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Five Simple Methods for the Determination of Sorghum Grain End-Use Quality (with Adaptations for Those without Laboratory Facilities)

John R.N. Taylor
University of Pretoria

Janet Taylor
University of Pretoria

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FIVE SIMPLE METHODS FOR THE DETERMINATION OF SORGHUM GRAIN END-USE QUALITY

(With adaptations for those without laboratory facilities)

John R N Taylor

Janet Taylor

University of Pretoria

South Africa

August 2008



**Department of Food Science
University of Pretoria
Pretoria 0002
South Africa**

**Tel +27 12 420 4296
Fax +27 12 420 2839
E-mail jtaylor@postino.up.ac.za**

(Amended January 2007)

Disclaimer – Neither the authors, nor the University of Pretoria, nor INTSORMIL accept any responsibility in respect of these methods or their application.

Readers are, however, welcome to contact the authors with regard to seeking clarity on how to perform any of the methods.

John R N Taylor

Janet Taylor

**August 2008
Pretoria South Africa**



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1.

Method:

Detection of Tannin in Sorghum Grain by the Bleach Test

1. Scope

Applicable to whole grain sorghum.

2. Definitions

Certain varieties of sorghum contain proanthocyanidins (commonly referred to as tannins or more strictly-speaking condensed tannins) in the seed coat layer beneath the pericarp (commonly referred to as the testa layer) of the grain. These varieties are variously referred to as: tannin, high-tannin, brown, bird-proof, bird-resistant, or bitter sorghums.

Varieties of sorghum not containing tannins are variously referred to as: non-tannin, low-tannin, condensed tannin-free, or sweet sorghums.

In this Standard the term “tannin sorghum” shall be used for those sorghums containing tannins and the term “non-tannin sorghum” used for those sorghums not containing tannins.

3. Principle

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.

4. Reagent

4.1 Bleaching reagent

Five g sodium hydroxide is dissolved in 100 ml of 3.5% sodium hypochlorite solution (commercial bleach). Reagent can be stored at room temperature in light-proof bottle for up to one month.

4.2 Sorghum standards

An appropriate tannin and non-tannin standard.

5. Apparatus

Glass beakers (50 ml)

Tea strainer

Aluminum foil

Paper towel

6. Reference

Waniska, R.D., Hugo, L.F. & Rooney, L.W. 1992. Practical methods to determine the presence of tannins in sorghum. *Journal of Applied Poultry Research* 1:122-128.

7. Procedure

7.1

Test must be performed in duplicate

7.2

Known tannin sorghum and non-tannin sorghum standards must be included each time the test is performed.

7.3

One hundred whole, sound sorghum grains are placed in a beaker.

7.4

Bleaching reagent is added to **just** cover the sorghum grains and close beaker with aluminum foil. Too much bleaching reagent will cause over bleaching and give false negative results. If in doubt repeat using less reagent.

7.5

Incubate beaker at room temperature (20-30°C) for 20 minutes, swirling contents of beaker every 5 minutes.

7.6

Empty contents of beaker into tea strainer, discarding bleaching reagent. Rinse sorghum grains in tea strainer with tap water.

7.7

Empty contents of tea strainer onto sheet of paper towel. Spread grains out into a single layer and gently blot them dry with another piece of paper towel.

7.8

Count tannin sorghum grains. Tannin sorghum grains are those grains that are **black over the entire surface of the grain**, with the exception of the where the germ is which is somewhat lighter in colour. Non-tannin sorghum grains are those which are either completely white, **or** are brown over **part** of the surface of the grain.

8. Presentation of results

8.1

Calculate tannin sorghum grains as percentage of total sorghum grains. Duplicate determinations should not differ by more than +/- 5 grains, for example first determination 90%, second determination 85%, or 95%. The mean of the duplicate determinations should be calculated.

8.2

Expression of results

Results should be expressed as:

Percentage tannin sorghum, e.g. 90% tannin sorghum

9. Recommended standards

It is recommended that:

Batches containing $\geq 95\%$ tannin or non-tannin sorghum be classified as Tannin or Non-tannin Sorghum, respectively

Where batches contain $< 95\%$ tannin (or non-tannin) sorghum and $> 5\%$ non-tannin (or tannin) sorghum, the batch be classified as Mixed Tannin and Non-tannin Sorghum and that the percentage tannin sorghum be given.

NOTES

1. A 5 ml medicine measuring spoonful may be used to measure out approx. 5 g of sodium hydroxide if a weighing balance is not available
2. Commercial caustic soda, sometimes marketed as drain cleaner, may be used
3. Measure using for example a 200 ml 'Buddy' soft drink bottle (after use wash out with water and then crush bottle before disposal) and use a 2 x 5 ml medicine spoon measuring spoonfuls of caustic soda.
4. Any clear glass or plastic beaker or container with a diameter of around 3 cm.

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Chairperson: Prof J R N Taylor, University of Pretoria, South Africa, jtaylor@postino.up.ac.za

2. Method: Classification of Sorghum Grain According to Colour

1. Scope

Applicable to whole grain sorghum.

2. Definitions

Sorghum grain colour is the overall visual perception of the colour of the grain as viewed with the naked eye, where the colour results from a combination of intrinsic factors, principally: pericarp colour, the presence or absence of a pigmented testa, endosperm colour.

Sorghum grain colour is important with regard to end-use, in particular for milling to produce meal for porridge making and for malting for use in opaque beer brewing.

3. Principle

Sorghum grains are viewed with the naked eye.

Sorghum grains are classified as being either “white” or “coloured”.

4. Apparatus

Sheets of white (A4) paper

5. Reference

Rooney, L.W. & Miller, F.R. 1982. Variation in the structure and kernel characteristics of sorghum. in: Mertin, J.V. (ed.). Proceedings of the International Symposium on Sorghum Grain Quality. ICRISAT, Patancheru, India, pp. 143-162.

6. Procedure

6.1

Test must be performed in duplicate.

6.2

Known white and coloured sorghum standards must be included each time the test is performed.

6.3

Count out 100 intact sorghum grains without glumes and spread evenly over the surface of the sheet of white paper so that none of the grains are touching each other.

6.4

Examine the grains and count the number of “white” or “coloured” grains, whichever is the least.

A “white” grain is coloured white all over its surface, irrespective of whether the grain is: “weathered” i.e. shows signs of mould on its surface, and/or has purplish anthocyanic blotches on its surface.

A “coloured” grain is coloured yellow, pink, red, brown, or purple (or combinations of these colours) all over its surface.

7. Presentation of results

7.1

Calculation

Calculate white (or coloured) sorghum grains as percentage of total sorghum grains. Duplicate determinations should not differ by more than +/- 5 grains, for example first determination 90%, second determination 85%, or 95%.

The mean of the duplicate determinations should be calculated as a whole number.

7.2

Expression of results

Results should be expressed as:

Percentage white (or coloured) sorghum, e.g. 90% White Sorghum

8. Recommended standards

It is recommended that:

Batches containing $\geq 95\%$ white (or coloured) sorghum be classified as White (or Coloured) Sorghum.

Where batches contain $< 95\%$ white (or coloured) sorghum and $> 5\%$ coloured (or white) sorghum, the batch be classified as Mixed White and Coloured Sorghum and that the percentage coloured sorghum be given.

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3.

Method:

Estimation of Sorghum Grain Endosperm Texture (revised 12/2007)

1. Scope

Applicable to whole grain sorghum.

2. Definitions

Sorghum grain endosperm texture is defined in terms of the proportion of corneous (horny/glassy/vitreous/steely) endosperm relative to floury (mealy/chalky/opaque) endosperm in the grain. Grains with a high proportion of corneous endosperm tend to be more resistant to breakage during decortication (dehulling) and milling than grains with a high proportion of floury endosperm.

Resistance of the grain to breakage is often referred to as grain strength or hardness.

Sorghum grain endosperm texture is of importance as “hard” grains tend to yield proportionally more clean (uncontaminated with bran) endosperm of large particle size during milling operations than “soft” grains.

“Hard” grains are also more resistant to insect and mould damage than “soft” grains.

3. Principle

Sorghum grains are cut into halves longitudinally.

One half is viewed with the naked eye and the relative proportion of corneous endosperm to floury endosperm is determined by reference to a standard.

On the basis of the relative proportion of corneous to flour endosperm, grains are classified into: corneous, intermediate and floury.

4. Apparatus

4.1

Sharp disposable scalpel or sharp single-edged razor blade

4.2

Blunt ended forceps

4.3

Magnifying glass

4.4

Sheets of (A4) paper, preferably dark coloured

4.5

Rubbery gum used to attach posters to walls (for example Prestik)

5. References

Chandrashekar, A. & Mazhar, H. 1999. Mini Review: The biochemical basis and implications of grain strength in sorghum and maize. *Journal of Cereal Science* 30 : 193-207.

Rooney, L.W. & Miller, F.R. 1982. Variation in the structure and kernel characteristics of sorghum. in: Mertin J.V. (ed.). *Proceedings of the International Symposium on Sorghum Grain Quality*. ICRISAT, Patancheru, India, pp. 143-162,

6. Procedure

6.1

Test must be performed in duplicate.

6.2

Press a small piece of “rubbery gum” (approximately the same size as a sorghum kernel onto the cutting surface (approximately 5 sheets of white paper). Push a sound sorghum grain, germ side up, into the side of the piece of “rubbery gum” to hold it in place. The germ side has a circular indentation at the end of grain.

6.3

Hold grain with forceps and cut the grain in two lengthwise, to produce two even size halves, so that each half contains an equal portion of the germ.

6.4

Repeat until 20 grains have been cut.

6.5

Compare one half of each grain against the illustration (Figure below) and classify it as:

Corneous – the endosperm is totally corneous (translucent) or most (>50%) of the endosperm is translucent

Intermediate – the outer, corneous endosperm is continuous, but comprises less than 50% of the total endosperm; the inner part of the endosperm being floury (having a chalky appearance)

Floury – the endosperm is totally floury or the outer, corneous endosperm is very narrow and incomplete.

7. Presentation of results

7.1 Calculation

Calculate the number of corneous, intermediate and floury grains as a percentage of total sorghum grains. Duplicate determinations should not differ by more than +/- 1 grain in each class.

The mean of the duplicate determinations should be calculated as a whole number.

7.2 Expression of results

Results should be expressed as:

Percentage of corneous, intermediate and floury sorghum grains, e.g.

| Grain endosperm texture | Corneous (%) | Intermediate (%) | Floury (%) |
|-------------------------|--------------|------------------|------------|
| Sample X | 85 | 10 | 5 |

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8. Recommended standards

It is recommended that:

Batches containing $\geq 90\%$ corneous (or intermediate or flourey) sorghum grains be classified as Corneous (or Intermediate or Flourey) Endosperm Texture Sorghum

Batches containing 100% of only corneous and intermediate sorghum grains should be classified as Intermediate Endosperm Texture Sorghum

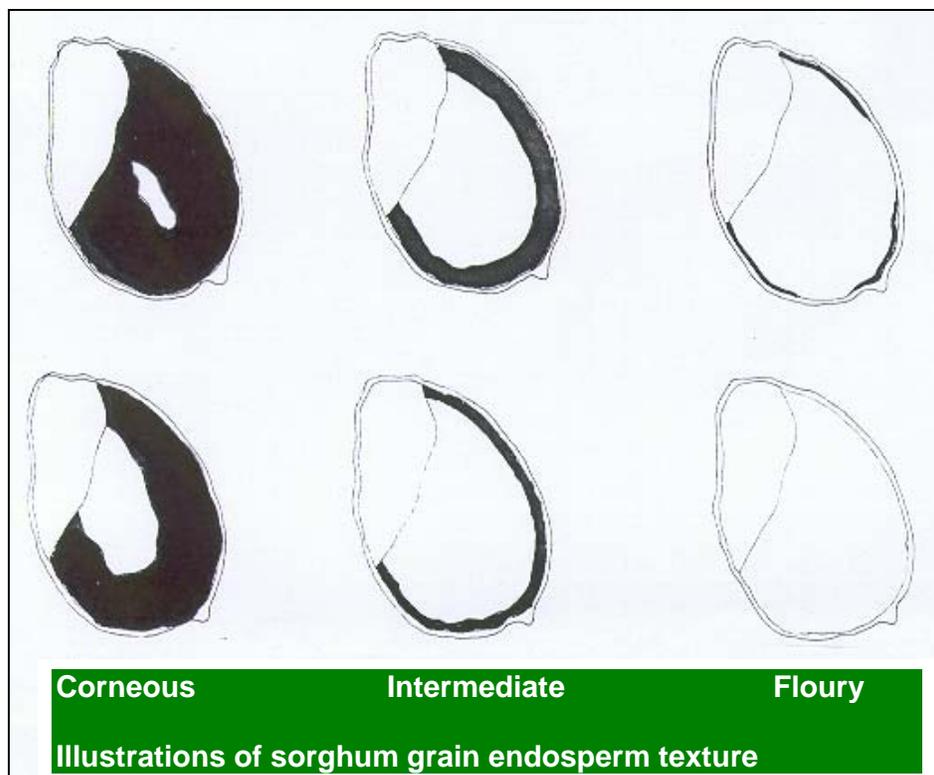
Batches containing $>10\%$ and $< 90\%$ flourey, or intermediate or corneous sorghum grains should be classified as Mixed Endosperm Texture Sorghum and the percentages of Corneous, Intermediate and Flourey Sorghum must be given.

NOTES:

A very sharp, narrow bladed knife may be used

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4.

Method:

Determination of Germinative Energy of Sorghum Grain (revised January 2007)

1. Scope

Applicable to whole grain sorghum.

2. Definitions

To produce sorghum malt, it is necessary that a high proportion of sorghum grains in a batch germinate.

Germinative Energy is the percentage of grains which can be expected to germinate if the batch is malted normally at the time of the test.

3. Principle

Sorghum grains are placed on damp filter paper in closed petri dishes and allowed to germinate at a set temperature for set periods of time.

The percentage of grains that have germinated at the end of each period is calculated.

4. Apparatus

4.1

Incubator set at 25°C and 100% relative humidity

4.2

Petri dishes (glass or plastic) 10 cm diameter with lids

4.3

Filter paper (Whatman No. 1) 9 cm diameter

4.4

Graduated pipette with 4 ml measure

4.5

Distilled water

5. Reference

European Brewery Convention. 1987. Method 2.6. Germinative Energy. in: Analytica-EBC, 4th Ed. Brauerei- und Getränke-Rundschau, Zurich, E 19-20.

6. Procedure

6.1

Test must be performed in duplicate.

6.2

Moisten the filter paper with 4 ml of distilled water.

6.3

Place two filter paper circles into the bottom of the petri dish.

6.4

Count out 100 intact sorghum grains and spread evenly over the surface of the moistened filter paper so that none of the grains are touching each other. Close the petri dish.

6.5

Place the filled petri dishes in the incubator.

6.6

After 24, 48 and 72 hours, the grains are examined. At each time interval, the germinated grains are counted and removed from the petri dish. Germinated grains are grains where the root has penetrated the pericarp, i.e. the grain has chitted.

7. Presentation of results

7.1 Calculation

At each time interval calculate the percentage germinated grains. Duplicate determinations should not differ by more than +/- 5 grains, for example first determination 95%, second determination 90%, or 100%.

Germinative Energy is the mean of the duplicate determinations, expressed as a whole number.

7.2 Expression of results

Results should be expressed as:

Germinative Energy (%) 24 hours, 48 hours, 72 hours, e.g.

| Germinative Energy | 24 hours (%) | 48 hours (%) | 72 hours (%) |
|--------------------|--------------|--------------|--------------|
| Sample X | 84 | 92 | 95 |

8. Recommended standard

It is recommended that sorghum grain for malting should have a Germinative Energy at 72 hours of $\geq 90\%$.

NOTES

1. A polystyrene box with close-fitting lid may be used. Incubation may be carried out at ambient temperature (20-30°C). High relative humidity is maintained in the box by placing two layers of thin cotton dishcloth saturated with water at the bottom of the box covering the entire surface area; and placing two layers of thin cotton dish cloth saturated with water covering the petri dishes. The cloths must be re-saturated with water each day of the test.
2. Any type of dish of similar diameter, such as a plastic lid, may be used. The dish may be covered with aluminum foil to close it.
3. Circles of newspaper (black printing on only) of diameter the same size as the internal diameter of the smaller of the dishes may be used.
4. When using newspaper circles, 5 ml of water should be used, which may be measured out using a 5 ml medicine measure, see also note 6.
5. Tap water may be used, but there may be greater variability between results of different operators.
6. Where newspaper and 5 ml of water are used, the number of circles of newspaper to be put in the dish has to be established prior to the test, since the thickness of newspaper is variable. The newspaper circles must be saturated with water, but there must be no free water on the surface, i.e. if for example it is found that after adding the 5 ml of water to two circles of newspaper there is still free water on the surface, additional circles must be added one at a time until such a number has been added that there is no free water.

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Chit

**Chitted (germinated) sorghum grain (left)
and unchitted (ungerminated grain (right)**

5.

Method:

Determination of Total Defects in Sorghum Grain (revised January 2007)

1. Scope

This method is applicable to determination of total defects in consignments of whole grain sorghum intended for human consumption.

2. Definitions

The term total defects applies to all components of a sorghum sample which differ from the normal basic variety, including extraneous matter, filth, blemished grains, diseased grains, broken kernels and other grains.

2.1 Extraneous matter

All organic and inorganic material other than sorghum, broken kernels, other grains and filth. Extraneous matter includes loose sorghum seedcoats.

2.2 Filth

Impurities of animal origin including dead insects.

2.3 Blemished grains

2.3.1 Grains which are insect or vermin damaged, of abnormal colour, sprouted, diseased, or otherwise materially damaged.

2.3.2 Diseased grains – grains made unsafe for human consumption due to decay, moulding, or bacterial decomposition, or other causes that may be noticed without having to cut the grains open to examine them.

2.3.3 Insect or vermin damaged grains – Kernels with obvious weevil-bored holes or which have evidence of boring or tunneling, indicating the presence of insects, insect webbing or insect refuse, or degermed grains, chewed in more than one part of the kernel which exhibit evident traces of an attack by vermin.

2.3.4 Grains having an abnormal colour – Grains whose natural colour has been modified by bad weather conditions, contact with the grain, heat, and excessive respiration. These grains may be dull, shriveled, swollen, puffed, or bloated in appearance.

2.3.5 Sprouted grains – Grains exhibiting obvious signs of sprouting.

2.3.6 Frost damaged grains – Grains which are damaged by frost and may appear bleached or blistered and the seed coat may be peeling. Germs may appear dead or discoloured.

2.4 Broken kernels

Sorghum and pieces of sorghum which pass through a 1.8 mm round-hole sieve.

2.5 Other grains

Edible grains, whole or identifiable broken, other than sorghum (i.e. legumes, pulses and other edible cereals).

3. Principle

The principle of the method is to separate all defects, defined under 3, from the normal basic grains by manual selection.

4. Reagents

No reagents are required for this determination.

5. Apparatus

5.1

Balance (precision 0.1 g)

5.2

Sheet of cardboard approximately A4 size on which is drawn a 20 x 20 cm square grid divided into 400 x 1 cm square blocks with a 10 x10 cm square insert marked in the corner of the larger square (Figure). If determination is to be carried out routinely, it is recommended that the square should be drawn on A4 sized piece of wood, metal or plastic, or on paper and then laminated.

5.3

Thin object with straight flat edge (for example 15 cm ruler)

6. References

Codex Alimentarius. 1995. Codex standard for sorghum grain. Codex Stan 172-1989 (Rev. 1-1995). in: Codex Alimentarius, Volume 7, Cereals, Pulses, Legumes and Derived Products and Vegetable Proteins. FAO/WHO, Rome, pp 37-42.

6. Association for Cereal Chemistry 1972. Determination of besatz of wheat. ICC Standard No. 102/1. In: ICC Standard Methods. International Association for Cereal Science and Technology, Schwachet, Austria.

7. Sampling

According to ICC Standard 101/1

8. Procedure

8.1

Test must be performed in duplicate.

8.2

Weigh out an average (final) sample of 25.0 g and empty sample onto A4 sheet.

8.3

Spread sample into a monolayer on the 10 x 10 cm square insert. The sample should approximately fill the square.

8.4

With the aid of the ruler move all defects described out of the 10 x 10 cm square insert.

8.5

When the entire sample has been carefully and completely sorted through and all defects have been moved out of the 10 cm square insert, collect all the defects and weigh the defects to one decimal place.

Alternatively, collect all the defects and with the aid of the ruler systematically fill the 1 cm square blocks with a monolayer of defects. There must be no space between the defects. Count the number of 1cm squares of defects. If there is a square that contains less than 1 square cm of defects it should be counted as a full square.

9. Presentation of results

9.1 Calculation

Express total defects as percentage by weight of the sample.

If defects have been estimated by area, the following formula should be used to convert the number of squares of total defects into percentage total defects.

% Total defects = number squares of defects x 0.5

Duplicate determinations should not differ by more than +/- 2 squares, for example first determination 10 squares (5%), second determination 8 squares (4%), or 12 squares (6%).

The mean of the duplicate determinations should be calculated.

9.2 Expression of results

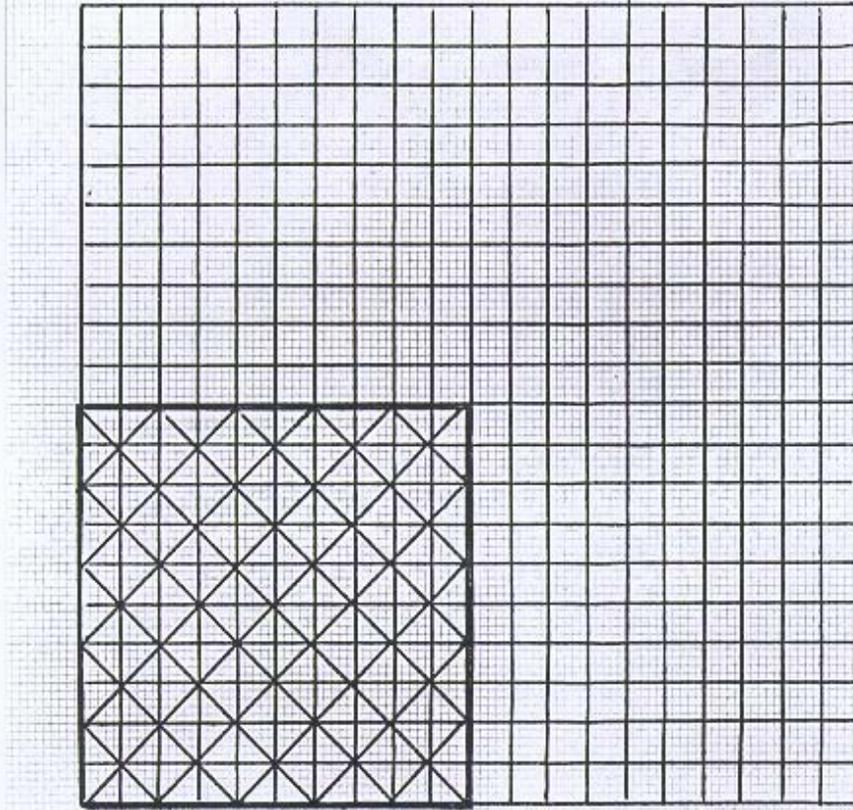
Results should be expressed as:

Percentage total defects of sorghum grain, e.g. 5%

10. Recommended standard

It is recommended that the maximum permissible total defects in sorghum grain for human consumption should not exceed 8%, as specified by Codex Alimentarius.

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20 x 20 cm square grid for sorting total defects from sound whole sorghum grains, with a 10 x 10 cm insert for checking sample size.