

# Methods

## GRADING:

Full grading was conducted in accordance with the Regulations relating to the grading, packing and marking of bread wheat intended for sale in the Republic of South Africa (Government Notice No. R. 1547 of 29 November 2019). Please see pages 104 to 115.

Hectolitre mass, screenings, protein and falling number were determined. The determination of deviations relating to wheat kernels comprised foreign matter including gravel, stones, turf and glass; other grain and unthreshed ears; damaged kernels including heat-damaged kernels, immature kernels, insect-damaged kernels and sprouted kernels; heavily frost-damaged kernels; field fungi; storage fungi; ergot; poisonous seeds; possible presence of undesirable odours and live insects.

**Hectolitre mass** means the mass in kilogram per hectolitre and was determined according to ISO 7971-3, 2019 by means of the Kern 222 instrument.

Hectolitre mass provides a measure of the bulk density of grain and is also useful as a guide to grain soundness and potential milling extraction (flour yield).

**Screenings** means all material that passes through a standard 1.8 mm slotted sieve. For the definition and description of the standard sieve please refer to the definitions of Regulation No. R. 1547 on page 106 of this report.

**Damaged wheat** means wheat -

- (a) which have been damaged by insects;
- (b) which have been distinctly discoloured (orange-brown, dark brown or black) by external heat or as a result of heating caused by internal fermentation in wheat with an excessive moisture content, excluding wheat kernels in respect of which the discolouration is confined to the germ end;
- (c) which are immature and have a distinctly green colour; and
- (d) in which germination has proceeded to such an extent that the skin covering the embryo has been broken or the developing sprouts and/or rootlets are clearly visible.

**Combined deviations** means the sum of the percentages screenings, other grain and unthreshed ears, foreign matter and damaged kernels.

## THOUSAND KERNEL MASS:

This is the weight in grams of one thousand kernels of wheat 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 SAGL Industry Accepted Method 008. Thousand kernel mass is reported on a 13% moisture basis.

## FALLING NUMBER MILLING:

At least 300 g of wheat is cleaned by using the standard 1.8 mm sieve and by removing coarser impurities by hand. The sample is then milled on a falling number hammer mill fitted with a 0.8 mm sieve.

## NEAR INFRARED SPECTROSCOPY (NIRS):

NIRS is a measurement technique based on the fact that the constituents to be measured, absorb electromagnetic radiation in the near infrared region of the electromagnetic spectrum. The moisture and protein content of the whole wheat flour as well as Quadromat milled flour samples are measured with a SpectraStar 2400 NIR Analyser RTW.

The calibration on the NIR was developed by the SAGL and is verified by analysing every fifth sample by means of the primary methods, described on the next page under Moisture and Protein.

## FALLING NUMBER:

This method is based upon the rapid gelatinisation of an aqueous suspension of meal or flour in a boiling water bath and subsequent measurement of the liquefaction of the starch paste by the  $\alpha$ -amylase in the sample. The method measures the enzyme activity, mainly the  $\alpha$ -amylase activity.

ICC Standard No. 107/1, latest edition is used to determine the falling number. The altitude-corrected value is reported on a 14% moisture basis.



### QUADROMAT JUNIOR MILLING:

Cleaned wheat samples are conditioned by adding 3 mL water per 100 g wheat, 18 hours prior to milling. The samples are then milled on a Quadromat Junior laboratory mill.

### BÜHLER MILLING:

Cleaned wheat samples are conditioned to between 15.0% and 16.0% moisture according to the wheat moisture and kernel hardness and allowed to stand for a minimum of 18 hours (18 - 24 hours). Samples are then milled on a Bühler MLU 202 mill and passed through a bran finisher.

### BÜHLER EXTRACTION:

The extraction represents the flour yield after milling plus flour obtained from bran that passed through a bran finisher. Flour extraction is calculated from the mass of the total products. The Bühler MLU 202 mill is set for South African wheat, mill settings and sieve sizes deviate from AACCI method 26-21.02, latest edition.

### MOISTURE:

ICC Standard No. 110/1, latest edition is used to determine the moisture content of wheat flour. This method determines moisture content as a loss in weight of a sample when dried in an oven at 130 °C for 90 minutes for flour or 2 hours for whole wheat flour.

### PROTEIN:

The Dumas combustion analysis technique is used, according to AACCI method 46-30.01, latest edition.

This method prescribes a generic combustion method for the determination of crude protein. Combustion of the sample at high temperature (1 100 °C) in pure oxygen sets nitrogen free, which is measured by thermal conductivity detection. The total nitrogen content of the whole wheat flour and flour samples are determined and converted to equivalent protein by multiplication with a factor of 5.7 to obtain the protein content.

### COLOUR:

Colour is one of the important properties of milled grains and the colour of wheat flour often affects the colour of the finished product. In general, a bright white colour flour is more desirable for most products.

The colour of wheat flour can be measured accurately and precisely with the Konica Minolta

CM-5 spectrophotometer. CIE L\*a\*b\* (CIELAB) is a colour model using lightness (L\*) and two colour values (a\* and b\*). The colour coordinates define where a specific colour lies in a Cartesian graph. L\* represents lightness (100 being white and 0 being black), a\* represents green to red variation and b\* represents variation from blue to yellow. The results reported are for the 10° observer and D65 illuminant.

### ASH:

Ash is defined as the quantity of mineral matter that remains as incombustible residue, after incineration of a sample in a muffle furnace by application of the described working method. The ash constituents of wheat are taken from the minerals of the soil. The total mineral content as well as the relative proportions of individual elements depend largely upon the soil, rainfall and other climatic conditions during growth.

Since the level of minerals present in flour is related to the rate of extraction, the ash content also indicates milling performance by indirectly revealing the amount of bran contamination. In-house method No. 011, based on the AACCI method 08-02.01 Rapid (Magnesium Acetate) method, is used for the ash determination.

### RAPID VISCO ANALYSER:

AACCI method 76-21.02, latest edition, is followed to prepare a complete pasting curve by means of the Rapid Visco Analyser (RVA). The RVA is a rotational viscometer, able to continuously record the viscosity of a sample (under controlled temperature conditions) as the starch granules hydrate, swell and disintegrate (gelatinisation and pasting), followed by possible realignment of the starch molecules during cooling (retrogradation).

Maximum viscosity before the onset of cooling (peak viscosity), time to peak viscosity, minimum viscosity after peak (trough) and final viscosity are measured and provide indications of the pasting properties of the samples and therefore its processing value for baking and other applications.

The results are reported in centipoise (cP) on a 14% moisture basis. Results can also be converted to RVU (rapid visco unit), 1 RVU = 12 cP.

### GLUTEN:

Wheat gluten is the water-insoluble complex protein fraction present in wheat flours. The ability



of wheat flour to produce dough with good gas retaining properties is attributed to gluten. Gluten is a plastic elastic substance composed principally of two functional protein components. Glutenin, the high molecular weight fraction, contributes elasticity (is less extensible) and Gliadin, the low molecular weight fraction, provides the viscous component (is highly extensible and less elastic).

The gluten content of wheat flour is determined by means of AACCI Method 38-12.02, latest edition. Wet gluten is washed from meal or flour by an automatic washing apparatus (Glutomatic).

The wet gluten is dried under standardised conditions in a Glutork to obtain the dry gluten. The total wet and total dry gluten contents are expressed as percentages of the sample on a 14% moisture basis.

Wet gluten content correlates to loaf volume and dry gluten content to the crude protein content. The difference between the wet and dry gluten contents is an indication of the water-holding capacity of the gluten proteins, which is in turn, related to flour water absorption.

The gluten index is the ratio of the wet gluten remaining on the sieve (after centrifugation) to the total wet gluten. The gluten index provides an indication of the gluten strength and is not influenced by the protein content.

### FARINOGRAPH:

AACCI method 54-21.02, latest edition constant flour weight procedure is followed, using 300 g of flour on a 14% moisture basis.

The farinograph measures and records the resistance of a dough to mixing, as it is formed from flour and water, developed and broken down. This resistance is called consistency. The dough is subjected to a prolonged, relatively gentle mixing action.

The water absorption is the amount of water required for a dough to reach a definite consistency (500 Brabender units). The amount of water added to the flour is expressed as a percentage of the flour mass and reported on a 14% moisture basis.

The development time, measured in minutes, is the time from the beginning of water addition until the dough reaches its optimum consistency and the point immediately before the first indication of weakening. A long mixing time can be associated

with flour with a high percentage of gluten-forming proteins.

The stability, measured in minutes, is the time during which the top of the curve intercepts a horizontal line drawn through the centre of the curve. This gives an indication of the dough's tolerance to mixing: the longer the stability, the longer the mixing time that the dough can withstand. Dough with a longer stability can also withstand a longer fermentation period.

The mixing tolerance index (MTI) value is the difference, in Brabender units (BU), between the top of the curve at the peak and the top of the curve measured 5 minutes after the peak is reached. This value provides an indication of the extent to which breakdown of the dough occurs. The higher the value, the more and the quicker the breakdown of the dough occurs. This value is similar to the mixogram tail height.

### EXTENSOGGRAPH:

The extensograph measures the resistance and extensibility of a fully mixed, relaxed flour-water dough, by measuring the force required to stretch the dough with a hook until it breaks. ICC Standard No. 114/1, latest edition is followed.

The strength, measured in  $\text{cm}^2$ , provides an indication of the total force (work) needed to stretch the dough and is represented by the area under the curve.

The maximum height/resistance, measured in BU, gives an indication of the dough's resistance to stretching and is measured as the mean of the maximum heights of the curves of the two test pieces.

The extensibility, measured in millimeters, is the mean length at the base of the two curves and indicates the stretch ability of the dough.

### ALVEOGRAPH:

The alveograph measures the resistance of the dough to stretching as well as the extensibility of the dough. The alveograph stretches the dough in more than one direction (as is happening during proofing), whereas the extensograph stretches the dough in only one direction. ICC Standard No. 121, latest edition is followed.

Strength (S): The area under the curve gives an indication of the dough strength and is measured in  $\text{cm}^2$ .



Stability (P): Obtained by multiplying the maximum height of the curve with a constant factor of 1.1. This value is an indication of the resistance of the dough to extension (force required to blow the bubble of dough) and is measured in millimetres.

Distensibility (L): The length of the curve in millimetres, measured along the base line, corresponds to the maximum volume of air that the bubble can withhold. This provides an indication of the extensibility of the dough.

P/L-value: This ratio is obtained by dividing the P-value by the L-value, thus providing an approximate indication of the shape of the curve that combines stability and extensibility (viscoelastic properties).

### MIXOGRAPH:

A 35 g mixograph is used. The amount of flour weighed is adjusted according to the flour moisture content and the amount of water added to the flour is adjusted according to the flour protein content. Industry Accepted Method 020 based on AACCI method 54-40.02, latest edition is followed.

Mixogram peak time, is the time measured in minutes, that dough takes to reach its maximum consistency or first indication of dough weakening. The peak time is a measure of optimum dough development and thus a measure of protein quality.

Mixogram tail height at 6 minutes, is the distance in millimetres measured from the base line of the paper at 6 minutes to the graph centre point at 6 minutes. This figure is an indication of the weakening effect of the dough. Higher values indicate flours that are more tolerant to mixing.

### 100 g BAKING TEST:

This procedure, according to Industry Accepted Method 022 based on AACCI Method 10-10.03, latest edition, provides an optimised bread-making method for evaluating bread wheat flour quality and a variety of dough ingredients by a straight-dough method in which all ingredients are incorporated in the initial mixing step.

### MYCOTOXIN ANALYSES:

Mycotoxins are secondary metabolites produced by fungi on agricultural commodities intended for human and animal consumption. These mycotoxins are potentially dangerous to humans and animals since they are, amongst other also carcinogens. 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 method using UPLC - MS/MS. 40 of the 335 wheat crop samples were tested for Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, Fumonisin B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, Deoxynivalenol, 15-ADON, HT2 Toxin, T-2 Toxin, Zearalenone and Ochratoxin A.

### AMINO ACID PROFILE:

The protein bound amino acids (Aspartic acid (Asp), Glutamic acid (Glu), Serine (Ser), Glycine (Gly), Histidine (His), Arginine (Arg), Threonine (Thr), Alanine (Ala), Proline (Pro), Tyrosine (Tyr), Valine (Val), Isoleucine (Ileu), Leucine (Leu), Phenylalanine (Phe) and Lysine (Lys)) were determined by using In-house method No. 028, (AccQ-Tag method).

Samples (200 mg) are hydrolysed with 6 N hydrochloric acid (HCl) for 24 hours and then derivatised with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) to produce stable derivatives. These amino acids are then analysed by a reverse phase UPLC method, using a Waters Acquity H-Class UPLC with Empower software (Waters, Millipore Corp., Milford, MA).

The determination of Cysteine (as Cysteic acid) and Methionine (as Methionine sulfone) were done by means of In-house method No. 15. The sample is first oxidised and dried and then analysed with liquid chromatography using a modified Pico-Tag method.

The determination of Tryptophan, according to In-house method No. 007, entails the hydrolysis of samples 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.

