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.

Once grading has been completed, 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 content of each container was divided 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 was more than one container per class and grade per silo bin, the combined contents of the containers were mixed thoroughly before dividing it with a multi slot divider to obtain the required 3 kg sample.

The samples were 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 were 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, Government Notice No. R. 473 of 8 May 2009 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 endosperm 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 pinked kernels in 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.

All samples were also inspected for the visual symptoms of Diplodia and Fusarium infection, which were 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.

The % Cobrot reported, represents the percentage maize kernels that are both Fusarium and Diplodia infected.

USA GRADING

USA grading was determined in accordance with the American Grading Regulations (United States Department of Agriculture).

The US grading system makes provision for three classes of maize/corn based on colour, namely Class White corn, Class Yellow corn and Class Mixed corn. Each class is divided into five U.S. numerical grades (Nos. 1 to 5) and U.S. Sample Grade. US No.1 is the most desirable grade followed by No. 2 down to sample 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 discoloured and damaged by heat, including material discolouration 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 according to procedures prescribed in Federal Grain Inspection Service (FGIS) instructions.

Foreign material is all matter that passes readily through a 6/64-inch round-hole sieve and all matter other than corn that remains on top of the 12/64-inch round-hole sieve after sieving according to procedures prescribed in FGIS instructions.

Broken corn and foreign material is all matter that passes readily through a 12/64-inch round-hole sieve and all matter other than corn that remains in the sieved sample after sieving according to procedures prescribed in FGIS instructions.

Bushel weight

The specific mass (or grain density) of maize/corn (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.

Test weight is the weight per Winchester bushel (2,150.42 cubic inches) as determined using an approved device according to procedures prescribed in FGIS instructions.

Other colour

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

PHYSICAL CHARACTERISTICS

Test weight

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.

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.

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. The results are reported on an "as is" basis.

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, routinely 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 (e.g. 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 fractions below the 6.35 mm and 4.75 mm sieves were collected and the percentages broken kernels smaller than (<) 6.35 mm and < 4.75 mm 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 a model developed on the Foss NIT Infratec 1241- Generation 3 Standard Version Grain Analyser where the NIT spectra were modelled against the Roff milling fractions. In the previous seasons (until 2015/16) the Milling index of the samples were determined with the calibration model developed by the Grain Crops Institute of the ARC. With this model, the average milling index of a sample with good milling characteristics is about 95 with a variation of about 55 (low milling quality) to about 115 (very good milling quality).

The SAGL was tasked by the Maize Trust to develop a new model for Milling Index using samples from maize cultivar trials supplied by the ARC-GCI and by commercial seed breeders over four seasons (from 2012/13 onward). The trials included a range of hardness levels. The New Milling Index (NMI) that was developed is similar to the original ARC formula but on a 14% moisture basis, and with the constants removed. The NMI model has improved precision compared to the older version, due to the almost tenfold increase in the number of samples used to build the calibration model.

During the fifth year, samples of commercial hybrids, selected imported maize samples and outlier samples from the 2014/15 and 2015/16 seasons were included to develop a robust model with the assistance of Foss to produce accurate results. The latest version of the improved new model, SAGL Milling Index 2020, includes two parameters, SAGL Milling Index (SAGL MI) as well as Grit Yield All (GYA).

SAGL MI indicates the relative ratio of total hard endosperm products (B2 grits, B3 fine grits and B3 coarse grits) to offal products (B1 fine flour and total chop/bran) as determined on a Roff mill and used for calibration of the NIT. It is expressed as a dimensionless index value according to the following scale:

SAGL MI	<40	40-60	60-80	80-100	>100
Description	Soft	Medium	Moderately hard	Hard	Very hard

GYA is defined as the sum of the mass fractions of the Roff B2 grits, B3 fine grits and B3 coarse grits fractions expressed as a mass percentage of the total mass of the whole maize before milling. GYA is linearly correlated with the SAGL MI and indicates the true amount of total hard endosperm that can be extracted from the maize during Roff milling. The NIT calibration value for GYA provides this estimate directly from the whole maize without need for further milling tests. GYA is also reported on a 14% moisture base.

The 2019/20 season maize samples were measured with the NIT on the SAGL Milling Index 2020 model.

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 chop, 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 percentages: Break 1 meal, Break 2 meal, Break 3 meal and Break 3 grits. Break 1, 2 and 3 chop are combined and then weighed for determination of % Chop. Break 3 grits are weighed for determination of % Grits. The percentage extraction total meal is determined as the sum of the percentages Break 1, 2 and 3 meal as well as the % Grits.

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.

NUTRITIONAL VALUES

The moisture, fat, protein, starch and crude fibre 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. The results are reported on a dry (moisture free) basis.

With the assistance of Foss, a calibration for crude fibre content was developed during the 2017/18 season. The calibration on the Infratec 1241 Grain Analyser (NIT), is updated annually by Foss using NIT spectra and international primary chemical method results of maize crop quality samples from the specific season under discussion, provided by SAGL.

The chemical methods used to check the calibration are:

- Moisture on whole maize kernels: Sample is dried in an oven for 72 hours at 103 °C (AACCI 44-15.02)
- Moisture on milled maize: Sample is dried in an oven for 1 hour at 130 °C (AACCI 44-15.02)
- Crude fat: Petroleum ether extraction (Soxhlet) method (In house method 024)
- Crude protein: Dumas (Leco) method (AACCI 46-30.01)
- Starch: Hydrochloric Acid dissolution method (Polarimeter) (In house method 019)
- Crude fibre: Loss on ignition of the dried residue after digestion of the sample with dilute and acid and alkali (In house method 020)

The results obtained by the Infratec 1241 Grain Analyser (NIT) on the 2019/20 season's samples, were checked by analysing every tenth sample by means of the primary methods.

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 890 maize crop samples were tested for Aflatoxin B_1, B_2, G_1, G_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 (In house method 026) using UPLC - MS/MS.
Limit of quantitation (LOQ) means the lowest concentration level that can be quantified with acceptable precision 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, latest edition was followed. Results were scanned and interpreted quantitatively with the EnviroLogix QuickScan system.

70 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).*

Cry1Ab	MON810	Tradename/Brand
Cry1Ab	MON810 Cry1A.105 Bt11	YieldGard®
Cry2Ab	MON89034	*in* SmartStax ™
CP4 EPSPS	NK603	Roundup Ready ®