Methods

1.Grading

1.1 RSA grading

RSA grading was done in accordance with the Grading Regulations for maize, as published in the Government Gazette No. 19131of 14 August 1998, regulation No. R.905.

Description of deviations relating to RSA grading

a. Defective maize kernels
The term "defective kernels" means all maize kernels and pieces of maize kernels which are shrivelled, obviously immature, frost-damaged, heat-damaged, mouldy or discoloured, have sprouted (including kernels whose growing point in the germ is visibly discoloured), have cavities in the germ or endosperm caused by insects or rodents, are visibly contaminated by smut, soil, smoke or coal-dust, can pass through the 6.35 mm round-hole sieve, are clearly of inferior quality and of subspecies other than ***Zea mays indentata*** or ***Zea Mays indurata***.

b. Foreign matter
The term "foreign matter" means all matter other than maize, glass, stone, coal, dung or metal.

c. Other colour
The term "other colour" means maize kernels of a colour other than white or yellow but excludes pinked maize kernels.

d. Total deviation
The term "total deviation" means the total defective kernels plus foreign matter plus other colour kernels.

e. Pinked kernels
The term "pinked kernels" means maize kernels whose endosperm is white or yellow and whose pericarp or part thereof is red or pink in colour.

The specification, according to the Grading Regulations for classes 1 to 3 of white and yellow maize is a maximum of 12 %.

Fungal infection

All samples were inspected for the visual symptoms of *Diplodia* and F*usarium cobrot*. There are four fungi which cause cobrot in South Africa namely *Stenocarpella maydis (Diplodia maydis)*, *Fusarium moniliforme, Fusarium graminearum* and *Stenocarpella macrospora(Diplodia Macrospora) Fusarium* spp infections are localized on the cob and discoloured maize kernels, which become reddish (light pink to lilac). *Diplodia maydis* normally rots the entire maize cob and infected maize kernels are recognized by a light ash colour to black colour that appears at the germ and can infest the whole kernel.

1.2 USA grading

USA grading was determined in accordance with the method of the American Grading Regulations (United States Department of Agriculture).
There are seven grades or standards in US grading, Grades nos. 1 through 5 and sample grade and mixed grade. No.1 is the most desirable followed by no. 2 down to sample grade and mixed grade.

Description of deviations relating to USA grading

a. Damaged kernels
Kernels and pieces of corn kernels that are badly ground-damaged, badly weather-damaged, diseased, frost-damaged, germ-damaged, heat-damaged, insect-bored, mould-damaged, sprout-damaged, or otherwise materially damaged.

b. Heat-damaged kernels
Kernels and pieces of kernels which are materially discoloured by excessive respiration, with the dark discoloration extending out of the germ through the sides and into the back of the kernel as well as

kernels and pieces of kernels which are puffed or swollen and materially discoloured by external heat caused by artificial drying methods.

b. Broken corn and foreign material
Broken corn is all matter that passes readily through a 12/64-inch (4.76 mm) round-hole sieve and over a 6/64-inch (2.38 mm) round-hole sieve.
Foreign material is all matter that passes readily through a 2.38 mm round-hole sieve and all matter other than corn that remains on top of the 4.76 mm round-hole sieve after sieving.
Broken corn and foreign material is all matter that passes readily through a 4.76 mm round-hole sieve and all matter other than corn that remains in the sieved sample.

c. Bushel weight
Test weight per bushel is the weight of grain required to fill a level Winchester bushel. Bushel weight is multiplied by the factor 1.2872 to get the hectolitre mass.
Bushel weight is done according to the Federal Grain Inspection Services' (FGIS) Grain Inspection Handbook II, Chapter 1, Section 1.11.

d. Other colour
Maize samples are deemed to be mixed grade when maize kernels of another colour for white maize exceeds 2 % and for yellow maize exceeds 5 %.

2. Nutritional value

The Infratec 1241 Grain Analyzer (Near Infrared) (NIT) was calibrated against the different international chemical methods for determining nutritional values.

The chemical methods used to establish a set of calibration samples were:
a) for fat, the petroleum ether extraction (Soxhlet) method (AACC 30-25, 1999),
b) for protein, the Dumas (Leco) method (AACC 46-30,1999), and
c) for starch, the Hydrochloric Acid dissolution method (Polarimeter) (ICC standard no. 123, 1976 - Revised 1994).

These sets of calibration samples were used to calibrate the Infratec 1241 Grain Analyzer (NIT).

3. Physical characteristics

Hectolitre mass
Hectolitre mass (grain density or bushel weight) means the mass in kilogram per hectolitre.

100 kernel mass - Industry accepted method 001

100 kernel mass is the weight in grams of one hundred whole maize kernels and provides a measure of grain size and density.

Kernel size - Industry accepted method 017

Kernel size is important to the sophisticated starch manufacturing industry as well as to the dry milling industry. Kernels that are too small hamper the separation of kernel fractions in the wet milling process. The result is a lower starch yield. A mixture of small and large kernels causes additional problems, as homogeneous steeping cannot be achieved. On the other hand, very large kernels can also cause problems since the ratio between volume and mass is unfavourable to proper steeping.

The dry milling industry also prefers fairly larger maize kernels. However, a uniform kernel size is of particular importance to this industry, as kernels that are too large create problems especially when mixed with smaller kernels.

Kernel size is less important to the animal feed manufacturing industry. Larger kernels are nevertheless preferred, as small kernels are easily lost during the screening stage of processing.
The determination of kernel size comprises

the sieving of a 100 g representative whole maize kernels for each sample through both 8 mm and 10 mm round-hole grading sieves, normally used in the seed industry.

Breakability - Industry accepted method 007

Maize is normally cleaned before processing. In the cleaning process, broken kernels are removed with other impurities, causing losses. Broken kernels are further broken during handling, resulting in much grain dust being generated. This creates the potential for dust explosions, health hazards, hygiene problems and so forth. Maize containing a high percentage of broken kernels tends to become insect infected more easily and is subject to general deterioration.

In the modern dry milling industry, maize is first cleaned and then conditioned by dampening before the germ is removed. Broken kernels cause many problems during these stages of processing. Broken kernels can also lead to a lower extraction of the so-called high-quality products, like samp and maize grits. The presence of many broken kernels cause problems with the fibre and fat content of other maize products, like the various grades of maize meal, because the quantity of germ required to be returned to the milled endosperm cannot be accurately determined.

In the wet milling process broken kernels steep more rapidly than whole kernels and by the time the whole kernels have been sufficiently steeped, the broken kernels have been over-steeped, causing an ineffective separation of protein and starch.

In the livestock feed industry breakability is not an important quality characteristic, except for dust and hygiene problems.

Every sample was subjected to a breakage susceptibility test. After the sample of whole maize kernels was propelled in a Stein Breakage tester for 4 minutes, the fraction below the 6.3 mm and 4.75 mm sieve was collected and the percentage broken kernels < 6.35 mm and < 4.75 mm was determined.

Stress cracks - Industry accepted method 006

Stress cracks are determined by visual inspection of a certain amount of whole maize kernels examined on top of a light box for small internal cracks in the endosperm. Some kernels may even have two or more internal cracks. Any form of stress may cause internal cracks, for example rapid moisture loss on the land, during harvest or during drying. Stress cracks are genetic and different cultivars will differ.

Milling index - Industry accepted method 015

Milling index is an indication of the milling abilities and milling quality of maize kernels where a higher milling index means a higher extraction of the high-grade and most profitable products like samp, maize rice and maize grits (degermed products) that are manufactured from the corneous part of the endosperm . The milling index is an indication of the relative differences between samples tested. The milling index is measured with the Infratec 1241 Grain Analyzer. The SAGL uses the calibration of the Grain Crops Institute of the ARC .

Whiteness index - Industry accepted method 004

Whiteness index of white maize meal was determined with the Hunterlab colorflex 45°/0°. Whiteness is associated with a region or volume in colour space in which objects are recognized as white. The degree of whiteness is measured by the degree of departure of the object from a perfect white. The higher the whiteness index value the whiter the meal.

Milling of maize on Roff maize mill - Industry accepted method 013

The Roff 150 Series maize mill is used to mill representative samples of 500 g. The mill should be pre-set to the following specifications: Break 1 roll nip - 0.3 mm, Break 2 roll nip - 0.18 mm and Break roll nip - 0.08 mm. These settings are according to the specifications in the method developed by the ARC Grain Crops Institute (GCI). Every mill has three separations, namely germ, grits and maize meal. The grits from Break 1 are transferred to the Break 2 rolls and the grits from Break 2 are transferred to Break 3 rolls.
The following fractions are weighed and determined as percentage:
Break 1 meal
Break 2 meal
Break 3 meal
Break 3 grits
Break 1, 2 and 3 germ are combined and then weighed
Break 1, 2 and 3 meal are combined to get the % extraction total meal.
Break 1, 2 and 3 meal are combined to get the % extraction total meal.

4. Mycotoxin analyses

The pathogenic nature of certain species of fungi to plants has been observed virtually since the beginning of agriculture. These plant pathogens can produce metabolites (mycotoxins) that show toxic effects when they are ingested.
The mycotoxin analyses were carried out in accordance with the Vicam immunoaffinity column technique using the different Vicam Instruction Manuals for the different mycotoxins. Detection of the toxins was done on a Fluorometer. 90 samples of the 900 maize crop samples were tested for Aflatoxin, Fumonisin, Deoxynivalenol, Zearalenone and Ochratoxin.

Fungi	Toxin	Method reference
Aspergillus flavus	Aflatoxin	Vicam Aflatest Instruction Manual May 5, 1999
Aspergillus ochraceus and several species of *Penicillium sp*.	Ochratoxin	Vicam Ochratest Instruction Manual May 4, 1999
Fusarium moniliforme	Fumonisin	Vicam Fumonitest Instruction Manual Nov 15, 2002
Fusarium graminearum	Zearalenone	Vicam Zearalatest Instruction Manual Nov 19, 1998
Fusarium graminearum	Deoxynivalenol (DON)	Vicam DONtest TAG Instruction Manual Apr 4, 2000

5. GMO (Genetically Modified Organisms)

90 samples of the 900 maize crop samples were tested for Bt and RUR Modified maize. Quantitative analyses for Bt maize were done using the ELISA Method, AACC Method 11 - 10 November 8, 2000. Cry 1 Ab protein in corn is produced from a gene derived from *Bacillus thuringiensis (Bt)*. This method is a quantitative enzyme-linked immunosorbent assay (ELISA) test for the determination of *Bt* modified corn in corn flour. Proprietary antibodies specific for Cry 1 Ab protein are used.

The GMO Soya test kit from Strategic Diagnostics Incorporated (SDI) were used to quantitatively determine Roundup Ready (RUR). The procedure was adapted by SDI for maize.