Methods

Sampling procedure

A working group determined the procedure to be followed to ensure that the crop quality samples sent to the SAGL by the various grain silo owners, were representative of the total crop.

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

After grading, the grading samples were placed in separate containers according to class and grade, per silo bin at each silo.

After 80% of the expected harvest had been received, the content of each container was divided with a multi slot divider in order to obtain a 3 kg sample.

If there were 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 number(s) represented by each individual sample as well as the class and grade and were then forwarded to the SAGL.

Grading

Full grading was done in accordance with the Regulations relating to the Grading, Packing and Marking of Sorghum intended for sale in the Republic of South Africa (Government Notice NO. R. 15 of 08 January 2016).

See pages 43 to 51 of this report.

Detection of tannin in sorghum grain by the bleach test

This method is applicable to whole grain sorghum.

Sorghum grain is immersed in a sodium hypochlorite solution (bleach) containing alkali. The solution dissolves away the outer pericarp layer of sorghum grain, revealing the presence of a black pigmented testa layer in the case of tannin sorghums, or its absence in the case of non-tannin sorghums.

Bleaching Reagent

- Prepare a 5.25% sodium hypochlorite solution by mixing 250 mL bleach (Jik) with 750 mL tap water.
- Weigh 10.0 g potassium hydroxide and dissolve this in 50 mL of 5.25% sodium hypochlorite solution.

Note: Prepare fresh when the tannin test is conducted. The 50 mL solution is enough for one sample.

Apparatus

- Glass beakers (50 mL)
- Tea strainer
- Aluminum foil
- Paper towel

Procedure

- The test must be performed in duplicate.
- *Known tannin sorghum and non-tannin sorghum standards must be included each time the test is performed.*
- Weigh 25.00 g sound sorghum grains of the sample in a beaker or smal glass bottle.
- Add the bleaching reagent to just cover the sorghum grains and close the beaker with aluminum foil. Too much bleaching reagent will cause over bleaching and give false negative results. If in doubt repeat using less reagent.
- Incubate beaker at room temperature (20-30°C) for 20 minutes, swirling contents of beaker every 5 minutes. White sorghum is incubated for only 5 minutes.
- Empty contents of beaker into tea strainer, discarding bleaching reagent. Rinse sorghum grains in tea strainer with tap water.
- Empty contents of tea strainer onto sheet of paper towel. Spread grains out into a single layer and gentle blot them dry with another piece of paper towel.

- Remove the tannin sorghum grains in the sample and weigh them. **Tannin sorghum grains are those grains that are black over the entire surface of the grain.**
- Weigh the non-tannin sorghum grain (remainder of the sample after removing the tannin sorghum grains). **Non-tannin sorghum grains are those which are either completely white or are brown over part of the surface of the grain.**

Calculation of results

Calculate tannin sorghum grains as percentage of total sorghum grains as follows:

- Percentage tannin sorghum = Mass of non-tannin sorghum / mass total sample) x 100
  Example: % tannin sorghum = (23.85 g tannin/25.00 g sample) x 100
  = 95.4% tannin sorghum
- Duplicate determinations should not differ by more than 5% (1.25g), for example first determination 90%, second determination 85%, or 95%. The mean of the duplicate determinations should be calculated.

Reporting of results

- Results should be expressed as: Percentage tannin sorghum, e.g. 90% tannin sorghum.
- Classification:
  - Sample containing ≥ 95% tannin sorghum is classified as Tannin sorghum
  - Sample containing ≥ 95% non-tannin sorghum classified as Non-tannin sorghum.
  - Sample containing < 95% tannin sorghum and > 5% non-tannin sorghum, the sample is classified as Mixed Tannin
  - Sample containing < 95% non-tannin sorghum and > 5% tannin sorghum, the sample is classified as Mixed Tannin

Test weight

Test weight, providing a measure of the bulk density of grain and oilseeds, was determined according to ISO 7971-3:2019, by means of the Kern 222 instrument.

To calculate the bulk density p, expressed in kilogram per hectolitre (kg/hl), the following equation was applied: p = 0.1002 m + 0.53. This is the equation used for wheat, since an equation for sorghum is not available.

The test weight analyses were done on unscreened sorghum samples.

Thousand kernel mass

This is the weight in grams of one thousand kernels of grain and provides a measure of grain size and density. This determination does not include kernels that are broken or chipped and is done according to Industry Accepted Method 008 using a seed counter. Thousand kernel mass is reported on a 14% moisture basis.

Determination of sorghum kernel size by means of image analysis

Sorghum kernels were photographed on a Panasonic Lumix digital camera (DNC-LX3). Photos were analysed afterwards, using Digimizer version 4.0 software supplied by Medcalc (www.digimizer.com), to measure the size of the sorghum kernels. Photos of the samples are stored in a database. The following measurements were taken:

- Length, measured in millimeters (mm)
- Width, calculated at a 90% angle from the maximum length of an object, measured in millimeters (mm)
- Elongation (% Width/Length or W/L%)
- Surface:Volume ratio (calculated from length and width data)

Milling

All samples requiring milling were milled on a Retch ZM 200 mill fitted with a 0.5 mm screen.

Moisture

The moisture content of the milled grain was determined using ICC Standard 110/1 (latest edition). This method determines moisture content as a loss in weight of a sample when dried in a hot air ventilation oven at 130 °C for 2 hours. Moisture content results were used to report % starch, % protein and % fat content on a dry basis (db).

Crude Protein Content

The Dumas combustion analysis technique was used to determine the crude protein content, according to AACCI method 46-30.01, latest edition.

This method prescribes a generic combustion method for the determination of crude protein. Combustion at high temperature in pure oxygen sets nitrogen free, which is measured by thermal conductivity detection. The total nitrogen content of the sample is determined and converted to equivalent protein by multiplication with a factor of 6.25 to obtain the crude protein content.

Total Starch Content

Determination of the total starch content was according to the SAGL In-house method 019, a polarimetric method based on the modified Ewers method. The starch content is released from the sample by boiling in dilute hydrochloric acid. The starch solution in the filtrate is determined by measuring the angle of polarisation or optical rotation of the filtrate with a polarimeter. The acid also helps to break down the endosperm tissue, ensuring complete release of the starch granules from the protein matrix. Substances, which may interfere with the measurement, are removed by filtration.

Crude fat Content

In-House method 024 was used for the determination of the crude fat content in the samples. After sample preparation, the fat is extracted by petroleum ether with the aid of the Soxhlet extraction apparatus, followed by the removal of the solvent by evaporation and weighing the dried residue thus obtained. The residue is expressed as % crude fat.

Mycotoxin analyses

Mycotoxins are fungal metabolites, toxic to animals and humans, that are produced by moulds commonly found in almost all types of grain. Aside from health risks, mycotoxin contamination can also reduce the value of the crops. Environmental factors such as temperature, humidity, soil and storage conditions influence toxin production.

SAGL implements a validated SAGL In-house multi-mycotoxin screening method using UPLC - MS/MS. A sub-sample of each sorghum sample was milled and tested for Aflatoxin B_1; B_2; G_1; G_2, Fumonisin B_1; B_2; B_3, Deoxynivalenol, 15-ADON, HT2 Toxin, T-2 Toxin, Zearalenone and Ochratoxin A.

Dehulling of samples

Each sorghum sample was sieved and the fraction below the 4 mm and above the 3.55 mm sieve was dehulled by means of a Barley pearler. This fraction was selected to obtain an indication of comparative hardness and to eliminate difference due to kernels size. Tests were conducted using 150 g of sample with a dehulling time of 70 seconds. These parameters are based on results obtained on the outcomes of a processing application project funded by the Sorghum Trust. Barley pearler fractions are sieved into three fractions:

> 1.8 mm slotted sieve
< 1.8 mm slotted sieve and > 2.38 mm round hole sieve, and
< 2.38 mm round hole sieve.
The colour determinations for this project was done on the first fraction (> 1.8 mm).

Determination of colour

- The Barley pearler fraction above the 1.8 mm slotted sieve was milled on a Retch mill through a 0.5 mm sieve. The milled samples' colour was determined with the Hunterlab ColorFlex EZ 45°/0° spectrophotometer with key parameters set on a 10° observer angle and daylight illuminant D65 according to SAGL Industry accepted method 004. The spectrophotometers operate in the Hunter L, a, b scale where:
- L measures lightness and varies from 100 for perfect white to zero for black, approximately as it would be evaluated by the eye. The chromaticity dimensions (a and b) give understandable designations of colour as follows:
- a measures redness when positive, grey when zero, and greenness when negative.
- b measures yellowness when positive, grey when zero, and blueness when negative.