.6^{defg}	30.5^{defg}	43.3^{fghi}	4.7^{bcdef}	92.3^{efg}	26.8^{i}
	Std Dev	12.5	15.9	10.7	9.6	13.2	0.6	11.8	4.0
15	Mean	45.2^{cdef}	51.1^{efgh}	31.8^{cdef}	31.5^{defg}	44.7^{efghi}	4.6^{f}	99.9^{cde}	29.3^{efghi}
	Std Dev	12.4	16.2	11.3	9.7	13.7	0.6	11.4	5.0
16	Mean	42.6^{efg}	52.0^{defg}	33.7^{bcd}	31.5^{def}	45.1^{efghi}	5.0^{ab}	106.9^{a}	31.4^{bcdef}
	Std Dev	7.7	12.1	8.8	7.2	11.5	0.8	12.6	5.5
17	Mean	38.4^{ghi}	47.8^{fghij}	28.6^{fg}	29.8^{efgh}	42.6^{ghij}	5.1^{a}	96.3^{efg}	28.3^{ghi}
	Std Dev	6.3	11.3	7.3	7.1	11.3	0.8	11.1	4.9
18	Mean	38.3^{ghi}	44.5^{ijk}	26.1^{gh}	27.9^{fghi}	40.2^{ij}	4.7^{cdef}	92.5^{g}	27.8^{hi}
	Std Dev	8.3	10.7	7.7	6.3	9.5	0.5	8.8	5.1
19	Mean	37.1^{hi}	44.2^{ijk}	26.1^{gh}	27.7^{ghi}	39.7^{ij}	4.9^{abcdef}	94.1^{fg}	27.5^{hi}
	Std Dev	6.3	9.5	6.5	5.8	9.7	0.6	11.4	4.3
20	Mean	40.4^{fgh}	46.0^{ghij}	27.9^{fgh}	28.6^{fghi}	41.1^{hij}	4.6^{ef}	96.9^{efg}	29.0^{fghi}
	Std Dev	8.1	9.8	7.3	5.7	8.4	0.4	9.7	4.6

Tra – translucent area without corrections
Trb – translucent area with thickness corrections
Trc – translucent area with thickness and exposure corrections
Tr1% - Translucent area % formula 1(thickness and exposure corrections)
Tr2% - Translucent area % formula 2 (thickness and exposure corrections)

Formula 1: Translucency 1 = $\frac{\text{True translucent area } (mm^2)}{\text{Whole kernel area } (mm^2)} \times \frac{100}{1}$

Formula 2: Translucency 2 = $\frac{\text{True translucent area } (mm^2)}{\text{Endosperm area } (mm^2)} \times \frac{100}{1}$

* Damaged kernels were excluded from a total batch of 50 measurements, causing final sample size to vary
** Means with different letters are statistically significantly different ($p<0.05$) within a column

4.5.1.2 Experimental maize milling data

Table 4.2 Floating number, fat content and product yield (calculated on a moisture-free base) obtained during experimental milling of 20 industrial white maize samples

Sample no	Floating number (weight %)	Fat content (%) of whole maize	Fat content of total semolina* (%)	Yield of semolina 1** (weight %)	Total semolina (grit) yield (weight %, grits between 250 and 1000 µm)	Total semolina (grit) yield (weight %, grits between 250 and 1000 µm, calculated at 0% fat)
1	58	3.67	1.19	35.6	62.3	61.6
2	72	4.02	1.09	35.2	60.4	59.7
3	71	3.98	1.14	34.4	60.7	60.0
4	74	3.76	1.15	34.6	62.2	61.5
5	74	3.48	1.22	35.3	60.9	60.2
6	57	4.36	1.32	36.1	61.4	60.6
7	68	4.22	1.35	35.0	60.4	59.6
8	74	4.05	1.26	34.1	60.4	59.6
9	82	3.72	1.04	33.0	58.4	57.8
10	83	3.81	0.96	34.2	59.6	59.0
11	59	4.42	1.15	38.0	61.0	60.3
12	66	3.88	1.13	35.8	60.8	60.1
13	69	3.96	1.17	34.9	61.6	60.9
14	79	3.78	1.18	34.2	59.7	59.0
15	78	3.75	1.09	34.6	59.9	59.2
16	64	3.97	1.25	36.4	61.5	60.7
17	77	3.80	1.09	35.9	60.4	59.7
18	87	3.70	1.06	34.7	60.3	59.7
19	83	3.30	1.11	34.9	60.7	60.0
20	84	3.52	1.19	34.0	58.9	58.2

* Total semolina refers to all cleaned grits between 250 and 1000 μm

** Semolina 1 is produced from the cleaned large vitreous endosperm grits obtained after the first breaking (rolls I and II, Figure 4.1). The clean grits were larger than 1mm before being reduced to the semolina 1 fraction. Semolina 1 grits were smaller than 767μm and bigger than 250 μm

Analytical results and experimental milling data of the maize samples before milling are summarised in Table 4.2. Fat contents are shown of the whole maize kernels before milling and of the combined total semolina (grits between 250 and 1000 µm) yield after milling. The total semolina yield is given including fat and also calculated on a fat free basis in order to determine if the fat content had a significant effect on the yield of the maize grits. All yields were calculated on a dry basis.

4.5.1.3 Correlations

Table 4.3 Product moment correlation coefficient (r) and R^2 matrixes for milled white maize products and image analysis translucency measurements, with and without corrections for thickness and exposure, experiment 1 (n = 20)

Treatment	Tr1a	Tr2a	Tr1b	Tr2b	Tr1c	Tr2c
Semolina 1(weight %) r	0.55*	0.50*	0.73***	0.69***	0.74***	0.70***
Semolina 1 (weight %) R^2	0.30	0.25	0.53	0.48	0.54	0.48
Total semolina (grits) yield, no fat corrections (weight %) r	0.18	0.21	0.37	0.41	0.48*	0.52*
Total semolina (grits) yield, no fat corrections (weight %) R^2	0.03	0.04	0.14	0.17	0.23	0.27
Total semolina (grits) yield, 0% fat (weight %) r	0.17	0.20	0.36	0.40	0.47*	0.50*
Total semolina (grits) yield, 0% fat (weight %) R^2	0.03	0.04	0.13	0.16	0.22	0.25
Floating number (weight %) r	-0.63**	-0.68**	-0.73***	-0.78****	-0.84****	-0.88****
Floating number (weight %) R^2	0.40	0.46	0.52	0.61	0.70	0.78

Tr1a – Translucency formula 1 without corrections
Tr1b – Translucency formula 2 without corrections
Tr2a – Translucency formula 1 with thickness corrections
Tr2b – Translucency formula 2 with thickness corrections
Tr1c – Translucency formula 1 with thickness and exposure corrections
Tr2c – Translucency formula 2 with thickness and exposure corrections

Formula 1: Translucency 1 = $\frac{\text{True translucent area (mm}^2\text{)}}{\text{Whole kernel area (mm}^2\text{)}} \times \frac{100}{1}$

Formula 2: Translucency 2 = $\frac{\text{True translucent area (mm}^2\text{)}}{\text{Endosperm area (mm}^2\text{)}} \times \frac{100}{1}$

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ for level of significance of the correlation coefficient

Average to good correlations (Table 4.3) were obtained between semolina 1 and translucency and these values increased significantly after the application of thickness and exposure corrections. Good correlations were obtained between floating number and translucency and the values also increased after corrections for thickness as well as exposure. Although initial correlations between total semolina yield (both with fat and without fat) and translucency were poor, significant increases were obtained after applying corrections for thickness and exposure, therefore producing significant correlations ($p<0.05$). Although small differences existed between correlation coefficients calculated based on translucency 1 (% of whole kernel) and translucency 2 (% of endosperm) as seen in Table 4.3, none of these differences were significant when the pairs of r-values were compared.

Scatterplots and fitted regression lines of all correlations between translucency and product yield except semolina yield at 0% fat content are given in Figures 4.3 to 4.8. The linear regression lines show the actual effect that correction factors had on the changing of slopes and correlation coefficients after corrections for thickness and exposure. The placing of the translucency measurements within the middle of the scale range after corrections for exposure compared to having the uncorrected measurements at the higher end of the scale is also clearly visible in all the Figures. Exposure correction had a greater effect on the increase of the slopes of the regression lines than the corrections for thickness (Figures 4.3 to 4.8), while the effect of thickness correction was generally greater on the increase of r-values than the effect of exposure (Table 4.3). The slope always increased after exposure correction, while with thickness correction, the slope either remained the same or increased only slightly. Both the slope and the r-value increases after applying the two correction factors indicated stronger relationships and the trend occurred in all cases.

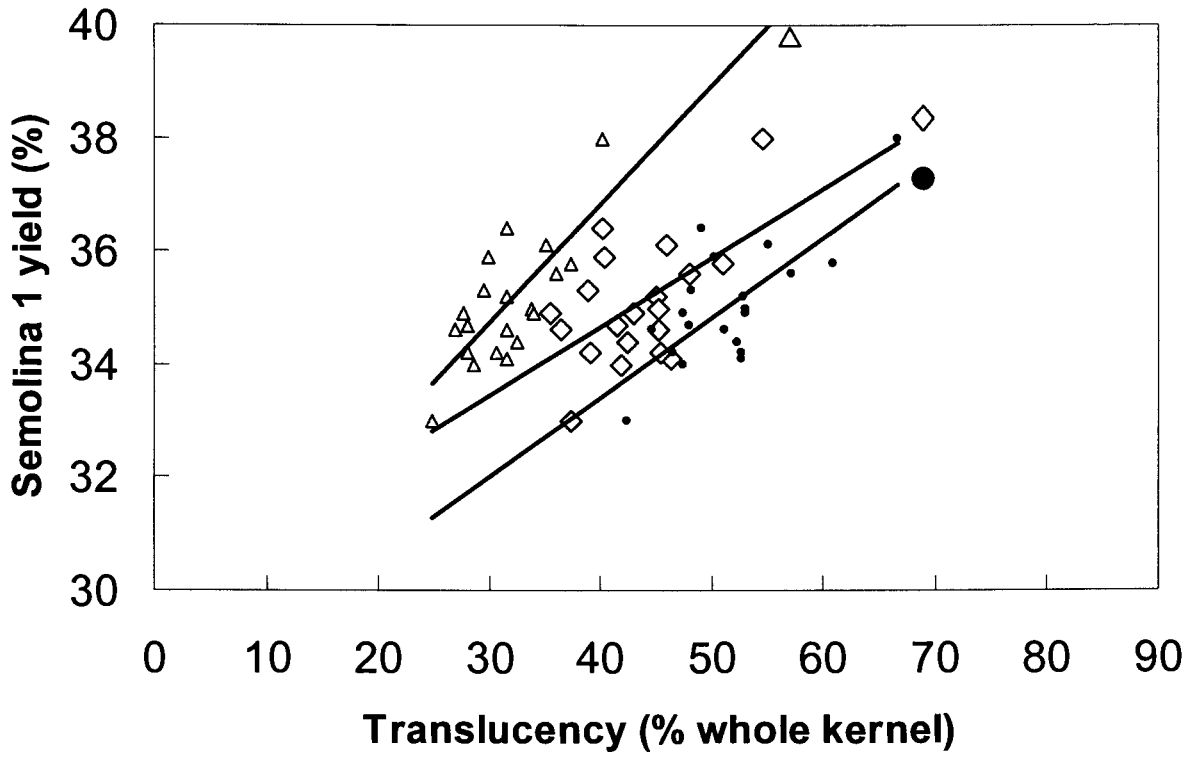


Figure 4.3 Effect of applying correction factors (CFs) on the relationship between the yield of semolina (grits) fraction 1 (mass %) as determined by laboratory milling (Experiment 1) and translucent area (% of whole kernel) of 20 industrial white maize batches determined by image analysis. "◊", before CFs (y = 0.12x + 30, r = 0.55); "●", after thickness CF (y = 0.14x + 28, r = 0.73); "△", after thickness and exposure CFs (y = 0.21x + 28, r = 0.74), n = 20. r-values did not differ significantly from each other ($p \geq 0.05$) (Fisher test)

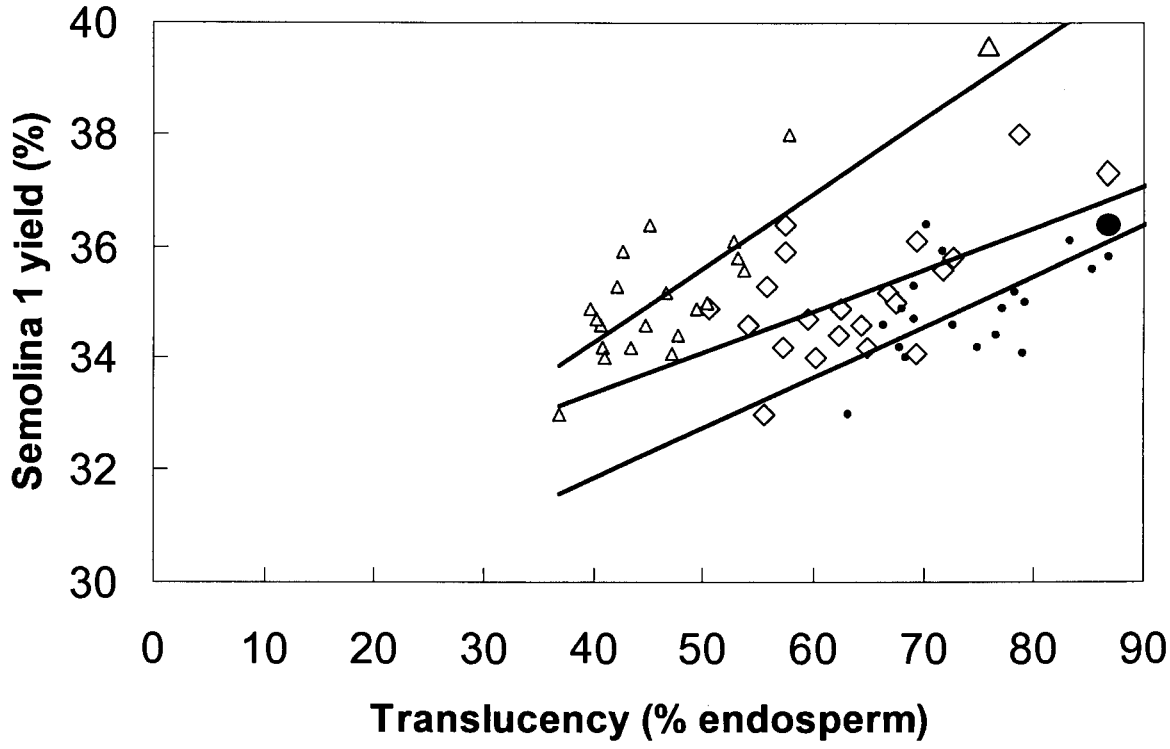


Figure 4.4 Effect of applying correction factors (CFs) on the relationship between the yield of the semolina (grits) fraction 1 (mass %) as determined by laboratory milling (Experiment 1) and translucent area (% of endosperm) of 20 industrial white maize samples as determined using image analysis. "◊", before CFs (y = 0.07x + 30, r = 0.50); "•", after thickness CF (y = 0.09x + 28, r = 0.69); "△", after thickness and exposure CFs (y = 0.13x + 29, r = 0.70), n = 20. r values of the three graphs did not differ significantly from each other ($p \geq 0.05$) (Fisher test)

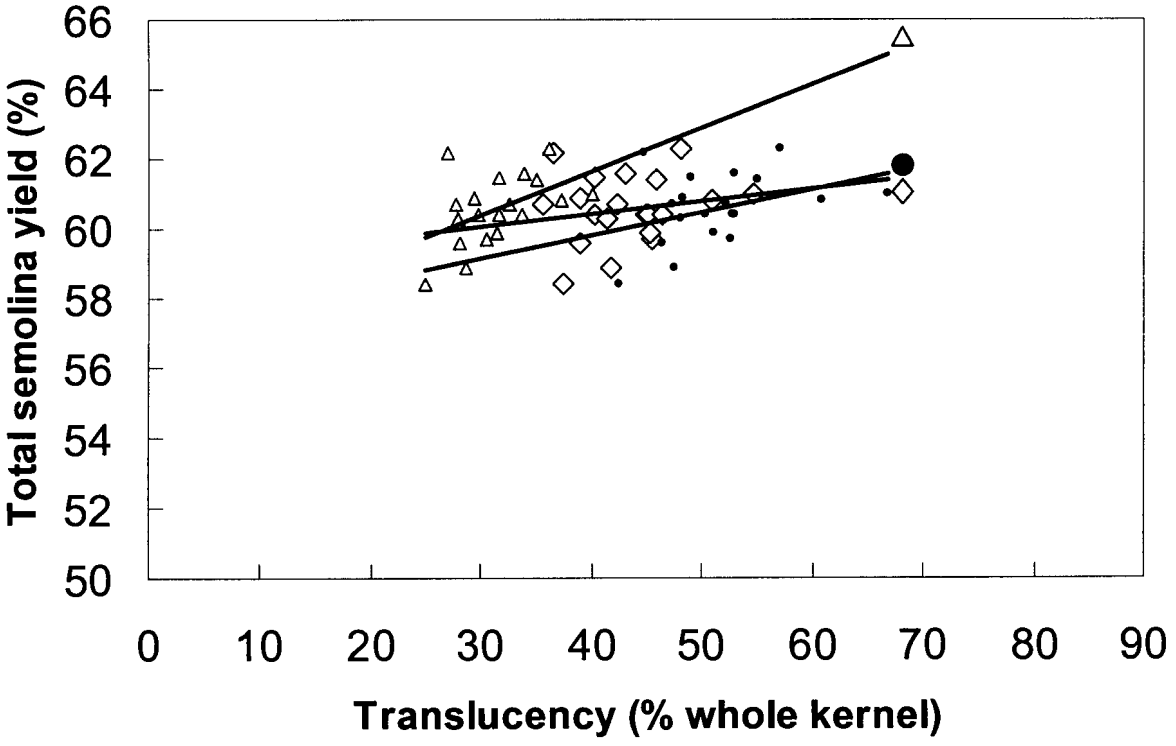


Figure 4.5 Effect of applying correction factors (CFs) on the relationship between the total yield of the semolina (mass %) before corrections for fat content as determined by laboratory milling (Experiment 1) and translucent area (% of whole maize) of 20 industrial white maize batches as determined using image analysis. "◊", before CFs (y = 0.04x + 59, r = 0.18); "●", after thickness CF (y = 0.07x + 57, r = 0.37); "△", after thickness and exposure CFs (y = 0.13x + 57, r = 0.48), n = 20. r-values did not differ significantly from each other ($p \geq 0.05$) (Fisher test)

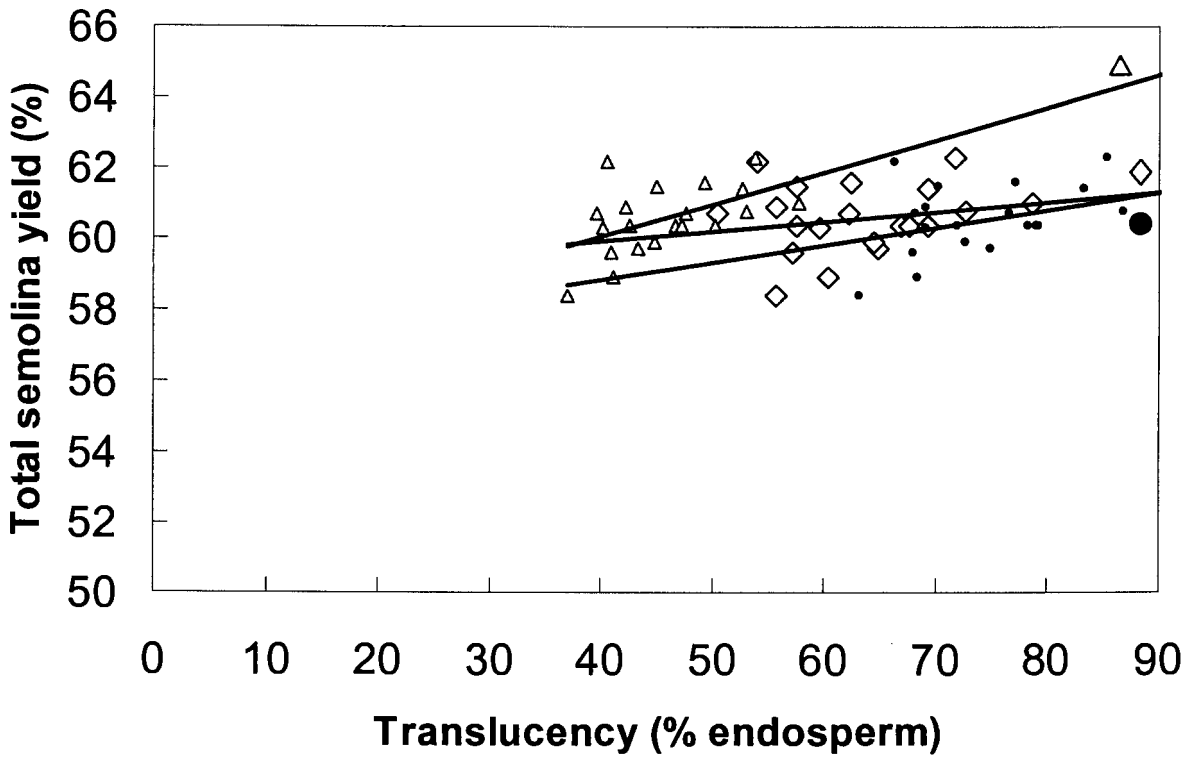


Figure 4.6 Effect of applying correction factors (CFs) on the relationship between the total yield of the semolina (mass %) on a fat free basis as determined by laboratory milling (Experiment 1) and translucent area (% of endosperm) of 20 industrial white maize batches as determined using image analysis. "◊", before CFs (y = 0.03x + 59, r = 0.21); "●", after thickness CF (y = 0.05x + 57, r = 0.41); "△", after thickness and exposure CFs (y = 0.09x + 56, r = 0.52), n = 20. r-values did not differ significantly from each other ($p \geq 0.05$) (Fisher test)

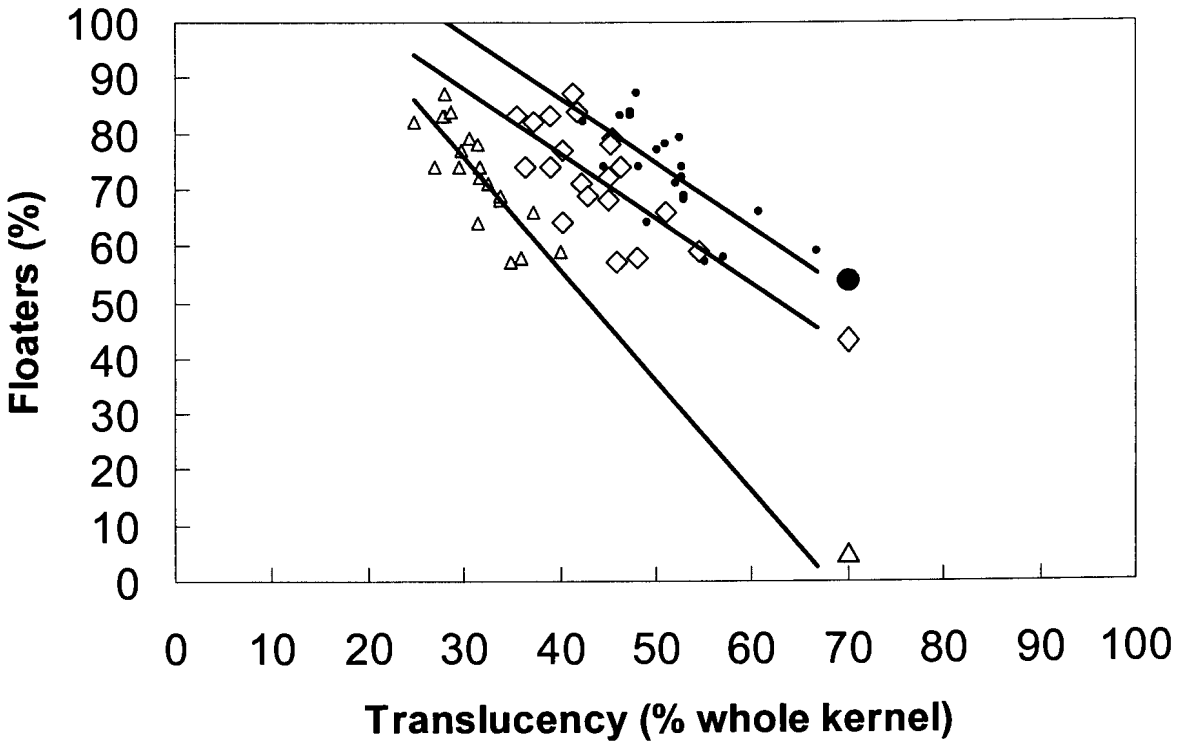


Figure 4.7 Effect of applying correction factors (CFs) on the relationship between floaters (mass %) and translucent area (% of whole kernel) of 20 industrial white maize batches determined by using image analysis. "◊", before CFs (y = -1.16x + 123, r = -0.63); "●", after thickness CF (y = -1.16x + 133, r = -0.73); "△", after thickness and exposure CFs (y = -2x + 136, r = -0.84), n = 20. r-values did not differ significantly ($p \geq 0.05$) (Fisher test)

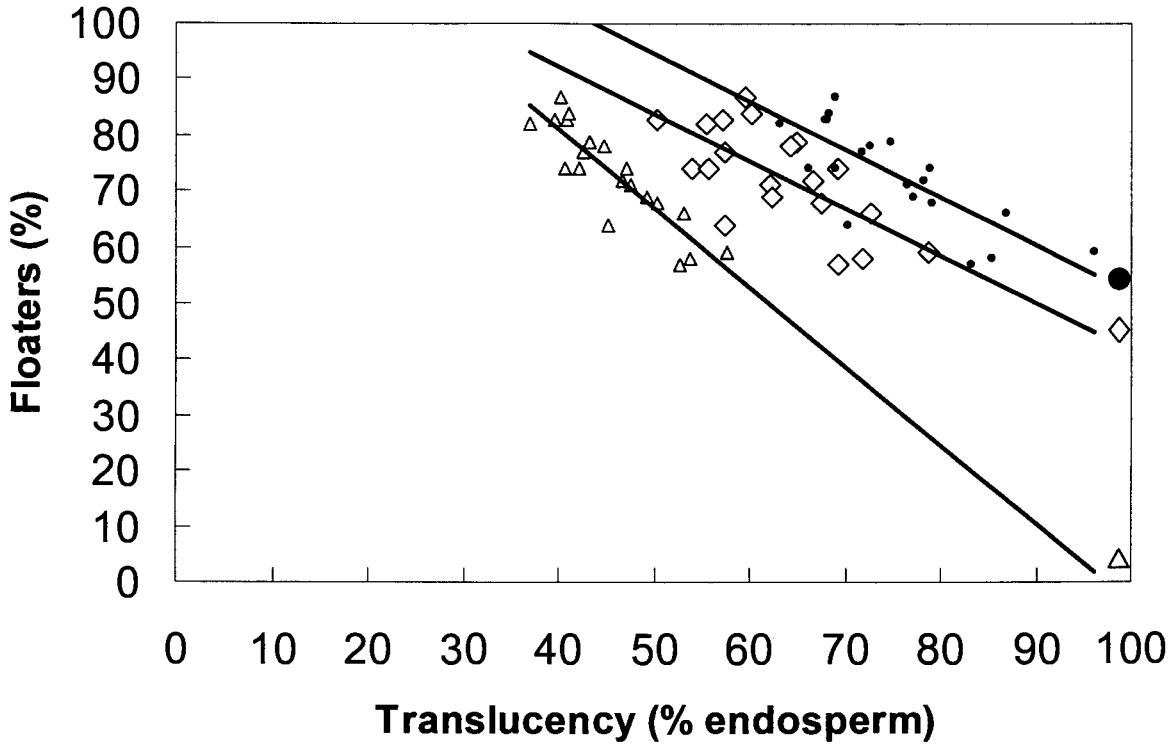


Figure 4.8 Effect of applying correction factors (CFs) on the relationship between floaters (mass %) and translucent area (% of endosperm) of 20 industrial white maize batches as determined by image analysis. "◊", before CFs (y = -0.84x + 126, r = -0.68); "●", after thickness CF (y = -0.86x + 138, r = -0.78); "△", after thickness and exposure CFs (y = -1.41x + 138, r =-0.88), n = 20. r-values did not differ significantly from each other ($p \geq 0.05$) (Fisher test)

4.5.2 Experiment 2

4.5.2.1 Image Analysis

Image analysis data are summarised in Table 4.4.

Table 4.4 Image analysis translucency and morphology measurements on eight industrial white maize samples and two industrial yellow maize samples (45 – 50* kernels per sample)

Sample no		Tra (mm^2)	Trb (mm^2)	Trc (mm^2)	Tr1 (%)	Tr2 (%)	Thickness (mm)	Total area (mm^2)	Germ area (mm^2)
		**							
1	Mean	57.3^{bc}	82.5^{ab}	53.5^{ab}	50.0^{ab}	72.0^{a}	6.0^{a}	106.1^{bc}	31.9^{bc}
	Std Dev	14.9	24.6	18.0	14.4	20.2	0.9	11.0	3.5
2	Mean	64.2^{a}	81.2^{ab}	54.1^{ab}	48.8^{ab}	63.4^{bc}	5.2^{cde}	110.0^{b}	24.9^{d}
	Std Dev	14.5	24.0	17.6	14.0	18.4	0.9	10.0	4.7
3	Mean	60.9^{ab}	75.4^{bc}	50.2^{bc}	45.2^{bc}	67.7^{abc}	5.1^{de}	109.4^{b}	35.2^{a}
	Std dev	14.9	21.5	17.4	12.0	17.1	0.8	14.8	6.2
4	Mean	52.0^{c}	63.8^{d}	41.5^{de}	38.6^{d}	55.5^{d}	5.1^{de}	106.7^{bc}	31.9^{bc}
	Std Dev	16.3	21.2	15.4	12.5	18.2	0.8	11.4	3.4
5	Mean	61.8^{ab}	80.4^{ab}	53.6^{ab}	48.2^{ab}	69.5^{ab}	5.5^{bc}	110.4^{b}	33.1^{b}
	Std Dev	16.2	26.6	19.5	15.6	22.4	0.8	12.4	4.4
6	Mean	64.0^{a}	88.9^{a}	58.2^{a}	53.7^{a}	70.2^{ab}	5.8^{ab}	107.4^{bc}	24.9^{d}
	Std Dev	14.7	23.5	17.1	13.7	17.8	1.1	9.4	4.6
7	Mean	52.9^{c}	69.0^{cd}	44.4^{cd}	42.0^{cd}	60.3^{cd}	5.4^{cd}	104.9^{bc}	31.4^{bc}
	Std Dev	14.5	22.3	15.8	13.2	19.5	0.7	9.1	5.1
8	Mean	41.0^{d}	50.1^{e}	35.2^{e}	29.5^{e}	39.7^{e}	4.9^{ef}	115.8^{a}	29.9^{c}
	Std Dev	16.2	23.5	18.7	13.3	17.1	0.8	18.6	4.7
9	Mean	55.7^{bc}	74.0^{bc}	47.1^{bcd}	45.2^{bc}	64.8^{abc}	5.5^{bc}	102.7^{c}	30.3^{c}
	Std Dev	9.8	20.8	16.3	11.8	15.9	1.1	13.3	6.3
10	Mean	31.9^{e}	36.2^{f}	24.1^{f}	24.7^{e}	37.5^{e}	4.6^{f}	97.0^{d}	32.7^{b}
	Std Dev	13.2	16.4	11.4	11.0	17.0	0.8	9.0	5.1

Tra – translucent area without corrections
Trb – translucent area with thickness corrections
Trc – translucent area with thickness and exposure corrections
Tr1% - Translucent area % formula 1(thickness and exposure corrections)
Tr2% - Translucent area % formula 2 (thickness and exposure corrections)
Samples 1 – 8, white cultivars
Samples 9 and 10, yellow cultivars

Formula 1: Translucency 1 = $\frac{\text{True translucent area } (mm^2)}{\text{Whole kernel area } (mm^2)} \times \frac{100}{1}$

Formula 2: Translucency 2 = $\frac{\text{True translucent area } (mm^2)}{\text{Endosperm area } (mm^2)} \times \frac{100}{1}$

* Damaged kernels were excluded from a total batch of 50 measurements, causing final sample size to vary
** Means with different letters are statistically significantly different ($p<0.05$) within a column

Statistically significant differences were found for Translucency 1 and 2 (Table 4.4) measurements between samples indicating that the data are suitable for further analysis by linear regression. Morphology data (thickness, total area and germ area) also showed statistically significant differences among the 10 samples.

4.5.2.2 Maize milling data

Experimental milling data are given in Table 4.5. Data include moisture contents after conditioning and before degerming, and yield of milled products (mass %) obtained during milling. The results represent mean values of 510 kg batches milled for each type of maize.

Table 4.5 Experimental milling data of 10 lots (22 sub-samples) of maize (eight white and two yellow) in a industrial 3 ton/hour Bühler dry maize mill

Sample no***		Whole kernel moisture before degermer (%)	Extraction at degermer (% overs, 5 mm sieve) ****	Break flour in thrus (%)*	Total maize meal extraction (%), as is	Total maize meal extraction (%), moisture free base
1	Mean	15.2	70.0^{a}	8.2^{bcde}	74.2^{c}	69.6^{bc}
	Std Dev	3.2	0.4	0.1	0.2	1.0
2	Mean	17.5	69.7^{ab}	7.9^{cde}	73.8^{c}	68.3^{c}
	Std Dev	0.6	3.6	0.5	0.6	0.8
3	Mean	17.3	72.1^{a}	7.5^{de}	76.0^{b}	71.0^{bc}
	Std dev	0.2	1.2	0.5	0.6	0.7
4	Mean	16.4	56.5^{cd}	10.2^{ab}	70.2^{d}	64.3^{d}
	Std Dev	1.8	3.3	1.4	1.2	2.2
5	Mean	15.6	69.7^{ab}	8.5^{bcd}	73.9^{c}	69.0^{bc}
	Std Dev	2.0	0.1	0.2	0.6	1.4
6	Mean	17.5	71.9^{a}	7.0^{de}	76.5^{b}	71.6^{b}
	Std Dev	0.8	2.4	0.7	0.4	0.7
7	Mean	15.5	59.5^{c}	9.8^{abc}	74.0^{c}	69.2^{bc}
	Std Dev	0.7	0.2	0.9	0.0	0.3
8	Mean	14.1	53.9^{d}	11.5^{a}	**	**
	Std Dev	0.7	3.6	1.9	**	**
9	Mean	16.9	64.6^{b}	6.3^{e}	80.0^{a}	76.1^{a}
	Std Dev	1.6	0.7	0.4	0.3	0.6
10	Mean	16.1	42.3^{e}	8.0^{cde}	68.7^{e}	62.2^{d}
	Std Dev	1.2	0.0	0.3	0.8	1.7

* Weight of fine flour (<500 μm) recovered after sieving the thru fraction obtained from the degermer
** Not determined due to mill breakdown
*** Samples 1 – 8, white maize, samples 9 – 10, yellow maize
**** Means with different letters are statistically significantly different ($p<0.05$) within a column

Statistically significant differences occurred among the yields of the various milled products for the 10 milled lots (Table 4.5). The different yields were in a narrow range for all the products except extraction at degermer, which showed a range of 29.8% for the mean of the yield between the highest and lowest values.

4.5.2.3 Correlations

Table 4.6 Product moment correlation coefficient (r) and R^2 matrixes for milled maize products and image analysis translucency measurements, with and without corrections for kernel thickness and light exposure, experiment 2 (n = 22, n includes all sub-samples of each lot, each sub-sample represents a separate milling trial)

Treatment	Tr1a	Tr2a	Tr1b	Tr2b	Tr1c	Tr2c
Extraction at degermer (weight %) r	0.91****	0.87****	0.91****	0.88****	0.93****	0.91****
Extraction at degermer (weight %) R^2	0.83	0.75	0.83	0.8	0.86	0.82
Total extraction of maize meal, no moisture corrections (weight %) r	0.61**	0.64**	0.75****	0.84****	0.68***	0.72****
Total extraction of maize meal, no moisture corrections (weight %) R^2	0.37	0.41	0.51	0.56	0.46	0.52
Total extraction of maize meal, 0% moisture (weight %) r	0.59**	0.64**	0.71****	0.75****	0.66***	0.72****
Total extraction of maize meal, 0% moisture (weight %) R^2	0.35	0.40	0.50	0.57	0.44	0.52
Break flour in the thrus (weight %) r	-0.46*	-0.48*	-0.50**	-0.52**	-0.50*	-0.50**
Break flour in the thrus (weight %) R^2	0.21	0.23	0.25	0.27	0.24	0.26

Tr1a – Translucency formula 1 without corrections
Tr1b – Translucency formula 2 without corrections
Tr2a – Translucency formula 1 with thickness corrections
Tr2b – Translucency formula 2 with thickness corrections
Tr1c – Translucency formula 1 with thickness and exposure corrections
Tr2c – Translucency formula 2 with thickness and exposure corrections

Formula 1: Translucency 1 = $\frac{\text{True translucent area (mm}^2\text{)}}{\text{Whole kernel area (mm}^2\text{)}} \times \frac{100}{1}$

Formula 2: Translucency 2 = $\frac{\text{True translucent area (mm}^2\text{)}}{\text{Endosperm area (mm}^2\text{)}} \times \frac{100}{1}$

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ for level of significance of the correlation coefficient

Correlation coefficients between extraction of milled maize products and maize kernel translucency (Table 4.6) were highly significant in general except for the break flour in the thrus, where the relationships were only significant at $p<0.05$. The values for extraction at degermer increased further after the application of thickness and exposure corrections. Although corrections for thickness significantly increased correlations for total extraction of maize meal and break flour in the thrus, corrections for exposure did not have the same effect (either stayed the same or decreased the correlations slightly.

Scatterplots and fitted regression lines of all correlations between translucency and product yield except extraction of total special maize meal at 0% moisture content are given in Figures 4.9 to 4.14. Scatterplots show the actual effect that correction factors had on the results such as changing slopes accompanied by changing correlation coefficients and levels of significance after corrections for thickness and exposure.

The placing of the translucency measurements within the middle of the scale range after corrections for exposure compared to having the uncorrected measurements at the higher end of the scale is clearly visible in all the Figures. Slopes did not always increase after corrections. In Figures 4.9 – 4.14, the slope decreased after corrections for thickness, but increased again after corrections for exposure. The slope always increased after exposure correction, while with thickness correction, the slopes decreased. Correlation coefficients either stayed the same or increased after thickness corrections, but either increased, stayed the same or decreased after corrections for exposure (Table 4.6). The combined effects of the slope and the r-value changes after applying the two correction factors produced stronger relationships in all cases.

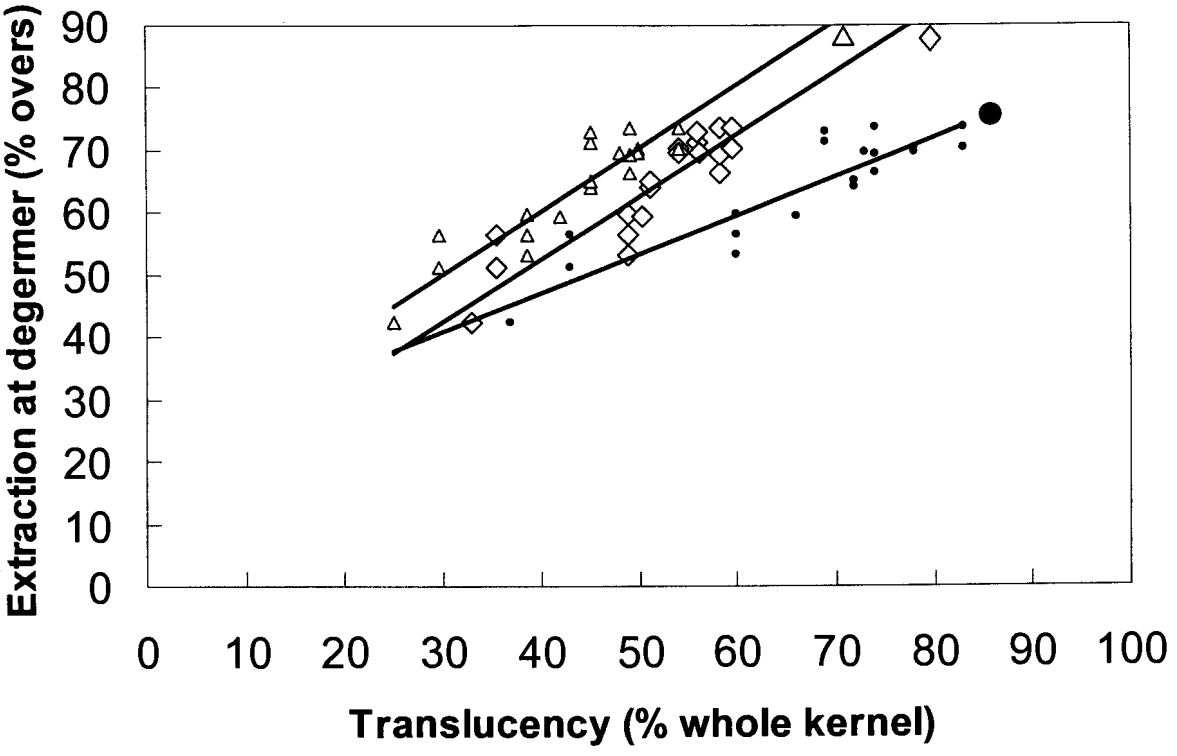


Figure 4.9 Effect of applying correction factors (CFs) on the relationship between extraction at degermer (mass %) and translucent area (% of whole kernel) of 8 industrial white maize batches and 2 industrial yellow maize batches as determined by image analysis. "◊", before CFs (y = 1.0x + 12.4, r = 0.91); "●", after thickness CF (y = 0.62x + 22.3, r = 0.91); "△", after thickness and exposure CFs, (y = 1.0x + 19.7, r =0.93), n = 22. r-values were not significantly different from each other ($p \geq 0.05$) (Fisher test)

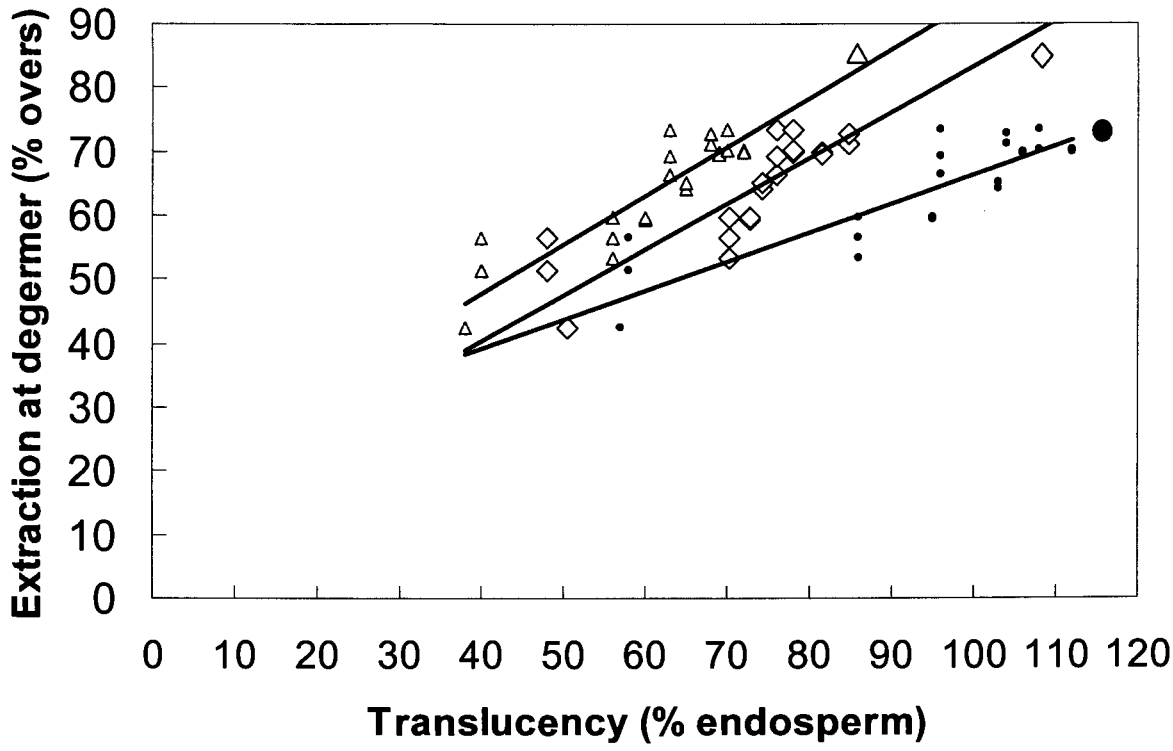


Figure 4.10 Effect of applying correction factors (CFs) on the relationship between extraction at degermer (mass %) and translucent area (% of endosperm) of eight industrial white and two industrial yellow maize batches as determined by image analysis. "◊", before CFs (y = 0.72x + 11.8, r = 0.87); "●", after thickness CF (y = 0.45x + 21.1, r = 0.88); "△", after thickness and exposure CFs, (y = 0.76x + 17.1, r =0.91), n = 22. r-values were not significantly different from each other ($p \geq 0.05$) (Fisher test), but were all highly significant at $p < 0.0001$

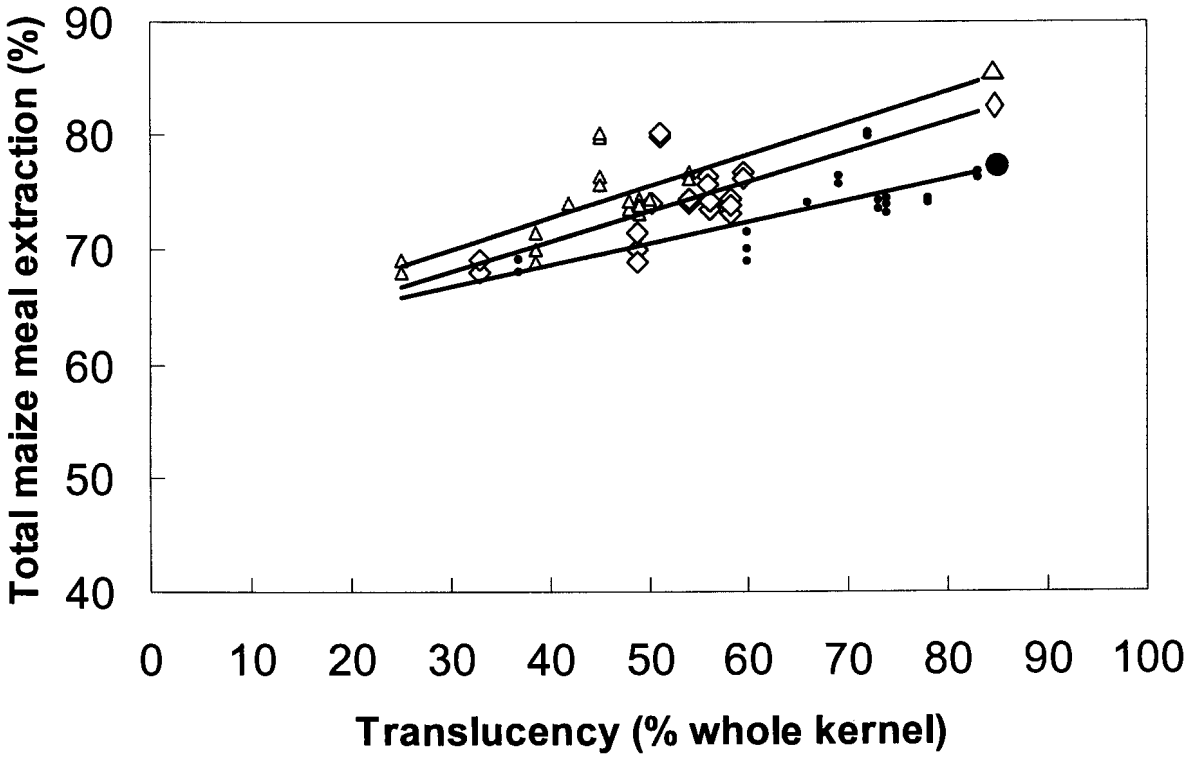


Figure 4.11 Effect of applying correction factors (CFs) on the relationship between total maize meal extraction (mass %) and translucent area (% of endosperm) of eight industrial white and two industrial yellow maize batches as determined by image analysis. "◊", before CFs (y = 0.26x + 60.2, r = 0.61); "●", after thickness CF (y = 0.19x + 61.3, r = 0.72); "△", after thickness and exposure CFs, (y = 0.28x +61.8, r = 0.68), n = 22. r-values were not significantly different from each other ($p \geq 0.05$) (Fisher test)

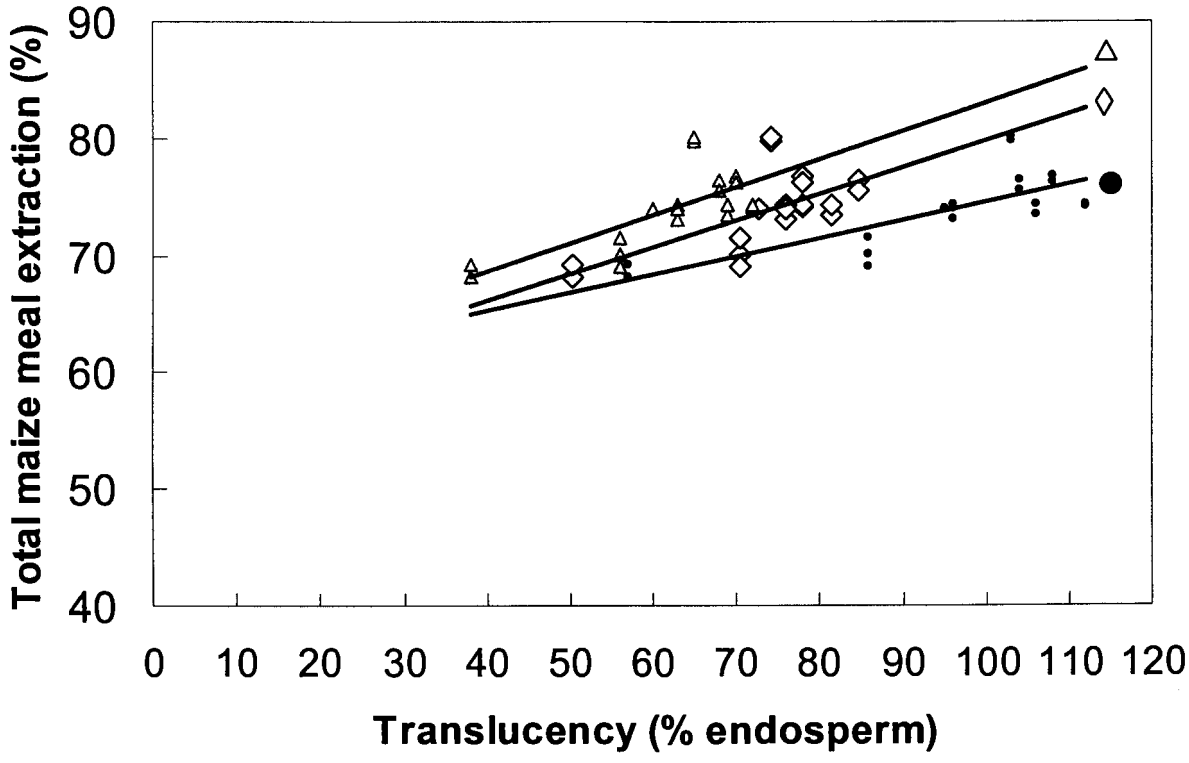


Figure 4.12 Effect of applying correction factors (CFs) on the relationship between total maize meal extraction (mass %) and translucent area (% of endosperm) of eight industrial white and two industrial yellow maize meal batches as determined by image analysis. "◊", before CFs (y = 0.23x + 57, r = 0.64); "●", after thickness CF (y = 0.16x + 59, r = 0.75);"△", after thickness and exposure CFs, (y = 0.24x + 59.1, r =0.72), n = 22. r-values were not significantly different from each other ($p \geq 0.05$) (Fisher test)

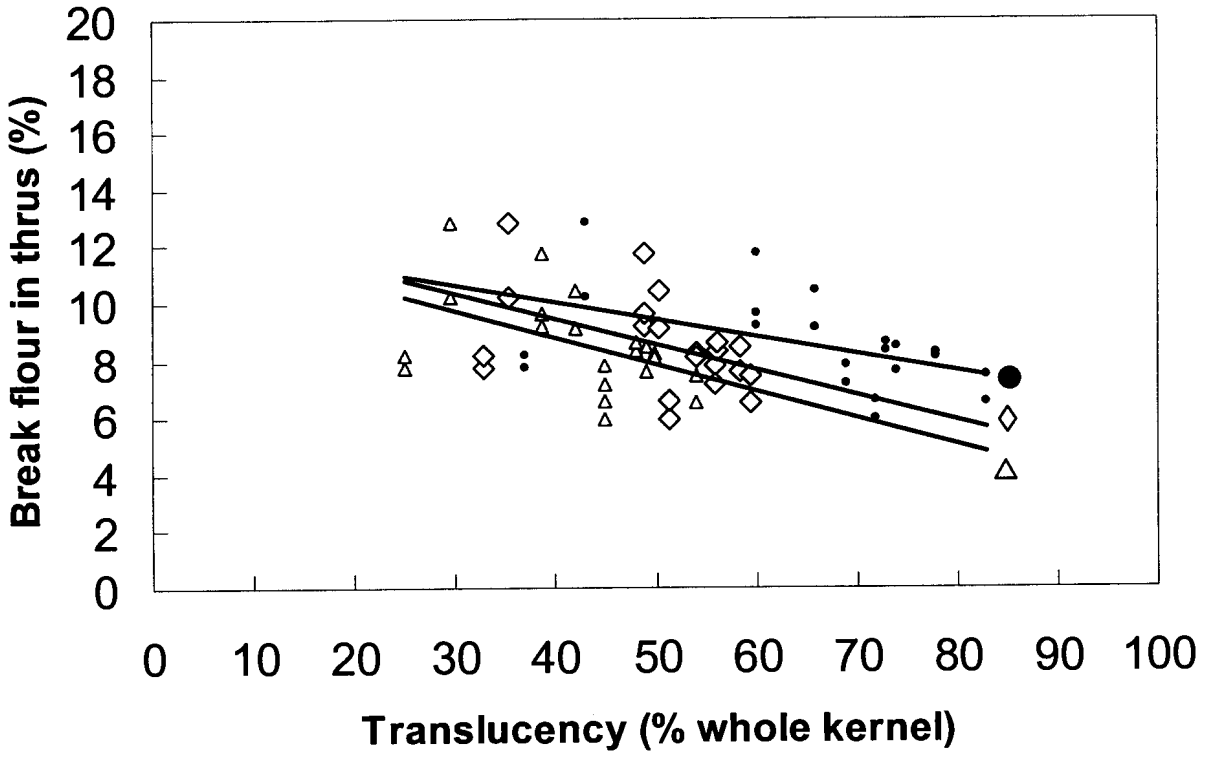


Figure 4.13 Effect of applying correction factors (CFs) on the relationship between break flour in thrus (mass %) and translucent area (% of whole kernel) of eight industrial white and two industrial yellow maize batches as determined by image analysis. "◊", before CFs (y = -0.09x + 13, r = -0.46); "•", after thickness CF (y = -0.06x + 12.5, r = -0.50); "△", after thickness and exposure CFs, (y = -0.1x + 12.5, r = -0.50), n = 22. r-values were not significantly different from each other ($p \geq 0.05$) (Fisher test)

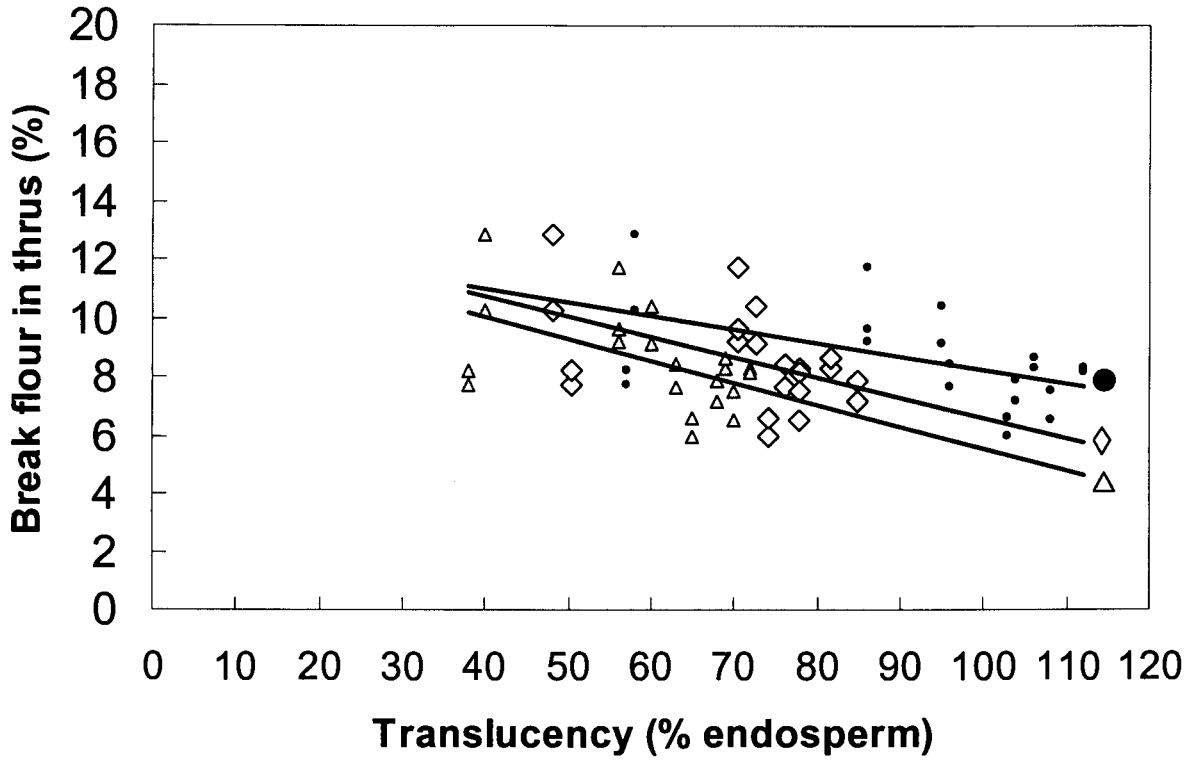


Figure 4.14 Effect of applying correction factors (CFs) on the relationship between the yield of break flour in thrus (mass %) and translucent area (% of endosperm) of eight industrial white and two industrial yellow maize batches as determined by image analysis. "◊", before CFs (y = -0.07x + 13.5, r = -0.48); "•", after thickness CF (y = -0.05x + 12.8, r = -0.52);"△", after thickness and exposure CFs, (y = -0.0.7x + 13, r = -0.50), n = 22. r-values did not differ significantly from each other ($p \geq 0.05$) (Fisher test)

4.5.3 Experiment 3

4.5.3.1 Image Analysis

Table 4.7 Image analysis translucency and morphology measurements on 12 samples of yellow maize (45 – 50* kernels per sample)

Sample no		Tra (mm^2)	Trb (mm^2)	Trc (mm^2)	Tr1 (%)	Tr2 (%)	Thickness (mm)	Total area (mm^2)	Germ area (mm^2)
		**							
1	Mean	34.2^{a}	51.7^{a}	33.2^{a}	35.7^{a}	45.5^{ab}	4.5^{d}	88.5^{bcd}	24.2^{d}
	Std Dev	12.0	14.7	10.7	9.7	14.0	0.4	11.9	5.1
2	Mean	40.6^{c}	40.1^{cd}	24.4^{e}	28.2^{cd}	42.2^{cde}	4.7^{c}	93.7^{e}	24.0^{c}
	Std Dev	15.2	13.1	8.1	9.3	15.6	0.4	8.1	4.1
3	Mean	30.5^{a}	51.3^{a}	32.1^{ab}	35.7^{a}	49.8^{a}	4.4^{d}	79.9^{cde}	22.6^{d}
	Std dev	11.2	13.4	9.9	8.8	13.4	0.4	8.4	4.6
4	Mean	35.3^{b}	46.9^{ab}	29.3^{abcd}	32.7^{ab}	46.2^{abc}	4.5^{d}	87.2^{de}	27.7^{d}
	Std Dev	9.9	13.5	10.2	8.8	12.9	0.4	10.0	5.1
5	Mean	46.4^{ab}	46.3^{ab}	30.1^{abc}	31.8^{abc}	43.2^{bcde}	4.4^{d}	92.2^{ab}	25.3^{d}
	Std Dev	12.9	15.6	10.9	10.5	15.3	0.4	8.6	4.0
6	Mean	41.7^{c}	40.4^{bcd}	25.2^{de}	28.9^{cd}	39.2^{de}	4.8^{bc}	88.1^{de}	25.4^{d}
	Std Dev	10.9	15.0	10.6	10.2	14.8	0.5	10.6	4.5
7	Mean	32.8^{c}	40.9^{bcd}	26.5^{cde}	28.2^{cd}	43.6^{abcde}	5.1^{a}	94.4^{ab}	32.5^{a}
	Std Dev	9.9	12.4	8.4	8.6	14.1	0.3	9.1	3.8
8	Mean	31.9^{c}	43.3^{bc}	28.9^{bcd}	29.4^{bcd}	45.8^{abcd}	5.3^{a}	93.1^{a}	33.2^{a}
	Std Dev	10.2	12.3	9.4	8.0	13.0	0.3	8.5	4.6
9	Mean	32.1^{c}	40.8^{bcd}	25.9^{cde}	28.3^{cd}	44.5^{abcde}	5.2^{a}	92.0^{bcd}	32.9^{a}
	Std Dev	11.3	13.5	8.8	9.3	15.5	0.4	8.9	4.7
10	Mean	30.7^{c}	41.7^{bcd}	26.8^{cde}	28.8^{cd}	45.2^{abcd}	5.2^{a}	93.4^{ab}	30.0^{a}
	Std Dev	8.7	10.1	7.1	7.0	12.3	0.3	10.0	4.2
11	Mean	33.1^{c}	41.1^{bcd}	26.4^{cde}	28.3^{cd}	44.6^{abcde}	5.2^{a}	97.0^{bc}	34.4^{a}
	Std Dev	10.3	12.1	8.3	8.2	13.9	0.4	9.6	4.1
12	Mean	32.0^{c}	37.1^{d}	23.9^{e}	25.6^{d}	38.4^{e}	4.9^{b}	93.2^{ab}	32.8^{b}
	Std Dev	9.3	11.3	7.7	7.7	12.7	0.3	10.7	4.1

Tra – translucent area without corrections
Trb – translucent area with thickness corrections
Trc – translucent area with thickness and exposure corrections
Tr1% - Translucent area % formula 1(thickness and exposure corrections)
Tr2% - Translucent area % formula 2 (thickness and exposure corrections)

Formula 1: Translucency 1 = $\frac{\text{True translucent area } (mm^2)}{\text{Whole kernel area } (mm^2)} \times \frac{100}{1}$

Formula 2: Translucency 2 = $\frac{\text{True translucent area } (mm^2)}{\text{Endosperm area } (mm^2)} \times \frac{100}{1}$

* Damaged kernels were excluded from a total batch of 50 measurements, causing final sample size to vary
** Means with different letters are statistically significantly different ($p<0.05$) within a column

Statistically significant differences were found for Translucency 1 and 2 (Table 4.7) measurements between samples indicating that the data are suitable for further

analysis by linear regression. Morphology data (thickness, total area and germ area) also showed statistically significant differences among the 12 samples.

4.5.3.2 Degerming data

Experimental degerming data are given in Table 4.8. Results include the yield of milled products (weight %) obtained during milling. The results represent mean values of three batches milled for each type of maize. Maize was degermed, sieved and aspirated.

Table 4.8 Yield of products after experimental degerming of 12 samples of yellow maize in a pilot scale Beall-type degermer followed by sieving and aspiration

Sample no		Flaking grits > 3.9 mm (weight %)	Coarse grits > 3.3 mm (weight %)	Fines (break flour) (weight %)	Offal* (weight %)	Total grits > 1.01 mm (weight %)
		**				
1	Mean	51.0^{bc}	60.1^{ab}	14.3^{a}	12.1^{a}	73.7^{a}
	Std Dev	2.4	4.4	1.2	5.9	4.8
2	Mean	50.7^{bc}	60.9^{ab}	13.8^{ab}	11.5^{a}	74.6^{a}
	Std Dev	1.0	2.1	1.6	5.4	3.8
3	Mean	53.6^{a}	64.0^{a}	11.7^{ab}	10.4^{a}	77.9^{a}
	Std dev	0.7	2.1	0.2	3.6	3.4
4	Mean	51.8^{ab}	63.1^{a}	12.0^{ab}	10.9^{a}	77.1^{a}
	Std Dev	1.1	2.1	0.7	4.5	3.9
5	Mean	50.9^{bc}	60.5^{ab}	13.6^{ab}	12.4^{a}	73.9^{a}
	Std Dev	0.6	1.9	1.4	4.7	3.3
6	Mean	49.8^{bc}	61.9^{a}	13.0^{ab}	11.3^{a}	75.7^{a}
	Std Dev	2.1	3.7	0.5	5.3	4.9
7	Mean	45.5^{d}	56.9^{b}	14.0^{ab}	12.0^{a}	74.0^{a}
	Std Dev	1.9	3.4	2.1	6.9	4.9
8	Mean	49.0^{c}	60.1^{ab}	12.0^{ab}	13.6^{a}	74.4^{a}
	Std Dev	0.3	0.9	0.7	3.0	2.3
9	Mean	48.9^{c}	60.0^{ab}	11.9^{b}	12.2^{a}	73.5^{a}
	Std Dev	1.6	2.2	0.8	5.4	3.1
10	Mean	50.5^{bc}	62.4^{a}	11.7^{ab}	11.9^{a}	76.4^{a}
	Std Dev	2.4	2.9	1.3	5.3	4.0
11	Mean	49.6^{bc}	59.0^{ab}	12.9^{ab}	13.5^{a}	73.6^{a}
	Std Dev	1.1	2.3	2.3	6.6	4.4
12	Mean	43.3^{d}	56.2^{b}	13.6^{ab}	10.2^{a}	76.2^{a}
	Std Dev	1.7	2.4	2.0	5.7	3.8

* Offal consisted of total combined separated bran and germ fractions
** Means with different letters are statistically significantly different ($p<0.05$) within a column

Statistically significant differences occurred among the yields of degermed products for the 12 samples for flaking grits, coarse grits and fines (Table 4.8). However, the samples of coarse grits and fines could only be divided into three groups. No significant differences occurred among the yields of offal and total grits > 1.01 mm.

4.5.3.3 Correlations

Table 4.9 Product moment correlation coefficient (r) and R^2 matrixes for image analysis translucency measurements and yield (weight %) of products from yellow degermed maize, with and without corrections for kernel thickness and exposure, experiment 3 (n = 36)

Treatment	Tr1a	Tr2a	Tr1b	Tr2b	Tr1c	Tr2c
Flaking grits > 3.9 mm (weight %) r	0.68***	0.48*	0.73****	0.51*	0.67***	0.44*
Flaking grits > 3.9 mm (weight %) R^2	0.46	0.23	0.53	0.26	0.44	0.20
Coarse grits > 3.3 mm* (weight %) r	0.71****	0.49*	0.68***	0.45*	0.58**	0.34
Coarse grits > 3.3 mm (weight %) R^2	0.5	0.24	0.46	0.21	0.33	0.11
Yield of grits > 1.01 mm* (weight %) r	0.14	-0.02	0.10	0.05	-0.03	-0.04
Yield of grits > 1.01 mm (weight %) R^2	0.02	0.00	0.01	0.00	0.00	0.00

Tr1a – Translucency formula 1 without corrections
Tr1b – Translucency formula 2 without corrections
Tr2a – Translucency formula 1 with thickness corrections
Tr2b – Translucency formula 2 with thickness corrections
Tr1c – Translucency formula 1 with thickness and exposure corrections
Tr2c – Translucency formula 2 with thickness and exposure corrections

$$\text{Formula 1: Translucency 1} = \frac{\text{True translucent area (mm}^2\text{)}}{\text{Whole kernel area (mm}^2\text{)}} \times \frac{100}{1}$$

$$\text{Formula 2: Translucency 2} = \frac{\text{True translucent area (mm}^2\text{)}}{\text{Endosperm area (mm}^2\text{)}} \times \frac{100}{1}$$

* Cumulative yields (all grits above the target particle size)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ for level of significance of the correlation coefficient

Correlation coefficients between extraction of milled maize products and maize kernel translucency (Table 4.9) were generally low indicating weak relationships, except for translucency 1 values against flaking grits and coarse grits, which were

slightly higher ($p<0.0001$). Correlations decreased after corrections for exposure in all cases.

Scatterplots and fitted regression lines of all correlations between translucency and product yield except the yield of grits larger than 1.01 mm are given in Figures 4.15 to 4.18.

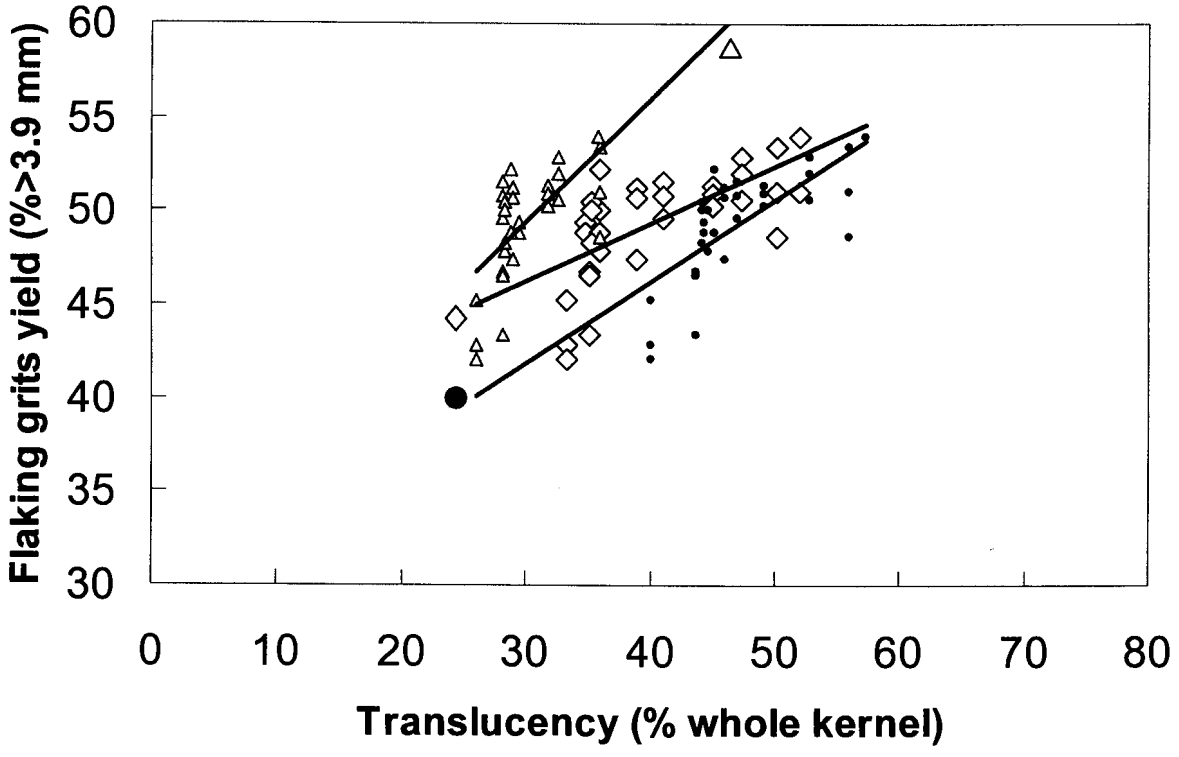


Figure 4.15 Effect of applying translucency correction factors (CFs) on the relationship between the yield of grits larger than 3.9 mm (mass %) and translucent area (% of whole kernel) of twelve industrial yellow maize samples as determined by image analysis. "◊", before CFs ($y = 0.31x + 39.9$, $r = 0.68$); "●", after thickness CF ($y = 0.44x + 28.7$, $r = 0.73$); "△", after thickness and exposure CFs, ($y = 0.66x + 29.6$, $r = 0.67$), $n = 36$. r-values did not differ significantly from each other ($p \geq 0.05$) (Fisher test)

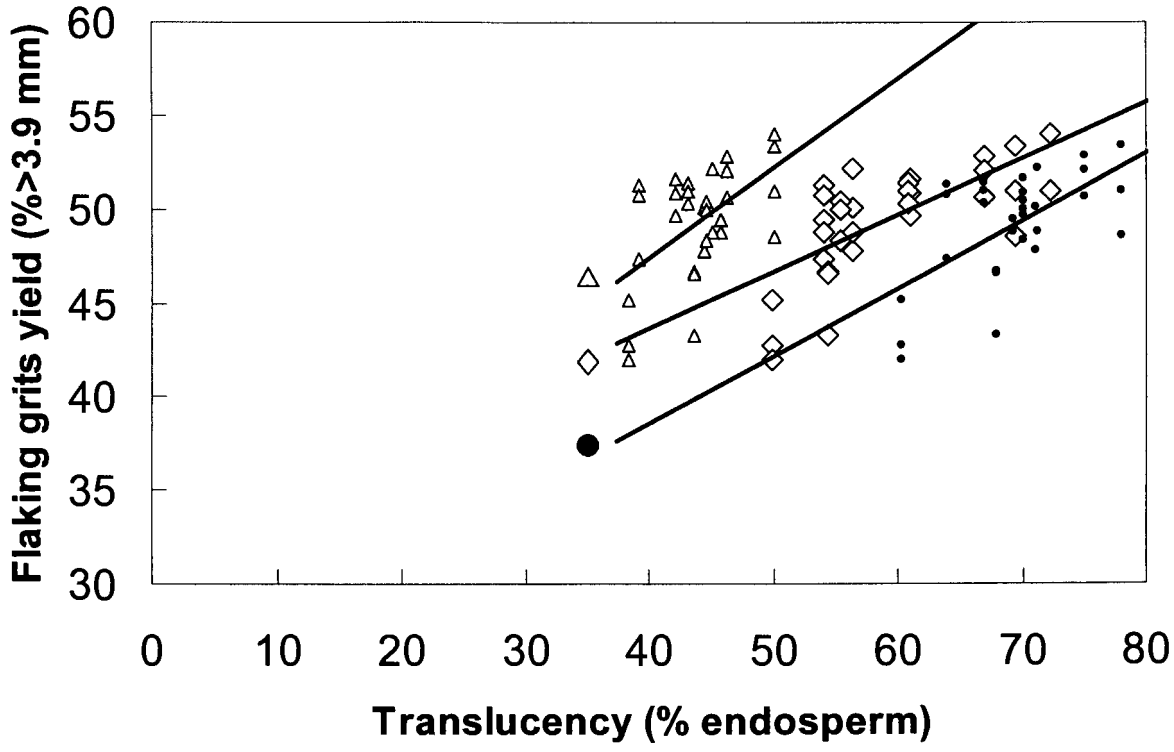


Figure 4.16 Effect of applying translucency correction factors (CFs) on the relationship between the yield of flaking grits larger than 3.9mm (mass %) and translucent area (% of endosperm) of twelve industrial yellow maize batches as determined by image analysis. "◊", before CFs (y = 0.24x + 50.5, r = 0.48); "●", after thickness CF (y = 0.33x + 44.8, r = 0.51); "△", after thickness and exposure CFs, (y = 0.47x + 46.2, r =0.44), n = 36. r –values did not differ significantly ($p \geq 0.05$) (Fisher test)

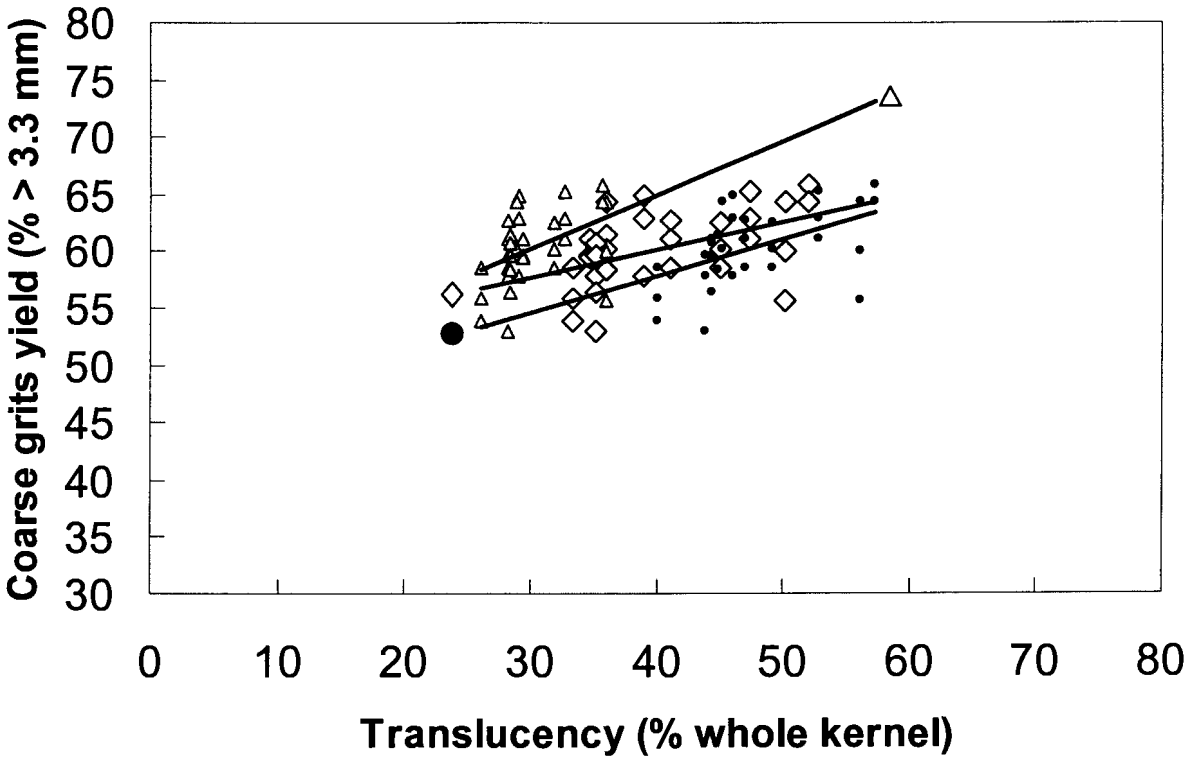


Figure 4.17 The effect of applying translucency correction factors (CFs) on the relationship between the yield of coarse grits larger than 3.3 mm (mass %) and translucent area (% of whole kernel) of twelve industrial yellow maize batches as determined by image analysis. "◊", before CFs (y = 0.3x + 31.6, r = 0.71); "●", after thickness CF (y = 0.36x + 24.1, r = 0.69); "△", after thickness and exposure CFs, (y = 0.48x + 28.2, r =0.58), n = 36. r-values were not significantly different from each other ($p \geq 0.05$) (Fisher test)

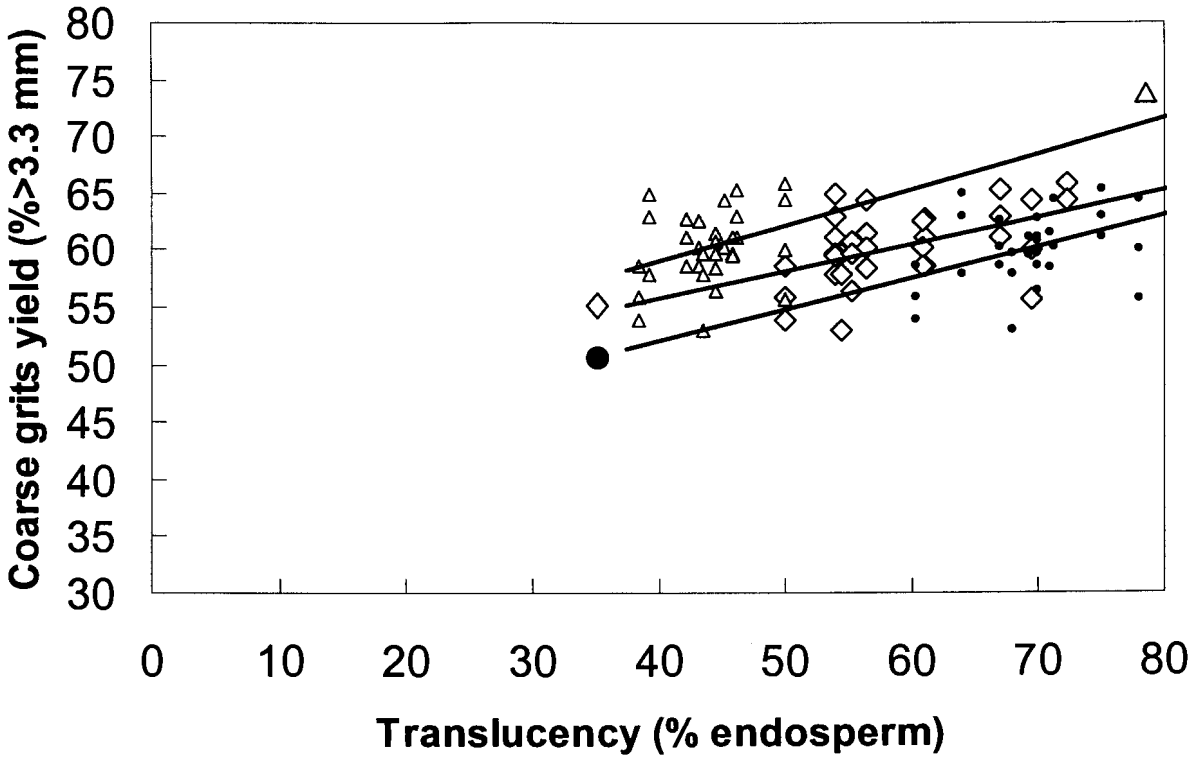


Figure 4.18 The effect of applying translucency correction factors (CFs) on the relationship between the yield of coarse grits larger than 3.3mm (mass %) and translucent area (% of whole kernel) of twelve industrial yellow maize batches as determined by image analysis. "◊", before CFs (y = 0.24x + 46.4, r = 0.49); "●", after thickness CF (y = 0.27x + 41.1, r = 0.45); "△", after thickness and exposure CFs, (y = 0.31x + 46.5, r =0.34), n = 36. r-values were not significantly different from each other ($p \geq 0.05$) (Fisher test)

No useful correlations were found between image analysis translucency measurements and the yield of grits larger than 1.01 mm and therefore graphs were not constructed. Slopes increased in all cases after thickness as well as after exposure corrections. Translucency values were also adjusted to the correct ranges after corrections for exposure (Figures 4.15 to 4.18). Corrections had a more consistent effect on slope (increased in all cases), than on the correlation coefficients (which increased, stayed the same or decreased in the various tests).

4.6 DISCUSSION

Experiment 1

Percent Translucency measured by image analysis correlated negatively with floating number at a very high significance level -0.84 and –0.88 for translucency 1 and 2, respectively, after both corrections for thickness and exposure were applied. Therefore, floating number may be an indication of kernel opaqueness. Translucency (expressed in terms of opaqueness which is the inverse value) could possibly be used as an indication of the floating number, depending on the consistency of other factors such as the occurrence of stress cracks (Watson 1987a). Floating number (also known as the flotation test) is a density-related property (Watson 1987a).

The density of maize kernels is the sum of the densities of the different components such as starch, protein, oil and water. Vitreous endosperm is very dense, while floury endosperm is full of void spaces in the cells trapping air and is less dense (Watson 1987a, Hoseney, 1994). This is illustrated clearly in Figure 2.2 where the loosely packed starch granules of the opaque (floury) endosperm are shown. The floating number (also known as percentage floaters) is widely used as an indication of the levels of vitreous endosperm (which is also translucent) (Gerstenkorn, 1991). The accuracy of the floating number test can be influenced detrimentally by kernel damage, allowing liquid to be absorbed into the voids of the opaque endosperm and therefore increasing the density of the maize kernels overall. The floating number value is also influenced by moisture content generally and accurate measurements can only be made if all samples are at the same moisture content (Watson 1987a). The 20 maize samples tested in this study were all produced under South African rain fed conditions with no artificial drying and therefore, stress crack levels and the development of large void spaces typically found in artificially dried maize were negligible and of no significance. In spite of general concerns around the accuracy of percent floaters, it is still widely used as a screening method for the selection of floury maize for the wet industry (Fox, Johnson, Hurburgh, Dorsey-Redding and Bailey 1992).

Experimental milling produced products from the different maize samples with a narrow range, for example a minimum of 33% and a maximum of 38% of semolina 1 yield (Table 4.2). The range for total semolina yield was even less, even after corrections were made for fat content. The primary difference between semolina 1 and total semolina was in the origin of the products. Semolina 1 only consisted of the reduced size grits obtained from large clean vitreous endosperm grits (larger than 1 mm) obtained after the first break (Figure 4.1). The large vitreous endosperm products obtained from the first break (rolls I and II, Figure 4.1) needed fewer steps to be cleaned and separated from contaminants such as bran and fat (maize germ particles). Smaller grits are more difficult to separate cleanly (Kent 1984) and more steps such as sieving and aspiration are needed. The reason for this is that the particle sizes of smaller grits and the small pieces of bran and germ in these fractions are similar and sieving does not separate them. Larger grits are heavier than the pieces of bran and germ, allowing for separation by aspiration where sieving did not produce the desired result. However, if the pieces of endosperm become too small, the weight differences are too small for separation by aspiration which results in fractions that are still contaminated with tiny pieces of germ and bran (Kent 1984). The semolina 2 and 3 fractions consisted of grits derived from smaller sized vitreous endosperm grits and were therefore more contaminated with fat and bran and more difficult to clean.

Differences in vitreous endosperm yield were expected to be more pronounced when comparing clean fractions than comparing fractions having a certain amount of bran and fat that could not be separated. The expected differences were clearly demonstrated with a wider range of yields for semolina 1 when compared to total semolina yield (the total of the three semolina fractions), as well as higher levels of statistical significance for semolina 1 when semolina yield and translucency were correlated. Semolina 1 was used as an indication of milling resistance (see section 4.3.1.2.4) as the fraction was obtained after the milling of cleaned endosperm pieces larger than 1 mm obtained after the initial milling stages (I and II followed by sieving) (Figure 4.1).

The grits (semolina) obtained were fine grits as all grits were reduced to have a particle size of less than 1000 microns. The final products resembled semolina (from

the wheat milling process) in appearance and consisted of clean vitreous endosperm particles with no visible break flour. Generally, the yield of such cleaned products is low (60%) when compared to other industrial milling yields of endosperm products (75%) (Fowler 1993). Floating number was in the range 58 to 87%.

Despite the narrow ranges for the milling yield data, significant correlations were found between translucency and the yield of some of the milled products (Table 4.3). Significant correlations were found between yield of semolina 1 and % translucency. The relationship between semolina 1 and % translucency both as a percentage of the whole kernel and the percentage of the endosperm was significant even before any corrections were made.

Levels of significance increased for both translucency 1 (translucent area as a percentage of whole kernel area) and translucency 2 (translucent area as a percentage of endosperm area) after corrections for thickness and exposure for all cases. The levels of significance for both translucency 1 and 2 increased in the same way. Correlation coefficients between translucency 1 and 2 were not significantly different from each other. The order of the correction applications did not have an affect on the final results and did not change the final values whether thickness or exposure corrections were done first.

A significant correlation was obtained at the 95% significance level for total semolina yield against % translucency only after all corrections were applied. The extremely narrow ranges of the data of the total yield of semolina influenced the overall significance level of the results. Narrow ranges of vitreousness in maize samples are a practical reality as the profitability of maize milling would have been seriously compromised if maize of variable quality is used. Millers search for maize of a consistent milling performance, even if up until now it has been primarily a matter of using the history of previously milled batches and linking it with factors such as cultivar, area, farmer and climatic condition. Over a number of years, cultivars tended to become similar in their performance purely by the continuous reactive selection by millers (personal communication, Viljoen, A., Research and Development Manager, Tiger Milling and Baking).

Providing that the dry milling fraction used for correlating yield is clearly defined, it is clear from these results that significant predictions are possible using % translucency as measured by image analyses according to the developed method. The definition of the fraction of choice for predicting milling yield is highly important. This is shown clearly in these results by the fact that the semolina 1 fraction gave significantly better correlations than the combined fractions did. This finding can be related to the action of the milling process. The milling process consists of gradual steps aimed at extracting a clean morphological fraction such as vitreous endosperm. The first fractions are easily separated, while successive fractions become more and more difficult to clean, causing a significant amount of other components (or contaminants) to be included and thereby reducing the accuracy of the final measurements. These components or contaminants include pieces of bran and germ fractured during the first stages of milling. If these contaminants have the same particle size or density as the endosperm particles, separation systems such as aspirators or gravitational systems cannot totally separate the contaminants from the endosperm (Kent 1984; Gerstenkorn 1991).

The correction factors increased the slope of the fitted curves and generally increased the level of significance of the correlation coefficients (Table 4.3). Although all correlation coefficients increased after corrections, the coefficients did not differ significantly from each other after comparing them using a z-transformation test. This can be attributed to the relatively low number of degrees of freedom available in the models. Only 20 different samples could be done due to time and cost constraints.

Experiment 2

Statistically significant differences by analysis of variance were found for all image analysis measurements on the 10 samples used for industrial milling (Table 4.4). Statistically significant differences were also found for all the milled products (Table 4.5). All these differences occurred at the $p<0.05$ level and indicated that despite large standard deviations in the image analysis data (Table 4.4), regression models could be developed. If no differences existed, regression modelling would have been impossible.

It is clear that there were excellent correlations between translucency 1 and 2 and the various products obtained from the mill (Table 4.6). Highly significant correlations ($p<0.0001$) were obtained between translucency and extraction at degermer, total extraction of special maize meal without moisture corrections and total extraction of maize meal on a dry basis. Generally, correlations were significant before corrections. It can be concluded that it is possible to predict the yield of specified milled products derived from vitreous endosperm by using % translucency as a predictor. Corrections for kernel thickness increased the level of significance of the correlation coefficients in all cases. Corrections for exposure had a limited effect on the correlation coefficients and slightly reduced the correlation coefficients between translucencies 1 and 2 and the total extraction of maize meal with and without moisture. However, the important effect of corrections for exposure were that they adjusted the translucency values into the correct range, i.e. the corrections took out overexposure. Slopes of the linear regression lines also increased in all cases after corrections for exposure and if correlation coefficients stayed the same, but the slope increased, it still indicated an overall improvement in the strength of the relationship. Overexposure have led to calculated translucency values (%) of more than 100. After correcting for thickness followed by correcting for overexposure, values were adjusted to within a realistic range. This was true for both positive and negative correlations. Significant negative correlations were found between break flour in the thrus and translucency. Break flour is mainly derived from opaque endosperm (Alexander 1987) and a negative correlation between translucency and break flour yield was expected, as break flour is not translucent. Although the corrections increased the levels of significance of the fitted regression curves, the r-values for each set of corrections did not differ significantly from each other following a z-transformation test. Again, the inability to show significant differences was the result of the low number of degrees of freedom due to practical problems limiting the actual number of samples that could be milled. In general, the slope of all the lines also increased with the application of correction factors. Translucency 2 (% of endosperm) apparently gave better correlations with total special maize meal extraction and than translucency 1, while translucency 1 and 2 had similar correlations with extraction at degermer and break flour in the thrus (also obtained directly after the first degerming step). These differences between translucency 1

and 2 were, however, not significant according to the z-transformation test for differences between r-values.

Experiment 3

Yellow maize samples showed statistically significant differences in translucency by analysis of variance at the $p<0.05$ confidence level (Table 4.7). However, after degerming, statistically significant differences were only found for flaking grits, coarse grits and fines (Table 4.8). The yields of offal and total grits were not significantly different from each other making it impossible to determine correlations between yields of these products and translucency (Table 4.9). Significant correlations were only obtained between % translucency and the yields of flaking grits and coarse grits. The yield of fines was not significantly correlated with translucency. Corrections for thickness and exposure did not increase the level of significance or the strength of the correlations. They either remained the same or were slightly reduced. The slopes of all lines increased after the application of the correction factors (Figures 4.15 – 4.18). Again, r-values were not significantly different from each other after the application of z-transformations and comparative analysis according to the Fisher test. The correlations became weaker as more of the grits at smaller particle sizes were added to the yield. This can be ascribed mainly to the fact that grits at smaller particle sizes could not be cleaned efficiently from contaminants such as pieces of opaque endosperm, bran and other particles using only sieving and aspiration after degerming. In order for these fractions to be separated completely, a series of further gradual steps of milling with rollers followed by sieving and aspiration must be used (Kent 1984). Roller milling flattens the small pieces of germ which can then be sieved out as their particle size will then differ from the reduced-size grits. After degerming only, small germ particles are produced that are similar in size to certain pieces of endosperm, making it impossible to separate out by sieving.

Particular problems were encountered with the degerming method. It was found during preliminary tests when the pilot-scale degermer was built and commissioned, that the standard error of measurements changed significantly with increased repetition of the process starting very high for three repetitions and reducing significantly with each additional repetition. Using the Fisher test (Diem and Seldrup

1982) to determine significant differences between standard deviations, it was found that the ideal number of repetitions for the degermer was 9 repetitions per cultivar or maize sample. From a practical perspective, this is undesirable especially when only limited sized samples are available from seed breeders. Yellow maize samples were grown specially for experiment 3 and only enough material for 4 repetitions per sample was available. This indicated that a high variability existed between repetitions and it was ascribed to variability induced by the degermer itself. When the degermer was studied to find a possible source of the induced variation, it was found that the motor tended to run at lower speeds due to the resistance of the kernels created during degerming. The average revolutions per minute (rpm) of the pilot-scale degermer running free without kernels was 1500 rpm. When running full of maize, the speed was significantly reduced and if the flowing speed into the degermer was too high, the machine came to a complete stop. The pilot-scale degermer was an experimental unit and it was found that the torque exerted from the motor was not sufficient to cope with the grinding resistance caused by the kernels when running full. By using a vibratory feeder to pour the kernels into the degermer at a fixed flow, it was possible to control the degermer speed between 200 and 600 rpm. Finer control was not possible and the variation in speed within the 200 to 600 rpm range would influence the torque on the kernels resulting in differences in the shattering of the vitreous endosperm. These problems with the pilot-scale degermer could have accounted for the fractions with no significant differences (Table 4.9), and unfortunately, it probably was also responsible for rendering the effect of the thickness and exposure correction factors on the data inconclusive, except in the case of translucency 1 and flaking grits, where the correlation coefficient and level of significance was increased.

4.7 CONCLUSIONS

In general, correlations of high significance were obtained using both methods of translucency calculation and the various products derived from the maize dry milling process. In all cases, better correlations were found between translucency and vitreous endosperm fractions obtained from the first break process (semolina 1 in experiment 1, extraction at degermer in experiment 2 and yield of large flaking grits > 3.9 mm in experiment 3) than with for fractions obtained from further milling steps.

The former fractions are usually easy to clean with only small quantities of opaque endosperm still attached to them. It was also easier to remove germ and bran from these fractions as the size of the coarse endosperm particles is still large and differ sufficiently in weight from germ and bran materials. As the separation of the fractions became more difficult, correlations with translucency became less significant, although still highly useful, for example the total extraction of maize meal in experiment 2, where r-values were 0.68 and 0.72 ($p<0.001$) after corrections.

Experiment 2 was done in an industrial mill with a degerming and full separation facility. This could therefore account for the better correlations obtained in this milling trial. Both the smaller-scale tests (experiments 1 and 3) gave smaller ranges of product yields, which can be attributed to the fact that small scale systems do not contain all the steps required to fully simulate the separation processes in a modern industrial mill.

CHAPTER 5: GENERAL DISCUSSION

Hoseney (1994) stated that vitreousness of maize is related to the translucency. Vitreousness, in turn, has been shown to be generally related to milling yield (Mestres, Louis-Alexandré, Matencio and Lahlou 1991; Paulsen and Hill 1985) and even with concerns about precision (Chandeshekar and Mazhar 1991), it was therefore expected to find a correlation in the work reported here of some description between translucency and milling yield, although the nature of these correlations were not known.

Vitreousness has been shown to be linked to the yield of certain product fractions during dry maize milling (Paulsen and Hill 1985; Watson 1987a, Louis-Alexandré, Mestres and Faure 1991). Vitreousness has been measured on single kernels based on the measurement of the vitreous and total endosperm areas by viewing sectioned kernels and these measurements were correlated with milling test data (Louis-Alexandré, Mestres and Faure 1991). Inevitably, there will be considerable variation between measurements made on single maize kernels due to the biological nature of the samples. Therefore, standard deviations of individual measurements tend to be quite large when compared with standard deviations obtained for other measurements such as moisture or fat contents of samples, where a selection of kernels is homogenised first by grinding and the analysis done on a sample taken from this mixture. In the work of Louis-Alexandré, Mestres and Faure (1991) it was found that standard deviations of kernels with smaller vitreous endosperm areas tended to be larger than those with large vitreous endosperm areas. This phenomenon was also found in the work of Kirleis, Crosby and Hously (1984) who used a similar technique to measure the amount of vitreous endosperm in sorghum. It was clear in their work that the standard deviations of samples with smaller amounts of vitreous endosperm were larger. In Tables 4.1, 4.4 and 4.7, standard deviations of the translucency measurements showed that in contrast with the published values (Louis-Alexandré, Mestres and Faure, 1991) for vitreousness indexes, the translucency values had similar standard deviations for samples with larger or smaller translucent areas. Standard deviations for translucency measurements as a percentage of the whole kernel (translucency 1) were similar in

comparison to those for vitreousness measurement on cut kernel surfaces published by Louis-Alexandré, Mestres and Faure (1991), but the standard deviations for translucency measured as a percentage of the endosperm (germ area removed, translucency 2) were higher. Both standard deviations (SDs) for the measured translucencies were generally lower than the SDs for translucency measurements in terms of grayscale indexes as published by Felker and Paulis (1993), except for the SD's of translucency 2 values in experiment 2 (Chapter 4), which were similar to those of Felker and Paulis. In the work published by Felker and Paulis, standard deviations varied between 20 and 50% from the measurement of only 10 individual kernels, while in this study standard deviations varied between 20 and 30% obtained from a minimum of 45 measurements per cultivar. Reasons for this difference is most probably linked to the selection of the samples at the initial stages of the experiment. Maize for dry milling purposes has been selected in South Africa for a number of years and individual kernels tend to be homogenous providing that damaged kernels are not included in the analysis. As only industrial samples were used in the milling experiments, it was expected that the homogeneity of the kernels would have been higher especially if produced under controlled industrial farming practices. Samples evaluated in Chapter 3 (method development) were selected from single cultivars produced under experimental farm conditions and a high level of homogeneity was also expected. Standard deviations were similar to the work described in Chapter 4.

It is interesting to note that in the work of Felker and Paulis (1993), standard deviations of grayscale indexes also increased after the area of the germ was removed, similar to the general increase in the variability found with the translucency 2 values (germ area removed) as shown in Tables 4.1, 4.4 and 4.7. Reasons for this are probably either errors in the translucency measurements due to the occurrence of partial translucency of the germ, or the difficulty in exactly measuring the germ area on the image analyser. Germ area had to be detected by marking of the boundaries by hand as the contrast between germ and the rest of the kernel was too small for automatic detection. In the work of Felker and Paulis, a fixed sized area including the germ was excluded and it also resulted in increased standard deviations of measurements. Similar trends were found in Tables 3.11 and 3.12, Chapter 3.

Felker and Paulis (1993) did not attempt to correlate their image analysis measurements of maize translucency with any milling test and therefore, no such correlation data exists for comparison with the work reported here. The closest data found were data on correlations between maize vitreousness as measured by detection of vitreous endosperm area on cut kernel surfaces with a selection of laboratory estimations of dry milling properties (Mestres, Louis-Alexandre, Matencio and Lahlou 1991). A correlation coefficient of 0.92 between vitreousness and kernel density was reported. In the work reported here, a correlation of –0.88 was found between the Floating Number and translucency (experiment 1, Chapter 4). This good correlation was obtained after applying kernel thickness and exposure correction factors (Table 4.3). Kernel density and floaters are inversely related (Chandrashekar and Mazhar 1991), providing that the influence of factors such as environmental conditions are taken into account and therefore, a significant negative correlation between translucency and % floaters was expected.

Percent floaters have been correlated with milling yields. Wu and Bergquist (1991) obtained correlation coefficients of as high as 0.89 ($p<0.01$) between corrected density values and total grits yield after milling maize according to a degermer/roller milling process described by Peplinski, Anderson and Eckhoff (1984). Peplinski, Anderson and Eckhoff (1984) obtained significant negative correlations between corrected (for moisture) maize kernel densities and % floaters. In the results of experiment 1, Chapter 4, the significant negative correlations obtained between % translucency and % floaters could indicate that % translucency can be a good indication of maize kernel density. The excellent correlation of 0.89 between corrected density and total grit yield obtained by Peplinski, Anderson and Eckhoff (1984), could be partially ascribed to a large range of samples in terms of differences in density and % floaters. Their samples varied between 19 and 100% for percent floaters with 100% floaters for "cornnuts 88" which was a cultivar with no vitreous endosperm. The samples analysed in experiment 1, Chapter 4 varied only from 58 to 87% floaters which gave a narrower range and therefore smaller r-values (Table 4.4).

In an industrial-scale milling trial by Paulsen and Hill (1985), 1780 ton samples of maize were milled for the production of large clean grits for cornflake manufacture. These grits contained less than 0.5% fat and less than 4% attached "hull" after degerming. Paulsen and Hill (1985) found a correlation coefficient of -0.98 between Floating Number and the yield of clean grits obtained after separation of the overtail stock at the degermer. As previously described, Floating Number and % translucency were significantly negatively correlated in Experiment 1, Chapter 4. It is possible to compare the r-value of Paulsen and Hill (1985) with the r-values obtained between extraction at degermer (an indication of flaking grits yield) and % translucency (an indication of % floaters) obtained in experiment 2, Table 4.6, which was an industrial milling trial. These r-values were obtained after application of both correction factors. Very high r-values were also obtained in experiment 2, Table 4.6 ($r = 0.93$) for extraction at degermer and % translucency (whole kernel). A trend in this study was observed, namely that larger milling trials using larger samples and more separation steps tended to produce better correlations between % translucency and kernel density and the yield of clean vitreous endosperm products. A similar trend was observed when comparing results of correlations between % floaters and the yield of dry-milled products in the published literature with values obtained by Paulsen and Hill (1985). These authors specifically mentioned that in order to produce high yields of flaking grits, it is preferable to use maize with high ratios of vitreous endosperm relative to floury endosperm, and that it is desirable to have complete separation of the endosperm fractions with the germ and bran. This emphasised the use of a larger, more complex milling system and this trend was clearly demonstrated in the results reported here. An interesting observation about the results of Paulsen and Hill (1985) is that the high correlations were found between % floaters and flaking grits, but the yields of so-called "white products" were similar regardless of the quality of the maize milled. "White products" represented all grits, flour and meal obtained by the addition of all the fractions after separation of the germ and "hull" portions. These results were similar to results obtained in all three experiments (Chapter 4) where correlation coefficients decreased significantly when finer fractions of clean products were added, for example the total maize meal extracted versus extraction at degermer (overtail flaking grits) in experiment 2, Chapter 4. A similar trend regarding correlations between % floaters and the yield of semolina was observed by Manoharkumar, Gerstenkorn, Zwingelberg and Bolling

(1978) where a higher correlation coefficient (r = 0.72) was found between % floaters and coarser semolina (>500 microns) than with finer semolina plus flour (r = 0.35).

Correlations between % vitreousness (as determined by measuring the percentage of vitreous endosperm on cut kernel surfaces) and the yield of vitreous endosperm following a micromilling process (similar to hand dissection) were determined by Louis-Alexandré, Mestres and Faure (1991). Correlation coefficients were similar to the values obtained in the work reported here, with better r-values obtained in experiment 2 (Chapter 4). However, Louis-Alexandré, Mestres and Faure (1991) did not do any further milling tests where the yields of products from milling were compared to the results obtained from the hand dissections. Li, Hardacre, Campanella and Kirkpatrick (1996) also measured the ratio of vitreous to opaque endosperm on cut kernel surfaces, but used vernier callipers and only measured 10 kernels per sample. They obtained correlation coefficients between vitreous/opaque endosperm ratio measurements and some milling properties obtained from the Stenvert Hardness Test. They found a r-value of 0.74 between vitreous/opaque ratio (as a percentage of the whole kernel surface) and milling energy and a r-value of 0.62 for resistance time using 38 cultivars of maize. They suggested that low to average correlations may have been due to inaccuracies during the measurement of the vitreous endosperm. They also suggested that the proportion of the vitreous endosperm at the measured section of the kernel was not an accurate estimation of the true volumes of vitreous and opaque endosperm which could have given rise to poor r-values. Their r-values were very similar to the values obtained in experiment 1 and experiment 3 (Chapter 4), but the r-values for experiment 2 (Chapter 4) were significantly better. Experiments 1 and 3 (Chapter 4) and also the trials done by Li, Hardacre, Campanella and Kirkpatrick (1996) were laboratory-scale milling assays, while experiment 2 (Chapter 4) was an industrial-scale experiment. It is not possible to simulate all the different milling, sieving, aspiration and cleaning steps possible in an industrial-scale test and better separations of the endosperm fractions are possible in a fully-operated mill. As no data on the correlation between the ratio of vitreous to opaque endosperm measured by cut kernel surface area ratios and the yield of dry-milled products obtained from an industrial mill exist, it is not possible to further compare the results of experiment 2 with any relevant data in the literature.

Mestres, Louis-Alexandré, Matencio and Lahlou (1991) also determined r-values between vitreousness (ratio of vitreous/opaque endosperm determined as area ratios on cut kernel surfaces) and the yield of semolina (maize grits) produced on a roller mill using 4–5 kg samples. Their samples were significantly smaller than those used in experiment 2 (510 kg each). They found a correlation of only 0.44 which was not statistically significant, but did indicate that there were problems during the sieving stages of the milling process. Unfortunately, no further comparisons can be made with this data due to the experimental problem mentioned.

Yuan and Flores (1996) estimated the ratio of vitreous to opaque endosperm by using hand dissection and weighing the fractions. They correlated the ratios with experimental milling data obtained from milling 500 g samples on a small laboratory-type horizontal drum degermer, followed by separating the germ and bran firstly by sieving and aspiration and then a further separation by flotation. During the flotation step, remaining germ and bran was removed by suspending the sieved maize grits in a $NaNO_3$ solution with a specific gravity of 1.22. They obtained a r-value of 0.44 between vitreous/opaque endosperm ratio and flaking grits (large grits), 0.58 between endosperm ratio and total grits (cleaned grits) and 0.61 between endosperm ratio and prime products. Only the mesh size of the prime products were given (3.5–25 mesh or 5.6–0.7 mm) with no further detail other than mentioning that this process was only a degerming process, but with an additional cleaning process using flotation. The results of Yuan and Flores (1996) can be compared to the results of experiment 3 (Chapter 4) based on the fact that translucency was correlated with hand dissection as previously described in Chapter 3. The correlations shown in Table 4.9 were similar to those found by Yuan and Flores (1996). It can thus be concluded that by using translucency measurements to estimate vitreous/opaque endosperm ratios, similar results can be obtained to results from using vitreous/opaque ratios from the hand dissection method when estimating the yield of maize grits from the degerming process on a laboratory scale. It also seems as if small-scale degerming of maize poses many problems as correlation coefficients are generally low due to the crudeness of the process. The samples in experiment 3 (Chapter 4) could not be completely cleaned as laboratory aspiration and sieving alone could not remove all the germ and endosperm particles left in the grits after degerming, resulting into experimental errors during weighing of the fractions.

Kirleis and Stroshine (1990) also used the ratio of vitreous/opaque endosperm determined as areas on cut kernel surfaces, but evaluated only three types of maize. They suggested a relationship between the ratio of vitreous/opaque endosperm and Stenvert Grinding time with the sample with the longest time having the highest vitreous/opaque ratio. Unfortunately, no comparisons can be made due to only three samples being tested with the result that r-values could not be determined statistically. However, it should be pointed out that their work focused on the effect of drying conditions on changes in dry milling properties and the correlation between milling properties and endosperm ratios was not the main focus of their work.

With respect to the work reported here, it can be said that good correlations between translucency and the yield of milled products were possible in all cases, providing that the end products were properly defined as being clean from contaminating fractions. Corrections for moisture and fat contents in end products did not seem to have significant effects on the correlation coefficients in general. For example correction for fat in experiment 1 (Chapter 4) and correction for moisture in experiment 2 (Chapter 4). The main reason for this is that all samples were treated exactly the same in each experiment, for example all samples being conditioned similarly. Therefore, the small differences in fat or moisture content as a result of individual differences between kernels did not seem to influence correlation coefficients to any significant extent (Tables 4.3 and 4.6). It can thus be concluded that the moisture and fat contents of cleaned vitreous endosperm products will not have a significant influence on the correlations between translucency and yield, as long as samples are prepared under the same conditions.

The correction factors developed using the hand dissection method described in Chapter 3 was applied to the results of the milling tests described in Chapter 4. In general, the correction factors had a similar effect on the measured translucency values both for hand dissection data (yields of vitreous and opaque endosperm) and the yields of milled products. In both cases, the correction factors improved the relationships between the calculated translucencies and the determined mass fractions of vitreous endosperm products.

Corrections for exposure generally caused the translucency values to become lower, and also to move into the correct ranges. Before corrections, % translucency could in some cases be more than 100%. This was due to overexposure, especially from maize kernels with a high percentage of translucent endosperm resulting in the germ area also being sensed by the computer as translucent. Corrections for exposure resulted in correcting the actual values of the translucency. It did not, however, always increase the significance levels of the r-values (for example experiment 2, Table 4.6). In some cases r-values remained unchanged, while in other cases they decreased or increased slightly. In the yellow maize samples, r-values decreased quite dramatically after corrections for exposure, while although small decreases in some of the white maize samples were observed, the decreases were so slight that they can be regarded as insignificant (Table 4.9). A possible explanation for the large effect with yellow maize is that the yellow colour of maize differs in intensity depending on the cultivar's genetic make-up (Zuber and Darrah 1987). These authors defined three distinct yellow intensity classes namely light, moderate and intense yellow based on the number of alleles for yellow obtained from the parents of the type of maize. Although an exposure correction factor was developed using three cultivars of yellow maize, it is possible that differences in the intensity of the yellow colour could have resulted in an incorrectly determined correction factor. Although all image analysis was done on the grayscale images, further work will be necessary to determine the effect of the different yellow intensity classes as described by Zuber and Darrah (1987) on the grayscale.

A possible solution for future application could be to investigate the use of a monochromatic light source in order to exclude the spectrum of light absorbed by the yellow colour in maize. In this research, the light source had a full visible wavelength spectrum. Although the white maize results were influenced to a lesser extent, it was also clear that there are visible colour differences between white maize cultivars as well and the extent of these differences on the measured gray levels is unknown. Further work is needed to find a wavelength that will not be absorbed by colour pigments in any of the white or yellow maize samples.

In most cases, thickness corrections increased the level of significance of the r-values. However, in experiment 2 (Table 4.6), thickness corrections had no

significant effect on the r-value obtained between % translucency and extraction at degermer, but did have a significant important effect on the r-value for the extraction of maize meal. The r-value between % translucency and extraction at degermer was very high before applying the correcton factors and the thickness effect could have been too small to be significant. In experiment 1 (Table 4.3), thickness corrections were very important resulting in significant increases in r-values. In experiment 3 (Table 4.9), thickness corrections resulted in significant increases in the correlation between % translucency and flaking grits > 3.9 mm. It had no effect on the other correlations. As stated, due to problems encountered with the degermer itself during operation, the sensitivity of the results could have been negatively influenced and although thickness may have had an affect on correlations between % translucency and all the fractions, it is possible that the data did not show it due to experimental errors. The significance of thickness corrections seems to be influenced by the range of the measurements of the dry milled products. For example, extraction at degermer (experiment 2) had a yield range of 29.8% and thickness correction did not increase the r-value significantly, but in the same experiment, it did have a significant effect on the r-value for the extraction of maize meal, where the yield range was only 11.3%. In experiment 1, thickness corrections had a major influence on the r-values for products such as the yield of semolina 1 (yield range of 5%) and total semolina yield (yield range of 3.9%). A similar trend was found in experiment 3 for the correlations between translucency and yield of flaking grits > 3.9 mm (yield range of 10.3%). This observation needs further investigation, but it is important from a practical viewpoint as there will definitely be a need to design an image analyser system capable of measuring kernel thickness as well as kernel area and kernel translucency.

In general, no statistically significant differences for r-values existed between the two ways of measuring translucency. Translucency 1, where translucency was measured as a percentage of the whole kernel, gave results almost identical to Translucency 2, where the translucency was measured as a percentage of the endosperm. However, as the germ size had to be drawn in by hand on the images for determining Translucency 2, errors could have occured as the distinctions between germ, tip cap and endosperm were not always clear on the images due to interferences from

adhering pieces of pedicel (remnants of the maize cob attached to the tip cap of a kernel).

The lack of significant differences (Fisher test) between r-values of Translucency 1 and Translucency 2 correlations with various milling properties in all experiments is an important finding, as it will lead to a reduction of the time needed for analysing the kernels. It will eliminate the need to measure the size of the germ and the tip cap of each kernel, which probably cannot be done automatically due to contrast problems and was prone to errors when estimated manually, as described.

By installing two cameras capable of detecting the top and side surfaces of each kernel, all required measurements are possible using the Translucency 1 calculation, simply by changing only the lighting set-up, followed by detecting appropriate images (including thickness). This can be programmed into the computer sequences including the sequence for changing of the lights. No manual calculation of data will be necessary and eliminates the use of the human eye which is subjective. A system with this type of design also has potential for use as an on-line measurement system. Autosampling is used for analysing specks of bran in wheat flour samples (Branscan, 2003), where on-line measurements of the desired property in the mill was found to be impractical. An autosampling system for maize kernels to be measured for translucency is a commercially viable option (as shown by the Branscan system for wheat flour), otherwise a custom-made conveyor system allowing kernels to be placed in specially designed slots for analysis can be designed.

The differences in the detected translucent areas when kernels are measured with the germ facing towards or away from the camera were not measured in this study and will need further investigation. Once known, the effect of germ position could be added to the calculations based on probability values for maize kernels sampled automatically with the germ facing towards or away from the camera.

The developed Image Analysis assay may have wide application in the field of seed breeding where non-destructive analyses of genetic material is advantageous. Additionally, it should have wide applications in maize processing quality control laboratories, ranging from analyzing incoming maize at silos to selecting preferred

batches of maize suitable for specific process applications such as the manufacture of corn flakes where large sized maize grits are desirable.

The use of this type of system could also find wide application in fields where the determination of vitreousness will lead to the prediction of a specific quality trait. For example, the use of Image Analysis to measure vitreousness in durum wheat was investigated by Novaro, Colucci, Venora and D'Egidio (2001) and Mahler, Beckmann and Ludewig, (2002), as non-destructive analytical techniques. In both these investigations, the percentage of vitreous kernels out of a total amount of randomly selected kernels was calculated by computer classification and counting in order to replace the visual classification tests. Although the actual formulas for measuring translucency differed from the work described in this study, they were based on similar principles demonstrating the potential usefulness of the assay for other cereals as well.

One of the potential applications for the developed image analysis technique is to measure the amount of opaque endosperm in maize used for wet milling. During wet milling, maize kernels are soaked in a mixture of sulphur dioxide and lactic acid in order to break disulphide bonds in the protein, as the main objective of wet milling is the extraction of pure starch. Better yields of starch are obtained during wet milling when kernels with higher percentages of opaque endosperm are used, which is usually measured by the percentage floaters (or Floating Number) test (Watson 1987a). As wet maize milling is a major industry world-wide, the new technique will have commercial potential in this area.

CHAPTER 6: GENERAL CONCLUSIONS AND RECOMMENDATIONS

Maize kernel translucency as determined by Image Analysis (IA) correlates significantly with laboratory and industrial dry milling yields of vitreous/primary products.

The IA method developed is rapid and non-destructive. Correction factors are applied to allow for constant illumination for each individual kernel taking into account kernel size variation as well as kernel thickness for white as well as yellow dent maize.

Maize kernel translucency as determined by IA is significantly correlated with Floating Number. It is suggested that translucency can potentially be used as a prediction method for the dry milling industry to replace the floating number test as an intake quality control screening method.

As very little sample preparation is necessary (only initial cleaning of kernels by removing damaged kernels or other foreign material), large numbers of individual kernels can be analysed quickly. Analysis rate will depend on the size and speed of the camera, computer software and the selected sampling technique.

The thickness measurement method developed will require some refining to confirm or adjust the current thickness correction factors. Suggested refining should include the effects of cultivar, relative size of germ, kernel shape and colour (for example different classes of yellow) and refinement of the preparation of samples for measuring the thickness effect. It is suggested to evaluate the thickness effect using specially-grown cultivars consisting of translucent endosperm only, and to evaluate the effect of light scattering caused by the distribution of opaque endosperm inside the kernels systematically.

The effect of the correction factors on the strength of the linear relationships is better demonstrated with the white maize cultivars than with the yellow cultivars. Differences in the intensity of the yellow colour in the yellow maize have an additional

influence on the measured translucency values. For future work, it is suggested to use different light sources such as monochromatic light at a wavelength that is not absorbed by the pigments in the maize kernels in order to reduce the effect of different endosperm colours.

The correction factor for exposure successfully addresses the problem of reduced contrast caused by excess light shining around the kernels by allowing the use of light areas of a fixed size smaller than the size of the kernels. This will successfully replace the use of modelling clay that was used previously for embedding of the kernels to exclude excess light.

The use of the correction factors can easily be programmed into computer software and therefore, the developed method has potential to be developed further as an on-line maize translucency detection method.

The differences between the correlations achieved when the translucency was measured as a percentage of the whole kernel and when it was measured as a percentage of endosperm only, are very small and not significant. To calculate translucency as a percentage of endosperm, the area of the germ and tip cap on the kernels is measured separately using a manual step (by hand with a computer mouse), while the calculation of translucency as a percentage of the whole kernel is done automatically using only computer software. As both methods produce similar results, the use of translucency as a percentage of endosperm can safely be discarded.

For the system to be successful as an on-line detection system, it is proposed to develop a future system consisting of two cameras at a 90° angle to each other. One camera will detect the features from the top or bottom of the kernel including the translucency measurements, while the second camera will detect the thickness of the same kernel. As the unique requirements of the lighting system will make direct detection on a conveyor belt not feasible, an autosampler system is proposed as the solution. In such a system, a specially adapted unit will have to be installed allowing for automatic sampling and spreading of kernels on a specially designed illumination

mask, possibly with indents or another device to allow for the kernels to be positioned on top of the holes. Further development work will be necessary in this area.
The developed method is also suitable for other applications. The most important potential application is for the prediction of the yield of starch with maize wet milling. Opacity of maize is linked to starch yield and the method can potentially easily be adapted for this purpose.

The measurement of translucency on other cereals is another potential application. Translucency in cereals such as wheat, rice and sorghum is a known phenomenon, but the precise use of it as a tool for predicting processing performance is only partially understood, mainly as it is not easily analysed. The newly developed method will allow for easier analysis of these samples leading to a better understanding of the translucency relationships.

CHAPTER 7: REFERENCES

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[1] LOUW, C., is the maiden name of ERASMUS, C.