

# METHODS

## SAMPLING PROCEDURE:

A working group determined the process which needs to be followed to ensure that the crop quality samples which were 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 are placed in separate containers according to class and grade.

After 80% of the expected harvest has been received, the content of each container is 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 is more than one container per class and grade, the combined contents of the containers are 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 number(s) represented by each individual sample as well as the class and grade and are then forwarded to the SAGL.

## GRADING:

Full grading was done in accordance with the Regulations relating to the Grading, Packing and Marking of soybeans intended for sale in the Republic of South Africa (No. R 225 of 6 March 2009).

Please see pages 25 to 32 of this report.

## CHEMICAL ANALYSIS:

### Milling

Prior to the chemical analyses, the soybean samples were milled on a Retch ZM 200 mill fitted with a 1.0 mm screen.

### Moisture

The method prescribed under the ISTA International Rules for Seed Testing, Section 9, latest edition was used to determine the moisture content of the soya samples. This method determines moisture content as a loss in weight of a sample when dried in an oven at 103 °C for 17 hours.

### Protein

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 protein content.

### **Fat**

In-House method 024 was used for the determination of the crude fat 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.

### **Ash**

Ash is defined as the quantity of mineral matter which remains as incombustible residue of the tested substance, after application of the described working method. In-house method No. 011, based on AACCI method 08-02.01 Rapid (Magnesium Acetate) method, was used for the determination.

### **GMO (Genetically Modified Organisms):**

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

### **AMINO ACIDS:**

Following acid hydrolysis of the samples, the protein bound amino acids were determined by using In-house method No. 009, liquid chromatographic analysis of amino acids using a modified Pico-Tag method.

In-house method No. 015, where the sample is first oxidized and dried, was followed for the determination of cysteine (as cysteic acid) and methionine (as methionine sulfone). The samples were then analysed with liquid chromatography using a modified Pico-Tag method as for the other protein bound amino acids.

For the determination of tryptophan according to In-house method No. 007, the samples are hydrolysed under alkaline conditions with a saturated barium hydroxide solution heated to 110 °C for 20 hours. The hydrolysate is analysed by reverse phase liquid chromatography with UV detection at 285 nm. All the samples were hydrolysed in duplicate.