IODINE AFFINITY

PRINCIPLE

Iodine complexes preferentially with the amylose (linear fraction) in corn starch. After defatting by solvent extraction, and drying, the sample is dispersed in alkali, and titrated with an iodine solution. The titration is monitored potentiometrically to determine the free or excess iodine when the end point is exceeded. Free iodine is plotted against bound iodine on a linear coordinate plot, and the iodine affinity (g of iodine bound by 100 g of dry, defatted sample) is deduced by extrapolation of the free iodine-bound iodine plot.

SCOPE

The method is applicable to all unmodified starches, starch fractions, and granular thin-boiling starches prepared by mild acid hydrolysis.

MEDIA AND REGEANTS

1. Heater: An electric hot plate is recommended having at least 300-watt capacity with continuously-variable regulation. Multi-unit extraction assemblies with rheostat-controlled electric heaters are available commercially.

2. Extraction Apparatus: This consists of a Butt-type extractor (see sketch), with a 34/45 female joint at the upper end for attaching a Friedrichs- or Hopkins-type condenser, and a 24/40 male joint at the lower end for attaching a 125-mL Erlenmeyer flask (available from SGA Scientific, 770 North Church Road, Elmhurst, Illinois 60126, as JE-7650 Extraction Tube).

3. Extraction Shells: Paper, 80 x 22 mm (Note 1)

4. Potentiometer: A precision potentiometer (for example, a Leeds and Northrup type K-2 bridge with a sensitive galvanometer), or a suitable pH meter reading to ± 1.0 milli-volt.

5. Electrodes: a platinum electrode and calomel reference electrode for operating with the potentiometer

IODINE AFFINITY — continued

6. Bath: A water bath operating at a temperature of 30.0 ± 0.1 °C

7. Stirring Motor: A suitable motor fitted with a propeller-type glass stirrer and equipped with a rheostat to control speed.

REAGENTS

1. Methanol Solution, 85%: Dilute 850 mL of reagent grade, anhydrous methanol to 1-liter volume with purified water.

2. Stock Iodine Solution, 2.000 mg/mL: Dissolve 166 g of reagent grade potassium iodide (KI), exactly 4.000 g of resublimed iodine (I_2) and 74 g of reagent grade potassium chloride (KCI) in a small amount of water in a 2-liter volumetric flask. Dilute to volume when the iodine has dissolved completely, and mix. Store in amber (low actinic glass) Pyrex bottle.

3. Standard Iodine solution, 0.2000 mg/mL: Pipet 50 mL of stock iodine solution into a 500-mL volumetric flask, dilute to volume, and mix. Make fresh daily.

4. Potassium Iodine Solution, 0.5 *N*: dissolve 41.5 g of reagent grade potassium iodide (KI) in purified water and dilute to volume in a 500-mL volumetric flask and mix. Store in an amber (low actinic glass) Pyrex bottle away from light. Discard solution when any perceptible trace of yellow iodine color develops.

5. Potassium Hydroxide solution, 1 *N*: Standard

6. Hydrochloric Acid Solution, 1 *N*: Standard

7. Methyl Orange Indicator, 0.1%

SAFETY

Person(s) performing this method should wear appropriate protective equipment. Glassware should be carefully inspected for defects before use. Analysts should also review the Manufacturer's Safety Data Sheets (MSDS) for all of the

IODINE AFFINITY — continued

chemicals required, however this review is not a substitute for adequate laboratory safety training.

PROCEDURE

A. Defatting: Place 5-10 g of starch on a 15-cm filter paper, roll paper tightly around the sample, and place in a paper extraction shell (Note 1). Plug the top of the shell with glass wool and place shell in the extractor. Pour 50-75 mL of 85% methanol solution into the 125-mL Erlenmeyer flask, connect the extraction apparatus, and extract sample for 24 hours. At the end of the extraction, turn off the heater ad remove the wrapped sample from the extraction thimble with tongs (Note 2). Transfer the alcohol wet cake to a beaker of water and stir for 15 minutes. Filter the slurry by suction using a Buchner funnel, wash with purified water and dry the cake overnight in a 50°C oven. Remove from oven and allow to stand uncovered to the air for 6-8 hours to regain moisture. Determine moisture by an approved method.

B. Calibration of EMF Against Free Iodine: An accurate calibration chart must first be prepared to relate EMF readings with the amount of free iodine in solution, under conditions identical with those employed in the starch sample titration. For this purpose dissolve 373 mg of reagent grade potassium chloride and 830 mg of reagent grade potassium iodide in 100.0 mL of purified water in a 250-mL beaker. Place the beaker in the 30.0 °C bath, insert the electrodes, and stir the contents mechanically at 300 rpm with a glass propeller for 15 minutes.

   Add standard iodine solution, from a buret in small increments every two minutes and take potentiometer readings 5-10 seconds before each incremental addition of iodine. The range of 230 to 285 mv is covered in this manner. Since the solution and the iodine reagent are both 90.05 *N* with respect to potassium chloride and potassium iodide, there is no change in salt concentration during the titration.

   Two calibration charts, A and B, are prepared from the graphed data. Chart A shows mg of free iodine against mv readings. Chart B shows mg of free iodine per 100 mL of

IODINE AFFINITY — continued

solution $\left[\left(\frac{\text{Titer} \times 0.2 \text{ mg/mL}}{100 + \text{Titer}}\right) \times 100\right]$ against mv

readings, and provides an alternate method of calculating free iodine to account for volume dilution when high titration values are obtained. Both charts show mg of free iodine for 1 mv readings from 230 to 260 mv and for 0.5 mv. New calibration charts are prepared when either the potentiometer or the calomel electrode is changed or replaced.

C. Sample Preparation: Weigh, by difference (to ± 0.1 mg), an appropriate amount (Note 3) of defatted and dried starch into a 250-mL beaker previously tared to 0.1 g by means of a torsion balance. Then add 5.0 mL of 1.0 *N* potassium hydroxide solution to the sample. Disperse the sample by breaking up any lumps or clots with a stirring rod, and place in a refrigerator overnight. Neutralize to a methyl orange indicator end point with 0.5 *N* hydrochloric acid. After the addition of 10 mL of 0.5 *N* potassium iodide solution, remove the stirring rod and rinse into the beaker. Add sufficient water to give a total weight of 100.9 g over the weight of the empty beaker. Except for the dissolved starch, solution composition is identical with that used in the calibration.

D. Sample Titration: Place the above solution in the water bath and stir t 300 rpm for 15 minutes. Titrate the sample with standard iodine solution by adding the iodine incrementally at 2-minute intervals, balancing the potentiometer, and taking EMF readings 5-10 seconds before the next iodine addition. Determine the EMF at 1- to 15 points between 230 and 285 mv. For each point between 230 and 285 mv in the titration data, calculate: (a) mg of total iodine in solution, free iodine in solution from Chart A using the appropriate mv readings. The difference between total iodine added and the free iodine in solution represents bound iodine. All values are in mg.

To compensate for milliliters of titrant added, calculate: (a) mg of total iodine per 100 mL of solution, and (b) free iodine in 100 mL of solution from Chart B using the appropriate mv readings. The difference between these two values represents bound iodine.

IODINE AFFINITY — continued

CALCULATION

Plot free iodine versus bound iodine on linear graph paper. The upper linear portion of this curve is extrapolated to zero free iodine on the bound iodine axis, and from this amount of bound iodine, calculate the iodine affinity of the sample (Notes 4, 5, 6, 7).

$$\% \text{ Iodine Affinity} = \frac{\text{mg Bound Iodine at Zero Intercept}}{\text{Dry Sample Weight, mg}} \times 100$$

REFERENCES

1. F. L. Bates, D. French, and R. E. Rundle, *J. A. Chem. Soc., 65,* 142 (1943)

2. T. J. Schoch, "Methods in Carbohydrate Chemistry," R. L. Whistler, ed., Academic Press Inc., New York, N.Y., *IV,* (1964) 157

NOTES AND PRECAUTIONS

1. Other extraction shells such as alundum thimbles are suitable.

2. At no time should the extracted starch be handled with the fingers.

3. From 40 to 50 mg of amylose (linear fraction), 200 mg of amylopectin (branched fraction), or 100 mg of common starch are recommended as sample weights.

4. In practice, the graphical method is used to draw the best straight line through the data points. This procedure is useful as it gives a good overall view of the accuracy of the analysis. If 3 or 4 data points do not fall on the line, the analysis should be rerun.

   The best line through the data points may be calculated by regression analysis.

   Where: $y = a + bx$

   $$a = \text{zero intercept} = \frac{\Sigma y}{n}$$

IODINE AFFINITY — continued

$$b = \text{Slope} = \frac{\Sigma(y - \bar{y})\,(x - \bar{x})}{\Sigma(y - \bar{y})^2}, \text{ or}$$

$$= \frac{n\Sigma\Sigma x - \Sigma x \Sigma y}{n\Sigma\Sigma^2 - (\Sigma\Sigma x^2}$$

n = number of points

x = free iodine

y = bound iodine

$\bar{x}$ = average free iodine

$\bar{y}$ = average bound iodine

The iodine affinity value of amylose (linear fraction) isolated from corn and purified by recrystallization from n-butanol should be 19.0 ± 0.1%.

5. Duplicate iodine affinity analyses should agree within ± 0.08%

6. At a 5% iodine affinity level a change of 1.0 °C may affect the level of binding by as much as 0.05%.

7. Amylose content is calculated frequently using the following equation:

$$\%\,\text{Amylose} = \frac{\%\ \text{Iodine Affinity} \times 100}{19.0}$$