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AVAILABLE CARBOHYDRATES

ASSAY PROCEDURE

K-AVCHO 01/2021

(100 Assays per Kit)

AOAC Method 2020.07

Megazyme

INTRODUCTION:

The concept of 'Available Carbohydrates' was introduced by McCance and Widdowson[1] in association with their efforts to provide information on available carbohydrate (AVCHO) values of foods for diabetics. They defined food carbohydrates as 'available carbohydrates' (total starch, sucrose, glucose and fructose) and 'unavailable carbohydrates'. Methodology to measure these individual components was described by Southgate.[2] Over the decades, these values have been, and still are, extremely valuable in completing nutrition tables. However, as our knowledge of dietary fiber (DF) and available carbohydrates has expanded, new fiber components have been developed and a new (physiological based) definition of DF was announced by Codex Alimentarius in 2009.[3] Consequently, analytical methodology for DF has been updated (AOAC Methods 2009.01, 2011.25 & 2017.16), and in parallel, methodology for AVCHO needs to be updated. In particular, accurate methodology is required to measure digestible starch, sucrose and lactose (and isomaltose).

The terminologies, rapidly digestible starch (RDS), slowly digestible starch (SDS) and amylase resistant starch (RS) were introduced by Englyst *et al.*[4] in 1992 to reflect the rate of in *vivo* digestion of starch. Their work, and that of Wahlquist *et al.*[5], Jenkins *et al.*[6] and others showed that the physiological form of food and the nature of the starch are major determinants of the rate of digestion of the starch. At the same time it was also shown that "digestible" starch can be hydrolysed and absorbed at the same rate as simple sugars. Englyst *et al.*[4] considered that resistant starch was that starch which resisted digestion by pancreatic α-amylase (PAA) plus amyloglucosidase (AMG) over a 120 min incubation period at 37°C. However, literature reports indicate that the average time of residence of food in the human small intestine is 4 ± 1 h. Consequently, in the current work, to distinguish between starch that is digested and that which is not (RS),[7,8] an incubation time with PAA/AMG of 4 h is employed.[7,8] Starch that is digested over a 4 h period is termed total digestible starch (TDS), and this is part of the carbohydrate that is available for digestion and absorption in the human small intestine.

Traditionally, sucrose has been measured as glucose plus fructose, following hydrolysis of the sucrose by invertase. However, invertase also hydrolyses lower degree of polymerisation fructo-oligosaccharides (FOS), resulting in overestimation of sucrose. Specific hydrolysis of sucrose in the presence of FOS can be achieved with the sucrase enzyme employed in AOAC Method 999.03.[9,10] Lactose is quantitatively hydrolysed to D-galactose and D-glucose using a β-galactosidase which has little action on galacto-oligosaccharides (GOS),[11] and isomaltose is hydrolysed to glucose with oligo-α-1,6-glucosidase.[12]

In the method described in this booklet,[10,12] available carbohydrate is measured as total digestible starch (TDS) plus maltodextrins, sucrose, lactose, isomaltose, D-galactose, D-glucose and D-fructose.

PRINCIPLE:

Digestible starch is hydrolysed to D-glucose plus traces of maltose using a mixture of pancreatic α-amylase and amyloglucosidase (PAA + AMG) according to the incubation conditions employed in AOAC Method 2017.16 for dietary fiber (1).

$$\text{(1)}\quad \text{Digestible starch} \xrightarrow{\text{(PAA + AMG, pH 6, 37°C, 4 h)}} \text{D-glucose}$$

Sucrose and maltose are specifically hydrolysed to D-glucose and D-fructose by sucrase/maltase enzyme (2), lactose is hydrolysed to D-galactose and D-glucose by MZ104 β-galactosidase (3) and isomaltose is hydrolysed to glucose by oligo-α-1,6-glucosidase (4).

$$\text{(2)}\quad \text{Sucrose + maltose} \xrightarrow{\text{(sucrase/maltase)}} \text{D-glucose + D-fructose}$$

$$\text{(3)}\quad \text{Lactose} \xrightarrow{(\beta\text{-galactosidase})} \text{D-galactose + D-glucose}$$

$$\text{(4)}\quad \text{Isomaltose} \xrightarrow{(\text{oligo-}\alpha\text{-1,6-glucosidase})} \text{D-glucose}$$

In the presence of the enzymes galactose dehydrogenase (GalDH) and galactose mutarotase (GalM) galactose is oxidised by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to galactonate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (5).

$$\text{(5)}\quad \beta\text{-D-Galactose} + NADP^+ \xrightarrow{\text{(GalDH/GalM)}} \text{D-galactonate} + NADPH + H^+$$

The amount of NADPH formed in this reaction is stoichiometric with the amount of D-galactose. It is the NADPH which is measured by the increase in absorbance at 340 nm.

D-Glucose and D-fructose are then phosphorylated by the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) to glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P) with the simultaneous formation of adenosine-5'-diphosphate (ADP) (6)(7).

$$\text{(6)}\quad \text{D-Glucose + ATP} \xrightarrow{\text{(HK)}} \text{G-6-P + ADP}$$

$$\text{(7)}\quad \text{D-Fructose + ATP} \xrightarrow{\text{(HK)}} \text{F-6-P + ADP}$$

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidised by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (8).

$$\text{(8)}\quad \text{G-6-P} + NADP^+ \xrightarrow{\text{(G6P-DH)}} \text{gluconate-6-phosphate} + NADPH + H^+$$

The amount of NADPH formed in this reaction is stoichiometric with the amount of D-glucose. It is the NADPH which is measured by the increase in absorbance at 340 nm.

On completion of reaction (6), F-6-P is converted to G-6-P by phosphoglucose isomerase (PGI) (9).

$$\text{(9)}\quad \text{F-6-P} \xrightleftharpoons{\text{(PGI)}} \text{G-6-P}$$

The G-6-P formed reacts in turn with $NADP^+$ forming gluconate-6-phosphate and NADPH, leading to a further rise in absorbance that is stoichiometric with the amount of D-fructose.

SPECIFICITY, SENSITIVITY, LINEARITY & PRECISION:

The assays specifically measure D-glucose, D-fructose and D-galactose derived from digestible starch plus maltodextrins, sucrose, lactose and isomaltose, as well as free D-galactose, D-glucose and D-fructose. If present in the sample, GOS will be partially hydrolysed leading to a slight overestimation of available carbohydrates.

The smallest differentiating absorbance for the assay is 0.010 absorbance units. This corresponds to 0.738 g/100 g available carbohydrates "as is", (measured as glucose) using a sample weight of 0.5 g and extract volume of 20.5 mL (Method 1). The detection limit is 1.475 g/100 g available carbohydrates "as is", which is derived from an absorbance difference of 0.020 (measured as glucose) with a sample weight of 0.5 g and extract volume of 20.5 mL (Method 1).

The assay is linear over the range of 4 to 80 μg of D-galactose, D-glucose or D-fructose per assay.

APPLICABILITY AND ACCURACY:

The method is applicable to samples containing > 3% w/w available carbohydrates). With such samples, standard errors of ± 5% are achieved routinely. Higher errors are obtained for samples with AVCHO contents of < 3% w/w.

INTERFERENCE:

If the conversion of D-galactose, D-glucose or D-fructose has been completed within the times specified in the assay, it can be generally concluded that no interference has occurred. However, this can be further checked by adding D-galactose plus D-glucose plus D-fructose standard (20 μg of each sugar in 0.10 mL) to the cuvette on completion of the reaction. A significant increase in the absorbance should be observed. Complete hydrolysis of sucrose, lactose and isomaltose can be confirmed by adding an aliquot of any of these sugars to the original sample extract, or to the assay cuvette before addition of sucrase/β-galactosidase/oligo-α-1,6-glucosidase.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website. **NOTE:** Susceptible individuals may be allergic to the powder form of pancreatic α-amylase and/or amyloglucosidase. If an analyst is allergic to powdered PAA and/or AMG, engage an analyst who is not allergic to prepare an ammonium sulphate suspension of the powdered enzyme mixture {see Note on page 5 in *[A(3)]*}.

KITS:

Kits suitable for performing 100 assays of each of available carbohydrates are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: **Imidazole buffer (11 mL, pH 7.6) plus magnesium chloride and sodium azide (0.02% w/v)** as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: **$NADP^+$ plus ATP.**
Stable for > 5 years stored dry below -10°C.

Bottle 3: **Mixture of purified PAA (40 KU/g) and AMG (17 KU/g)** (5.2 g).
Stable for > 5 years stored dry below -10°C.

Bottle 4: **Sucrase/maltase plus β-galactosidase and oligo-α-1,6-glucosidase.** Suspension (2.25 mL).
Stable for > 2 years at 4°C.

Bottle 5: **Galactose dehydrogenase/galactose mutarotase.** Suspension (2.25 mL).
Stable for > 2 years at 4°C.

Bottle 6: **Hexokinase plus glucose-6-phosphate dehydrogenase.** Suspension (2.25 mL).
Stable for > 2 years at 4°C.

Bottle 7: **Phosphoglucose isomerase.**
Suspension (2.25 mL).
Stable for > 2 years at 4°C.

Bottle 8: **D-Glucose, D-fructose plus D-galactose standard solution** (5 mL, 0.2 mg/mL of each sugar).
Stable for > 2 years; stored sealed at 4°C.

Bottle 9: **Available carbohydrates control** (~ 10 g).
AVCHO value shown on the label.
Stable for > 5 years at room temperature.

A. REAGENT SOLUTIONS:

1. Use the contents of bottle 1 as supplied.
Stable for > 2 years at 4°C.

2. Dissolve the contents of bottle 2 in 12 mL of distilled water. Divide into appropriately sized aliquots and store in polypropylene tubes below -10°C between use and keep cool during use if possible. Once dissolved, the reagent is stable for > 2 years below -10°C.

3. **Stock PAA/AMG solution.**— PAA (0.8 KU/mL) plus AMG (0.34 KU/mL). Immediately before use, add 0.5 g of PAA/AMG powder mixture (Bottle 3, page 4) to 25 mL of sodium maleate buffer *[B(a)]* and stir on a magnetic stirrer for 5 min. Store on ice during use. Use within 4 h of preparation. **NOTE:** If an analyst is allergic to powdered PAA and/or AMG, engage an analyst who is not allergic to prepare the powdered enzymes as an ammonium sulphate suspension as follows: gradually add 2.5 g of PAA/AMG powder mix (PAA 40 KU/g plus AMG 17 KU/g; **Bottle 3**) to 35 mL of cold, distilled water in a 100 mL beaker on a magnetic stirrer in a fume cupboard and stir until the enzymes are completely dissolved (approx. 5 min). Add 17 g of granular ammonium sulphate and dissolve by stirring. Adjust the volume to 50 mL with ammonium sulphate solution (50 g/100 mL) *[B(c)]*. Stable for 3 months at 4°C. ***Alternatively*** (and recommended), the suspension can be stored in appropriate volumes in polypropylene tubes below -10°C for > 3 years.

4, 5, 6, 7 & 8 Use the contents of bottles 4, 5, 6, 7 & 8 as supplied.
Stable for > 2 years at 4°C.

9. Use as supplied. Stable for > 5 years stored sealed at room temperature.

B. REAGENT (not supplied):

a. ***Sodium maleate buffer*** **(50 mM, pH 6.0) plus 2 mM $CaCl_2$.**— Dissolve 11.6 g of maleic acid in 1600 mL of deionised water and adjust the pH to 6.0 with 4 M (160 g/L) NaOH solution. Add 0.6 g of calcium chloride dihydrate ($CaCl_2.2H_2O$), dissolve and adjust the volume to 2 L. Store in a well-sealed Duran® bottle and add two drops of toluene to prevent microbial infection. Stable for ~ 1 year at 4°C.

b. ***Sodium maleate buffer (50 mM, pH 6.5) containing BSA (0.5 mg/mL) and sodium azide (0.02% w/v).***— Dissolve 5.8 g of maleic acid in 800 mL of deionised water and adjust the pH to 6.5 with 4 M (160 g/L) NaOH solution. Add 0.5 g of BSA and 0.2 g of sodium azide and dissolve by stirring. Adjust the volume to 1 L. Store in a well-sealed Duran® bottle. Stable for ~ 1 year at 4°C.

c. ***Ammonium sulphate solution, 50% w/v.***— Add 50 g of ammonium sulphate to 70 mL of distilled water and dissolve by stirring. Adjust volume to 100 mL with distilled water. Store in a Duran bottle. Stable for > 2 years at room temperature.

d. ***Ethanol (or IMS) 95% v/v.***

C. APPARATUS REQUIRED:

a. ***Grinding mill.***— Centrifugal, with 12-tooth rotor and 0.5 mm sieve, or similar device. Alternatively, a cyclone mill can be used for small test laboratory samples provided the mill has sufficient air flow or other cooling to avoid overheating of samples.

b. ***Meat mincer.***— Hand operated or electric, fitted with a 4.5 mm screen.

c. ***Water bath.***— To accommodate a 2mag Mixdrive 15® submersible magnetic stirrer with an immersion heater (e.g. Lauda Alpha® Figure 2). Alternatively, a shaking water bath (Grant OLS 200) set in linear motion at 100 rpm can be used. In this case, tubes must be attached sideways in the direction of motion.

d. ***Submersible magnetic stirrer.***— 2mag Mixdrive 15® submersible magnetic stirrer set at 170 rpm.

e. ***Spectrophotometer.***— capable of operating at 340 nm, (10 mm path length); e.g. Megaquant™ Wave Spectrophotometer (Megazyme cat. no. **D-MQWAVE**).

f. ***Analytical balance.***— 0.1 mg readability, accuracy and precision.

g. ***Dry-block heater.***— e.g. Stuart® Block Heater SBH130D. Fisher Scientific cat no. 11476568, or similar.

h. ***Microfuge centrifuge.***— Capable of 13,000 rpm.

i. ***Disposable 2.0 mL polypropylene microfuge tubes.***— e.g., Sarstedt cat. no. 72.691. Sarstedt Ltd., Drinagh, Co Wexford, Ireland.

j. ***pH meter.***— e.g. Seven Easy pH Mettler Toledo.

k. ***Vortex mixer.***— e.g. Daihan Scientific VM-10.

l. ***Moisture analyser.***— e.g. OHAUS MB45.

m. ***Magnetic stirrer.***— e.g. IKA KMO 2 basic stirrer.

n. ***Magnetic stirring bars.***— e.g. Fisherbrand™ PTFE Stir Bars 30 x 6 mm ridged and 15 x 5 mm ridged.

o. ***Digestion Bottles.***— 250 mL Fisherbrand™ soda glass, wide mouth bottles with polyvinyl lined cap (cat. no. FB73219).

p. ***Laboratory timer.***

q. ***Micro-pipettors.***— e.g. Gilson Pipetman® (100 μL), Woodside Industrial Estate, Dunstable, United Kingdom.

r. ***Positive displacement pipettor***.— e.g. Eppendorf Multipette®
- with 25 mL Combitip® (to dispense 2.5 or 5 mL aliquots of PAA/AMG preparation, 4 mL aliquot of IMS or 50% IMS).
- with 5.0 mL Combitip® (to dispense 0.1 mL of AMG solution).

s. ***Dispensers.***— e.g. Brand HandyStep dispensette® S Digital 2.5-25 mL, cat. no. 4600351.

t. ***Polypropylene tubes.***— Sarstedt polypropylene tube; 40 mL, 30 x 84 mm (cat. no. 62.555).

u. ***Polypropylene sheet with precision cut holes.***— to hold and align 40 mL polypropylene tubes on the stirrer plate of the 2mag Mixdrive 15® submersible magnetic stirrer (Figure 2, page 9).

D. PREPARATION OF TEST SAMPLES:

Collect and prepare samples as intended to be eaten, *i.e.* baking mixes should be prepared and baked, pasta should be cooked *etc.* Defat per AOAC 985.29 if fat content is > 10% w/w. For high moisture samples (> 25%) it is desirable to freeze dry. Grind ~ 50 g in a grinding mill *[C(a)]* to pass a 0.5 mm sieve. Transfer all material to a wide mouthed plastic jar, seal, and mix well by shaking and inversion. Store in the presence of a desiccant. Grind wet samples (e.g. wet pasta) in a meat mincer to an homogeneous paste. Remove a representative sample for analysis and record weight. Separately, determine the moisture content of the wet sample and allow for the liquid volume in the calculations.

E. ENZYME DIGESTION OF SAMPLES:

Method 1. Procedure for analysing ~ 0.5 g of sample.

(a) ***Accurately weigh*** approx. 0.5 g sample, correct to the third decimal place, into a 30 x 84 mm (40 mL) polypropylene tube *[C(t)]*. Record the weight. Add a 20 x 6 mm stirrer bar *[C(n)]* to each tube.

(b) ***Sample equilibration.***— Wet the sample with 0.5 mL of 95% v/v ethanol (or IMS) *[B(d)]* and add 17.5 mL of maleate buffer, pH 6.0 *[B(a)]* to each tube. Cap the tubes and place them in the special polypropylene holder *[C(u)]* (Figure 2) on a 2mag Mixdrive 15® submersible magnetic stirrer *[C(d)]* in a water bath and allow the contents to equilibrate to 37°C over 5 min with stirring at 170 rpm.

(c) ***Incubation with PAA/AMG solution.***— Add 2.5 mL of PAA/AMG solution *[A(3)]*, cap the tubes and incubate the reaction solutions at 37°C and at 170 rpm on the 2mag Mixdrive 15® submersible magnetic stirrer. **NOTE:** If using an $(NH_4)_2SO_4$ suspension of this enzyme preparation *[A(3)]*, add 1.0 mL of enzyme suspension and 1.5 mL of sodium maleate buffer *[B(a)]*.

Method 2. Procedure for analysing ~ 1 g of sample (using the incubation arrangemant employed for AOAC Method 2017.16 for TDF; RINTDF). [**NOTE:** with Method 2, the number of samples that can be analysed with a kit will be halved].

(a) ***Accurately weigh*** approx. 1 g sample, correct to the third decimal place, into a 250 mL Fisherbrand glass® bottle *[C(o)]*. Record the weight. Add a 30 x 6 mm stirrer bar *[C(n)]* to each bottle (Figure 3, page 14).

(b) ***Equilibration with buffer.***— Wet the sample with 1.0 mL of 95% v/v ethanol (or IMS) *[B(d)]* and add 35 mL of maleate buffer *[B(a)]* to each bottle. Cap the bottles and place them on a 2mag Mixdrive 15® submersible magnetic stirrer *[C(d)]* and allow the contents to equilibrate to 37°C over 5 min with stirring at 170 rpm.

(c) ***Addition and incubation with PAA/AMG solution.***— Add 5 mL of PAA/AMG solution *[A(3)]*, cap the bottles and incubate the reaction solutions at 37°C and at 170 rpm on the 2mag Mixdrive 15® submersible magnetic stirrer. **NOTE:** If using the $(NH_4)_2SO_4$ suspension of this enzyme preparation *[A(3)]*, add 2.0 mL of enzyme suspension and 3.0 mL of sodium maleate buffer *[B(a)]*.

F. DETERMINATION OF AVAILABLE CARBOHYDRATES:

(a) Carefully remove 1.0 mL of the reaction solution at 240 min and transfer to 25 mL of cold water in a 40 mL polypropylene tube [C(t)]. Cap the tube, mix the contents thoroughly and store the tube at 4°C awaiting analysis.

(b) Transfer 2.0 mL of this solution to 2.0 mL polypropylene microfuge tubes *[C(i)]* and centrifuge at 13,000 rpm for 5 min.

(c) Analyse 0.1 mL aliquots of the supernatant solution as described in the table below. In this assay, lactose is hydrolysed to D-glucose plus D-galactose by β-galactosidase, sucrose is hydrolysed to D-glucose and D-fructose by sucrase (which has no action on fructo-oligosaccharides; FOS), isomaltose is hydrolysed to glucose by oligo-α-1,6-glucosidase and remaining maltose is hydrolysed to D-glucose by maltase. Free D-galactose, D-glucose and D-fructose are then determined.

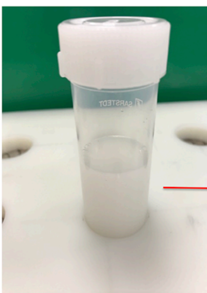

Figure 1. Procedures employed in the measurement of available carbohydrates in food samples according to Method 1.

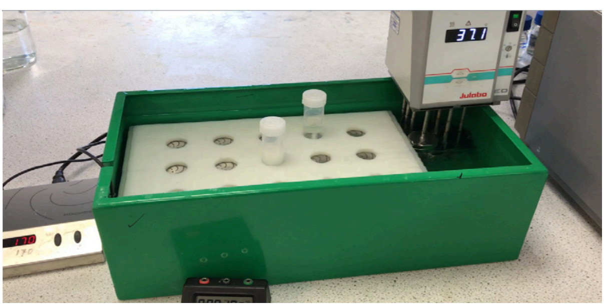

Figure 2. Incubation Method 1. Samples (~ 0.5 g) in 20 mL of buffer plus enzymes in 30 x 84 mm polypropylene tubes in a designed polypropylene tube holder (Megazyme cat. no. D-PPTH) on a 2mag Mixdrive 15® submersible magnetic stirrer in a custom made water bath (Megazyme cat. no. **D-TDFBTH**).

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 30°C
Final volume: 2.54 mL (D-galactose)
2.56 mL (D-glucose)
2.58 mL (D-fructose)
Sample solution 4-80 μg of D-galactose/D-glucose/D-fructose
Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample
sample in water maleate buffer *[B(b)]* suspension **Bottle 4** (sucrase, β-galactosidase, maltase & oligo-α-1,6-glucosidase)	- - -	0.10 mL 0.20 mL 0.02 mL
Ensure that all of the solutions are delivered to the bottom of the cuvette. Mix the contents by gentle swirling, cap the cuvettes and incubate them at ~ 30°C for 30 min. **Then add:**		
distilled water (at ~ 30°C) buffer **Bottle 1** (imidazole buffer) solution *[A(2)]* ($NADP^+$/ATP)	2.32 mL 0.10 mL 0.10 mL	2.00 mL 0.10 mL 0.10 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 3 min and start the reactions by addition of:		
suspension **Bottle 5** (GalDH/GalM)	0.02 mL	0.02 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 3 min). **Then add:**		
suspension **Bottle 6** (HK/G6P-DH)	0.02 mL	0.02 mL
Mix* and read the absorbances of the solutions (A_3) at the end of the reaction (approx. 5 min). **Then add:**		
suspension **Bottle 7** (PGI)	0.02 mL	0.02 mL
Mix*, read the absorbances of the solutions (A_4) at the end of the reaction (approx. 10 min).		

* for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

G. CALCULATION:

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-galactose}}$.

Determine the absorbance difference (A_3-A_2) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-glucose}}$.

Determine the absorbance difference (A_4-A_3) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of sample, thereby obtaining $\Delta A_{D\text{-fructose}}$.

The values of $\Delta A_{D\text{-galactose}}$, $\Delta A_{D\text{-glucose}}$ and $\Delta A_{D\text{-fructose}}$ should as a rule be at least 0.100 absorbance units to achieve sufficiently accurate results.

The concentration of D-galactose, D-glucose and D-fructose can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A \times D \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of D-galactose, D-glucose or D-fructose [g/mol]
ε = extinction coefficient of NADPH at 340 nm
= 6300 [$l \times mol^{-1} \times cm^{-1}$]
d = light path [cm]
v = sample volume [mL]
D = dilution factor (26-fold)

It follows for D-galactose:

$$c = \frac{2.54 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{D\text{-galactose}} \times 26 \quad [g/L]$$

$$= 18.885 \times \Delta A_{D\text{-galactose}} \quad [g/L]$$

for D-glucose:

$$c = \frac{2.56 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{D\text{-glucose}} \times 26 \quad [g/L]$$

$$= 19.034 \times \Delta A_{D\text{-glucose}}$$

for D-fructose:

$$c = \frac{2.58 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{D\text{-fructose}} \times 26 \quad [g/L]$$

$$= 19.183 \times \Delta A_{D\text{-fructose}} \quad [g/L]$$

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of D-galactose:

$$= c_{D\text{-galactose}}\,[g/L] \times \frac{EV}{1000} \times \frac{1}{W} \times 100 \quad [g/100\,g]$$

Content of D-glucose:

$$= c_{D\text{-glucose}}\,[g/L] \times \frac{EV}{1000} \times \frac{1}{W} \times 100 \quad [g/100\,g]$$

Content of D-fructose:

$$= c_{D\text{-fructose}}\,[g/L] \times \frac{EV}{1000} \times \frac{1}{W} \times 100 \quad [g/100\,g]$$

where:

$c_{D\text{-galactose}}$ [g/L] = concentration of D-galactose per L of undiluted extraction solution

$c_{D\text{-glucose}}$ [g/L] = concentration of D-glucose per L of undiluted extraction solution

$c_{D\text{-fructose}}$ [g/L] = concentration of D-fructose per L of undiluted extraction solution

EV = volume of solution used in the initial extraction (*i.e.* 20.5 or 41 mL)

EV/1000 = adjustment from g/L of undiluted extraction solution to g/volume of extraction solution actually used

W = weight of sample analysed in g

Available carbohydrates (g/100 g)
= ***D-galactose*** (g/100g) + ***D-glucose*** (g/100g) + ***D-fructose*** (g/100g)

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

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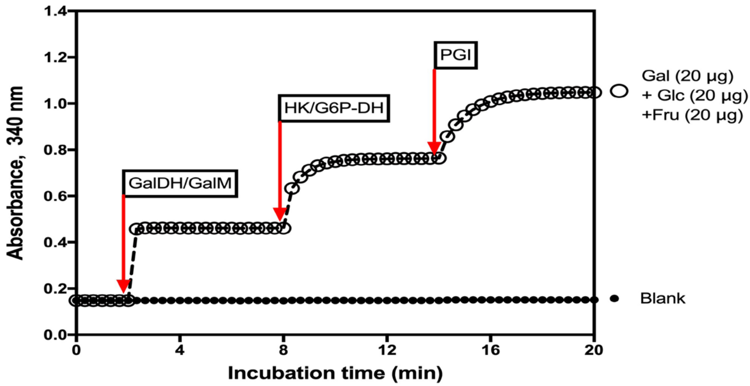

Figure 3. Sequential measurement of D-galactose, D-glucose and D-fructose. Absorbance increases on incubation of a mixture of D-galactose (20 µg), D-glucose (20 µg) and D-fructose (20 µg) with galactose dehydrogenase/galactose mutarotase followed by hexokinase and glucose 6-phosphate dehydrogenase and finally phosphoglucose dehydrogenase in the presence of $NADP^+$ and ATP.

Table 1. D-Glucose, D-fructose and available carbohydrates determined for a range of samples using the Available Carbohydrates assay method.

Samples	D-Glucose (g/100g)	D-Fructose (g/100g)	Available Carbohydrates (g/100g)
Kellogg cornflakes	76.1	2.8	78.9
Kellogg All Bran	37.9	7.1	45.0
Weetabix	68.1	1.1	69.2
Kellogg Special K	69.4	4.2	73.6
Kellogg Frosties	67.1	13.4	80.5
Roma Maceroni pasta	69.1	0.0	69.1
Rooster potato	50.9	0.8	51.7
Sweet potato	46.6	17.6	64.2
Red onion	28.2	24.2	52.4
Cauliflower	11.8	11.4	23.2
Celery	12.1	9.5	21.6
Brocolli	7.1	6.2	13.3
Carrott	26.8	18.3	45.1
Swede	33.8	17.4	51.2
Red pepper	24.3	37.9	62.2
Mushroom	3.1	0	3.1
Ripe banana	39.8	29.4	69.2
Red kidney beans (not cooked)	12.1	2.6	14.7
Soya bean (not cooked)	3.9	3.2	7.1
Heinz baked beans	43.2	3.5	48.7
Ryvita dark rye crackers	68.7	4.8	73.5
Wheat starch	86.3	0.0	86.3
Hylon VII	33.4	0.0	33.4
Potato amylose	59.4	0.0	59.4
Regular maize starch	83.7	0.0	83.7

Table 2. Repeatability of the Available Carbohydrates assay method for measurement of available carbohydrates in a range of samples.

Sample	Available Carbohydrates, % (w/w)[a], mean[b] ±2 SD, (%RSD$_r$[c] %)				Interday mean, ±2 SD, (%RSD$_r$)
	Day 1	Day 2	Day 3	Day 4	
Wheat Starch	87.3 ± 2.1	90.2 ± 2.2	88.2 ± 0.6	90 ± 1.1	88.9 ±2.8
	1.21	1.21	0.34	0.61	1.60
All Bran	43.2 ± 2.2	45.4 ± 0.4	43.2 ± 1	44.5 ± 0.2	44.1 ±2.2
	2.55	0.40	1.20	0.27	2.53
Sweet Potato	59.6 ± 0.2	60.7 ± 1.3	58.2 ± 2.1	60.4 ± 1	59.7 ±2.3
	0.14	1.10	1.80	0.81	1.92
Ripe Banana	65.4 ± 0.4	70 ± 0.3	67.1 ± 1	66.8 ± 0.6	67.3 ±3.6
	0.28	0.19	0.77	0.46	2.68
Carrot	53.7 ± 0.9	57.4 ± 0.6	55.1 ± 1.5	55.3 ± 0.3	55.4 ±2.9
	0.87	0.54	1.36	0.23	2.58
Red Pepper	51 ± 0.5	55.4 ± 2.5	53.8 ± 3.1	52.9 ± 1.9	53.2 ±3.8
	0.49	2.25	2.92	1.83	3.58
Ryvita	60.6 ± 1.3	61.5 ± 1.3	61 ± 2.4	62.6 ± 0.7	61.4 ±2
	1.07	1.04	1.96	0.60	1.61
Swede	55 ± 4.3	53.6 ± 0.4	54.2 ± 1.3	54.1 ± 2	54.2 ±2.1
	3.92	0.34	1.19	1.82	1.96

where:

a all results are presented as available carbohydrates on a dry weight basis;

b on each day samples of each material were analyzed in duplicate;

c RSD$_r$ of the available carbohydrates assay was assessed using 8 samples.

The available carbohydrate content of the samples tested covered a working range of 44.1 to 88.9% (w/w). The repeatability (%RSD$_r$) across this sample data set was less than or equal to 3.58% for all samples.

Megazyme

Bray Business Park, Bray,
Co. Wicklow,
A98 YV29,
IRELAND.
Telephone: (353 1) 286 1220
Facsimile: (353 1) 286 1264)
Internet: www.megazyme.com
E-Mail: info@megazyme.com