METHODS

SAMPLING PROCEDURE

A working group determined the process to be followed to ensure that the crop quality samples sent to the SAGL by the various grain silo owners/ agricultural businesses, are representative of the total crop.

Each delivery is sampled as per the grading regulations for grading purposes.

After grading, a sub-sample of each of these grading samples are placed in separate containers according to class and grade, per silo bin at each silo.

After 80% of the expected harvest has been received, the silo divides the content of each container with a multi slot divider in order to obtain a 3 kg sample (this should be done for each class and grade separately).

If there is more than one container per class and grade, the combined contents of the containers is mixed thoroughly before dividing it with a multi slot divider to obtain the required 3 kg sample.

The samples are marked clearly with the name of the depot, the bin/bag/bunker/dam number(s) represented by each individual sample as well as the class and grade and are then forwarded to the SAGL.

RSA GRADING

RSA grading was done in accordance with the Grading Regulations for maize, as published in the Government Gazette No. 32190 of 8 May 2009, Regulation No. R.473 and amended by Industry-Wide Dispensation REF No: 20/4/14/1, dated 15 April 2010.

Description of deviations relating to RSA grading:

Defective maize kernels

The following definition of Defective maize kernels is quoted from the Grading Regulations:

"Defective maize kernels" means maize kernels and pieces of maize kernels –

(a) that are shrivelled, obviously immature, frost-damaged, heat damaged, water damaged, mouldy or chalky;

(b) that are discoloured by external factors such as water and sun: Provided that discoloration on both sides of the maize kernel limited to less than a quarter from the bottom tip of the maize kernel shall not be considered as defective, oxidation stained maize kernels, coffee stained maize kernels and pinked maize kernels shall not be considered as defective;

(c) that have sprouted, including kernels which the shoot (plumule) in the germ is visibly discoloured;

(d) that have cavities in the germ or endosperm caused by insects or rodents;

(e) that are visibly soiled (smeared) or contaminated by smut, fire, soil, smoke or coal-dust;

(f) all matter that can pass through the 6.35 mm round-hole sieve; and

(g) that are of subspecies other than *Zea mays indentata* or *Zea mays indurata*.

Provided that –

(i) irregularity of shape and size of maize kernels shall not affect the grading thereof;

(ii) chipped or cracked maize kernels or pieces of maize kernels which are in a sound condition and which appear in a sample of maize, but which do not pass through a 6.35 mm round-hole sieve, shall not be regarded as defective maize kernels under these regulations."

Foreign matter

The term "foreign matter" means all matter above the sieve other than maize, glass, stones, coal, dung or metal.

Other colour

"Other colour maize kernels" in relation to -

(a) white maize, means maize kernels or pieces of maize kernels of which the endosperm as a result of genetic (characteristics) composition have another colour than white, excluding pinked maize kernels;

(b) yellow maize, means maize kernels or pieces of maize kernels of which the endopserm as a result of genetic (characteristics) composition have another colour than yellow.

Combined deviation

The term "combined deviation" means the sum of defective kernels (above and below the 6.35 mm sieve), foreign matter and other colour kernels.

Pinked kernels

The term "pinked maize kernels" means kernels and pieces of kernels of white maize of which the pericarp or part thereof is shaded red or pink in colour.

The specification, according to the Grading Regulations for classes 1 to 3 of white maize is a maximum of 12%. No specification for yellow maize according to the Grading Regulations.

Fungal infection

Kernels which are mouldy (fungi infected) are reported as defective kernels according to the grading regulations.

"Mouldy" means kernels and pieces of kernels that –
(a) are visibly infected by fungi and are characterised by black, blue, green, yellow or white fungi growth anywhere on the kernel, or are characterised by fungi growth underneath the bran layer of the kernel;
(b) are infected by ear-rot and are characterised by red, pink or brown discolorations. The kernel are partially to completely infected.

For this survey all samples were also inspected for the visual symptoms of Diplodia and Fusarium infection and reported separately.

Fusarium spp infections are localized on the cob with 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.

% Cobrot reported is the percentage maize kernels that are both Fusarium and Diplodia infected.

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 to 5, 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:

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.

Heat damaged kernels

Kernels and pieces of kernels which are materially discolored 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 discolored by external heat caused by artificial drying methods.

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 after sieving.

Bushel weight

The specific mass (or grain density) of maize (expressed as test weight or bushel weight) is a quality characteristic which is important to some maize consumers and is applied as a grading factor in the USA grading regulations.

The Test weight per bushel apparatus is used to determine the approximate weight of a bushel of a particular lot of grain.

Bushel weight in pounds (lbs), was determined on the maize crop samples and the results converted to test weight, reported in kilogram/hectoliter (kg/hl), by multiplication with a factor of 1.2872.

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%.

NUTRITIONAL VALUES

The fat, protein and starch contents are measured with an Infratec 1241 - Generation 3 Standard Version Whole Grain Analyser. The measurements are based on the fact that the constituents to be measured in the grain, absorb electromagnetic radiation in the near-infrared region of the spectrum. Since the Infratec 1241 Grain Analyser uses transmission absorption, the test is done on intact maize kernels.

Foss updated the calibration on the Infratec 1241 Grain Analyser (NIT) during 2016, using NIT spectra and international primary chemical method results of maize crop quality samples from the 2012/2013 to 2014/2015 seasons, provided by SAGL.

The chemical methods used to check the calibration were:

a) Crude fat: Petroleum ether extraction (Soxhlet) method (In house method 024)
b) Crude protein: Dumas (Leco) method (AACCI 46-30.01)
c) Starch: Hydrochloric Acid dissolution method (Polarimeter) (In house method 019)

The results obtained by the Infratec 1241 Grain Analyser (NIT) on the 2015/2016 season's samples, were checked by analysing every tenth sample by means of the primary methods.

PHYSICAL CHARACTERISTICS

Test weight

Test weight is reported in kilogram per hectolitre. The specific mass (or grain density) of maize expressed as test weight is influenced by amongst other, factors like cultivar, moisture content, foreign matter, other grain and damaged kernels like insect damaged and immature kernels.

Bushel weight in pounds (lbs) was determined on the maize crop samples and the results converted to test weight, reported in kilogram/hectoliter (kg/hl), by multiplication with a factor of 1.2872.

Hundred (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. 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, uniform kernel size is of particular importance to this industry, since too large kernels 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 sample through both 8 mm and 10 mm round-hole grading sieves, normally used in the seed industry.

Breakage susceptibility - Industry accepted method 007

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

In the modern dry milling industry, maize is cleaned first 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 causes problems with the fibre and fat content of maize products, for example the various grades of maize meal, because the quantity of germ required to be returned to the milled endosperm cannot be determined accurately.

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 reasons.

All samples were 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 6.35 mm and 4.75 mm sieves 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 in the field, during harvest or during drying.

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 - Generation 3 Standard Version Grain Analyser.

In previous seasons (up to 2012/2013) the samples were analysed by means of the calibration model developed by die Grain Crops Institute of the ARC. The last three seasons' samples were analysed by means of the new version of the milling index model developed by the SAGL. The NMI (New Milling Index) model was developed on data acquired from analyses performed on maize cultivar trials over four seasons. These trials included a range of hardness levels. The NMI model has improved precision compared to the older version, due to the almost tenfold increase in sample numbers used to build the model. Samples were supplied by the ARC-GCI and by commercial seed breeders for inclusion in the statistical modelling.

Calibrations were done between NIT spectra and various Roff Milling parameters including the ARC Roff milling formula. From these, the best solution was selected based on multivariate regression (Partial Least Square Regression). The samples used for the model were all pure cultivar samples.

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 is pre-set to the following specifications: Break 1 roll nip - 0.3 mm, Break 2 roll nip - 0.18 mm and Break 3 roll nip - 0.08 mm. These settings are according to the specifications in the method developed by the ARC Grain Crops Institute. 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 and Break 3 grits. Break 1, 2 and 3 germ and bran are combined and then weighed for determination of Bran/Germ %. Break 3 grits are weighed for determination of % Grits. Break 1, 2 and 3 meal are weighed for determination of % extraction total meal.

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 sample.

Whiteness index was done on unsifted and sifted maize meal obtained from Break 2 and 3 of the Roff mill. The sifted samples were obtained by sieving the unsifted samples through a 300 µm sieve. The fractions on top and below the sieve were then combined to result in sifted samples that contain 87% of maize meal > 300 µm and 13% of maize meal < 300 µm.

MYCOTOXIN ANALYSES

Mycotoxins are fungal metabolites, toxic to animals and humans, that are produced by moulds commonly found in almost all types of grain.

350 of the 920 maize crop samples were tested for Aflatoxin G_1; B_1; G_2; B_2, Fumonisin B_1, B_2 and B_3, Deoxynivalenol, 15-ADON, HT-2 Toxin, T-2 Toxin, Zearalenone and Ochratoxin A by means of a multi-mycotoxin screening method using UPLC - MS/MS.

Limit of quantitation (LOQ) means the lowest concentration level that can be quantified with acceptable presicion and accuracy by the mass spectrometer. A concentration measured below the LOQ is reported as <LOQ.

Limit of detection (LOD) is the lowest concentration level that can be detected but not quantified and is 50% of the LOQ of each mycotoxin. A concentration measured below the LOD is reported as not detected (ND).

GMO (Genetically Modified Organisms)

The EnviroLogix QuickComb kit for bulk grain was used to quantitatively determine the presence of genetically modified maize. The kit is designed to extract and detect the presence of certain proteins at the levels typically expressed in genetically modified bulk maize grain. The procedure prescribed in the EnviroLogix - QuickScan Instruction Manual, Rev 10-04-10 was followed. Results were scanned and interpreted quantitatively with the EnviroLogix QuickScan system.

100 crop samples were tested for Cry1Ab, Cry2Ab and CP4 EPSPS modified maize. Cry1Ab protein in maize is produced from a gene derived from *Bacillus thuringiensis (Bt)*.

GMO Protein/Trait	Event	Trade name / Brand
Cry1Ab	MON810 MON89034 Bt11	YieldGard®
Cry2Ab	MON89034	*in* Genuity™ VT Triple PRO™ SmartStax™
CP4 EPSPS	NK603	Roundup Ready ®