Subject: N352014
Work: No.3 – Starch
Lab: Starch Technology (B47)
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A) The Determination of moisture content in powdery samples by moisture analyser

Task: Determine the dry matter content in the sample of wheat flour using the moisture analyzer.

The principle of method:

The method is based on the thermogravimetric determination of moisture content. The instrument includes two quartz halogen lamps, each rated at 200 W. The drying temperature can be set between 40 to 250 ° C, step 1 °C. The program of the drying to constant weight is fully automatic.

Procedure:

Weigh out 2 g of sample to the aluminum dish on the moisture analyser. Close the lid and press the button "**START →**". After 10 or 15 minutes the symbol + and content of dry matter are lighting on the display only. Dry matter content determine twice.

B) The Determination of wet gluten quantity and quality gluten index according to Perten

Task:
Determine the wet gluten quality, gluten index and the dry gluten quality in flour sample.

The principle of method:

This method is based on the Glutomatic Gluten Washer and the Gluten Index Centrifuge and provides information on both quantity and quality of wet gluten.
Wet gluten in wheat flour is a plastic-elastic substance consisting of the proteins gliadin and glutenin, obtained after washing out the starch from wheat flour dough.
Gluten separated from whole wheat meal or wheat flour by the Glutomatic is centrifuged to force wet gluten through a specially constructed sieve under standardized conditions. The special sieve allows the collection of both parts of gluten, the gluten remains on the sieve and the part which passes through the sieve. The total weight of the gluten is defined as gluten quantity. The percentage of wet gluten remaining on the sieve after centrifugation is defined as the Gluten Index. When gluten is very weak, all gluten may pass through the sieve, then the Gluten Index is 0. When no substance passes through the sieve, the Gluten Index is 100.

Procedure for flour sample:

1 Assemble the Glutomatic wash chamber with the fine 88 micron polyester sieve. Centre

the sieve over the unmarked sieve holder and press the plastic chamber firmly over the sieve. Use the white plastic block. Turn the plastic chamber to fasten the sieve. Stretch the sieve by pulling carefully at the edges if necessary.

2 Weigh out 10 ± 0.01g (w) wheat flour and transfer it into the test chamber. Shake the wash chamber gently so as to spread the flour out evenly.

3 Add 4.8 ml of the 2 % sodium chloride solution from the dispenser. Hold the chamber at a slight angle and direct the water stream from the dispenser against the plastic chamber side wall, so that the water stream does not go directly through the sieve. Shake the test chamber gently so that the water is spread evenly over the flour.

6 Place the wash chamber with the flour and the mixing water into the working position and fix it in the bayonet fastening.

7 Press the green START button. The mixing/washing sequences are proceeding automatically now.

8 When the washing cycle in the Glutomatic is completed, put the gluten ball gently into the sieve cassette. Do not divide the gluten in parts but put a gluten sample in each cassette.

9 Close the cover, and press the GREEN start button. The centrifuge will start and run for one minute at a speed of 6.000 rpm and stop automatically

10 After centrifugation, remove the Gluten Index sieve cassette. Check that no gluten remains in the centrifuge. Using the stainless steel spatula, carefully scrape off all gluten, which has passed through the sieve.

11 pull out all gluten remaining on the sieve using tweezers. Weigh this portion of gluten and record the result (m_1). Add the gluten pass through the sieve to the balance to achieve total gluten weight (m_G).

12 Open the Glutork and place the wet gluten in the centre of the bottom plate. Close the Glutork. Press the red push button after 4 minutes the red push button light goes off and indicates the end of the drying cycle.

13 Open the Glutork, remove and weigh the dry gluten (m_{DG}). Close the Glutork again. The gluten index (GI) wet gluten quantity (WG) and dry gluten quantity (DG) is calculated from equation 1, 2, 3.

Calculation:

$$GI = \frac{100 \times m_1}{m_G} \qquad (1)$$

$$WG = \frac{100 \times m_G}{w} \qquad (2)$$

$$DG = \frac{100 \times m_{DG}}{w} \qquad (3)$$

GI gluten index [1]
m_1 weight of gluten remaining on the sieve [g]
m_G total gluten weight [g]
WG wet gluten quantity [%]
w the flour sample weight [g]

DG dry gluten quantity [%]
m_{DG} dry gluten weigh [g]

C) The Starch determination by Ewers method

Task: Determine starch content in wheat flour by Ewers method.

The principle of method:

Ewers polarimetric method is currently used as the official method for the measurement of starch purity. Ewers method also commonly applies for the starch determination in cereals and tubers. The method is based on the specific rotation of polarized light of the starch dissolved in acid solution. The starch solution is clarified using Carrez solution according the equation:

$K_4Fe(CN)_6 + 2\ ZnSO_4 = 2\ K_2SO_4 + Zn_2Fe(CN)_6$

Carrez I: 30 % zinc sulfate solution
Carrez II: 15 % potassium ferrocyanide solution

Procedure:

A known weight of starch 2 g was treated with hydrochloric acid (1.124%, 50 ml) in a 100 ml volumetric flask with constant shaking to prevent the formation of lumps. When the starch was well soaked the flask immersed in a boiling water bath at 100°C for 15 min with constant shaking to prevent gelation of the starch. The flasks were cooled rapidly to 20°C and distilled water (30 ml) added. Each solution was then treated with Carrez I solution (1 ml) followed by Carrez II solution (1 ml) and made up to a final volume of 100 ml with distilled water. These aqueous suspensions were then filtered and the angle of rotation of plane polarized light of the filtrates recorded on a Anthon Paar MCP 500 polarimeter at the D-line of sodium using a cell of 200 cm path length. Calculate starch content in percent using equation 4.

$$x = \frac{5 \times p \times f}{w_1} \qquad (4)$$

x starch content [%]
p polarization [°Z]
f starch factor (wheat starch *f* = 1.898)
w_1 wheat flour sample weight [g]

The obtained results should be converted into the dry matter of the original sample. Use the dry matter content determine by the moisture analyzer.

D) MEASUREMENT OF THE STARCH GRANULES PARTICLE SIZE DISTRIBUTION USING IMAGE ANALYSIS

Task:

Use the image analysis system NIS Elements V.2.3 for measurement of particle size distribution for 3-4 samples of starch and create the frequency diagram (i.e. histogram).

Method principle:

Measure samples of starch suspension in distilled water by the image analysis. To achieve optimal image, it is necessary to stain starch granules with iodine tincture and add glycerol to prevent starch migration caused by Brownian motion. Equivalent diameter is the measured and evaluated parameter.

Sample preparation:

1) Prepare a suspension of starch in cold distilled water; weigh 0.50 grams of starch, add 5 ml of distilled water and stir for 1 minute using the magnetic stirrer.
2) Drop the suspension on a slide, add 1 drop of iodine solution. Drain an excessive solution using filtration paper, then add a drop of glycerol and cover with a coverslip.
3) Place the prepared slide in the image analyses system NIS Elements V.2.3 and measure.

Measurement:

1) Adjust the brightness and sharpness. You can see how to change **contrast,** and **define threshold**, which mark all scanned particles with a chosen colour.
2) Scan the image for digital processing by the software NIS Elements V.2.3.
3) Remove improper objects and separate connected particles.
4) Commands **SCAN OBJECT** and **OBJECT DATA** in the menu **MEASURE** provide the measurement and complete the table with results.
5) Run the macro, separate objects (connected particles) and remove improper objects.
6) Export the frequency diagrams, and the tables of measured values into MS Excel.
7) In the first column, the table shows intervals of equivalent diameters (calculated from area of measured particles) and the numbers of particles of a certain equivalent diameter (frequencies) in the second column.
8) Carry out further processing in MS Excel. Create the graphs where equivalent diameters are on the x-axes and volume fractions on the y-axis.
9) Work-out a written record of the measurement.

Data evaluation:

A volume of particle *i*:

$$V_{ekv,i} = (\pi\ (d_{ekv,i})^3)/6 \qquad (5)$$

Volume ratio:

$$x_V = (f_i \,.\, V_{ekv,i} \,.\, 100)/(\ \Sigma(\ f_i \,.\, V_{ekv,i})) \qquad (6)$$

Abbreviation used:

$d_{ekv,i}$	equivalent diameter of the particle *i* [μm]
$V_{ekv,i}$	volume of sphere of given equivalent diameter $d_{ekv,i}$ [μm]
x_V	volume ratio [%]
f_i	number of particles of the chosen equivalent diameter $d_{ekv,i}$ [1]