Laboratory Standard Operating Procedure

SOP for Determination of Starch & Sugar through Acid Hydrolysis

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Ethics: The activities, which led to the production of this manual, were assessed and approved by the CIRAD Ethics Committee (H2020 ethics self-assessment procedure). When relevant, samples were prepared according to good hygiene and manufacturing practices. When external participants were involved in an activity, they were priorly informed about the objective of the activity and explained that their participation was entirely voluntary, that they could stop the interview at any point and that their responses would be anonymous and securely stored by the research team for research purposes. Written consent (signature) was systematically sought from sensory panelists and from consumers participating in activities.

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RTBfoods

WP2: Biophysical characterization of quality traits

SOP: Determination of starch and Sugar through acid hydrolysis

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1 SCOPE AND APPLICATION

This method seeks to determine the Starch and Sugar content of starchy RTB food samples.

2 REFERENCES

The following references were used in the method:

Dubois M., Gilles, K.A Hamilton, J.K, Rebers, P.A. and Smith, F.(1956). Colorimetric method for Determination of Sugars and related substances. Anal. Chem. 28: 350-356.

McCready, R.M. (1970). Determination of starch and dextrins. In Methods in Food Analysis 2nd Ed. A series of monographs ed. By Joslyn A.M. Academic press N.Y. 522-557

BeMillerJ.N., Huber, J.K. (2017) Carbohydrates (Chap 4) In: Damodaran, S, Parkin, K.L., Fennema, O.R (eds) Food Chemistry, 5th edn. Marcel Dekker, New York

Nielsen, S.S. (2017). Carbohydrate analysis In: Food Analysis (Chap 19) (5th edn) Springer , Switzerland

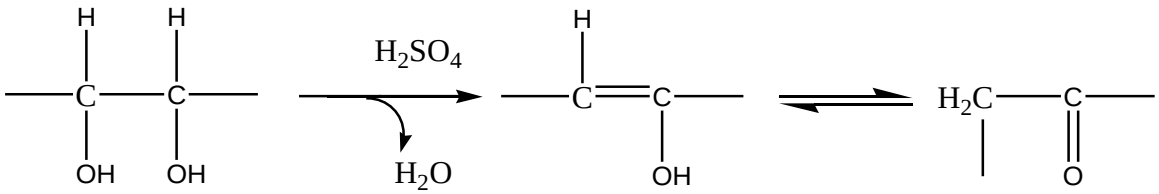
3 DEFINITIONS

Starch is a major structure forming food hydrocolloid that occurs in granules within plant cells. It is essentially a mixture of linear (amylose) and branched (amylopectin) α-glucans. Starch content of most foods cannot be determined directly because it is contained within a structurally and chemically complex food matrix. It is therefore necessary to isolate starch from the other components present in the food matrix prior to carrying out a starch analysis. Food samples are normally dried, ground and then dispersed in hot ethanol solutions. The monosaccharides and oligosaccharides are soluble in the ethanol solution, while the starch is insoluble. Hence, the starch can be separated from the sugars by filtering or centrifuging the solution. In acid hydrolysis methods, the starch is hydrolysed to glucose which is subsequently assayed.

4 PRINCIPLE

Dried homogenous food samples are dispersed in hot Ethanol solutions. The monosaccharides and oligosaccharides in the sample are soluble in the ethanol solution, while the starch is insoluble. Starch in the sample is then separated from the sugars by filtering and centrifuging the solution. The supernatant is used for Sugar analysis while the sediment is used for Starch analysis. In this method, hydrolysis of starch to glucose is achieved using a strong acid like perchloric acid. In the presence of this acid the glycosidic bond between monosaccharide residues in a polysaccharide is cleaved. During this reaction, one molecule of water is consumed for every glycosidic linkage cleaved. The hydrolysed saccharides are then dehydrated by sulphuric acid to furfural or furan derivatives (such as furanaldehyde and hydroxymethyl furaldehyde) (Figure 1). These furan derivatives then condense with themselves or phenolic compounds such as phenol, resorcinol, orcinol, α-naphthol, and napthoresorcinol to form coloured compounds that absorb UV-VI light. The absorbance is

proportional to the sugar concentration in a linear fashion. An absorbance maximum is observed at 490 nm for hexoses and 480 nm for pentoses and uronic acids. D-glucose in the presence of strong acids (concentrated Sulphuric acid) is dehydrated to produce furan derivatives 5-hydroxymethylfurfural (HMF). The furan derivatives then condense with Phenol to form yellow-gold or yellow-orange compounds. The absorbance of these yellow to gold or yellow to orange compounds is measured with a spectrophotometer at a wavelength of 490 nm (absorption peaks of glucose, fructose and galactose is at 490 nm). This method is non-stoichiometric; therefore, it is necessary to prepare a Standard calibration curve using glucose as a standard. The starch is converted to glucose by multiplying with 0.9. This calculation accounts for the removal of one molecule of water for each molecule of glucose during the covalent bonding of glucose molecules to form starch.



Sugar **Furan derivative**

Figure 1. Dehydration reaction of Sugars with Sulphuric acid

5 REAGENTS

- 95% Ethanol:
    - (To prepare 100 ml of 95% of ethanol, Check the Purity of the Ethanol from the bottle e.g if the purity is 99.7%, then 100 ml of 95%Ethanol will be: 95% divided by Percent purity (99.7%) multiplied by 100ml:
      95% X 100= 95.28
      99.7%
      Measure 95.28ml of 99.7% ethanol and make up to 100 ml with distilled water
- 5% Phenol: To 5g of Phenol add 100 ml of distilled water)
- Concentrated Perchloric acid
- Concentrated H_2SO_4 (> 95 % min)

6 APPARATUS

- High precision (0.01 mg) balance
- Centrifuge
- Centrifuge tubes
- Test tubes
- Dispenser (To dispense the Acid, Phenol)
- Spectrophotometer.

7 PROCEDURE

(A) Glucose Standard Calibration curve

1. Weigh 10 mg of D-Glucose into a 100ml Volumetric flask and make up to 100 ml mark with distilled water
2. Pipette 0, 0.1, 0.2, 0.3 or 0.4 ml of glucose solution into test tubes coded as 0, 1, 2, 3 and 4. Make up to 1.0 ml with distilled water. This will correspond to 0 (Blank), 10, 20, 30 and 40 µg glucose/ml.
3. Add 0.5ml of phenol, followed by the addition of 2.5 ml concentrated sulphuric acid to each test tube
4. The solution should be mixed thoroughly by vortexing, and allowed to cool to room temperature for 10 minutes
5. Read absorbance at 490 nm on the spectrophotometer and construct a calibration standard curve of absorbance against glucose concentration from which the proportion (%) sugar and starch in the sample will be calculated.

(B) Sample analysis

1. Weigh 20-25 mg of milled sample (see specific sampling/preparation SOP) into a centrifuge tube
2. Add 1ml of ethanol and 2.0 ml of distilled water to wet sample
3. Add 10 ml of hot ethanol (Put the ethanol in a boiling flask or beaker, heat the ethanol on a hot plate till it boils) then add to the sample in the centrifuge tube (No 1 above), mix thoroughly by vortexing and centrifuge at 2000*g*for ten minutes.
4. Decant the supernatant into a boiling tube. This is the sugar extract; this will be used for sugar analysis.
5. To the sediment add 7.5 ml of Perchloric acid and leave for one hour at room temperature (for starch hydrolysis)

(C) Sugar analysis

1. To the sugar extract from point 4. of section (B) above, add 9 ml of distilled water and mix thoroughly using a vortex mixer
2. Pipette 0.2 ml of this extract and make up to 1 ml with distilled water
3. Add 0.5 ml of Phenol (vortex properly)
4. Add 2.5 ml con H_2SO_4 (vortex properly)
5. Leave to cool to room temperature for 10 minutes (The samples can be kept in the fume hood or cool with ice water or leave at room temperature)
6. Vortex the tubes again and read the absorbance at 490 nm.

(D) Starch analysis

a. Filter the sediment in point 5. of section (B) above using a whatman no 4-filter paper
b. Add 17.5 ml of distilled water and vortex thoroughly
c. Pipette 1ml of extract and mix with 1ml of distilled water
d. From the extract in (c) pipette 0.05ml for assay and make up to 1 ml with distilled water (that is 0.95ml of distilled water will be added)
e. Add 0.5 ml of Phenol (vortex properly)
f. Add 2.5 ml con H_2SO_4 (vortex properly)
g. Leave to cool to room temperature for 10 min
h. Vortex the tubes again and read the absorbance at 490 nm.

8 METHOD OF CALCULATION AND FORMULAE

8.1 Calculation

$$Sugar\ (\%, wet\ basis) = 100 * \frac{(A - I) * DF1 * V1}{B * W * 10^6}$$

$$Starch\ (\%, wet\ basis) = 100 * \frac{(A - I) * DF2 * V2 * 0.9}{B * W * 10^6}$$

A= Absorbance of sample

I =Intercept of standard curve

D.Fi = Dilution factor based on aliquot of sample extract taken for assay.

Vi = Total extract volume (mL).

B = Slope of the standard curve (mL/µg).

W = Sample weight (mg).

8.2 Repeatability

The colour of the reaction is stable for several hours and it is advisable that the sample be weighed in duplicates and the duplicates be replicated twice so that in total we have 4 replicates; the accuracy of the method is within ± 2% under proper conditions.

9 CRITICAL POINTS OR NOTE ON THE PROCEDURE

- it is important for the sample to be homogenous
- Mix coloured complex very well to get a reproducible absorbance
- Allow samples to cool to room temperature before measuring absorbance
- Caution: Perchloric acid and concentrated sulphuric acid are very corrosive

10 TEST REPORT

The test report shall indicate the method used and the results obtained. In addition, it shall mention all operating conditions not specified in the international procedure, or regarded as optional, as well as any circumstances that may have influenced the results.

The test report shall include all details necessary for the complete identification and preparation of the sample.

11 REVISION RECORD

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