# CARBOHYDRATE ANALYSIS(Ch. 11+12)

I. Analysis of Sugars

A-Qualitative Detection Methods

B-Quantitative Analyses

II- Analysis of Nonsugars (polysaccharides)

A- Nutrient Polisaccharides (starch, dextrins, glycogen etc.)

-Qualitative analyses

-Quantitative analyses

B- Dietary Fiber or Structural Polysaccharides (cellulose, hemicellulose etc.)

a. Gravimetric methods:

1.Crude fiber

2. Detergent methods

3. AOAC method

b. Chemical Methods

# Composition and Occurrence

Carbohydrates are the most abundant and most widely consumed(>%70) macro food component. They provide satiety, and modify human physiological processes.They are a very heterogeneous group in structure-all contain carbon, hydrogen and oxygen, and are almost exclusively of plant origin(exceptions: lactose of milk and glycogen in meat).

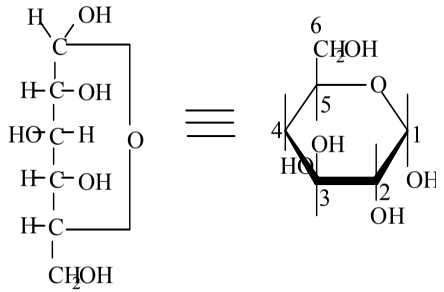
CLASSIFICATION:

**I. Mono and Oligo sachharides (Sugars)**: Sugars have nutritional and metabolic functions;they govern rheological properties, cause browning reactions, have role in food stability, are raw materials for fermentations.

a. Monosacharides: Polyhydroxy aldoses and ketoses

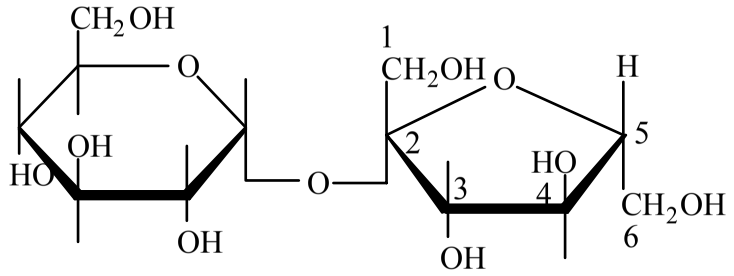
Pentoses: Xylose,arabinose($C_5$)

Hexoses: Glucose, fructose($C_6$ )



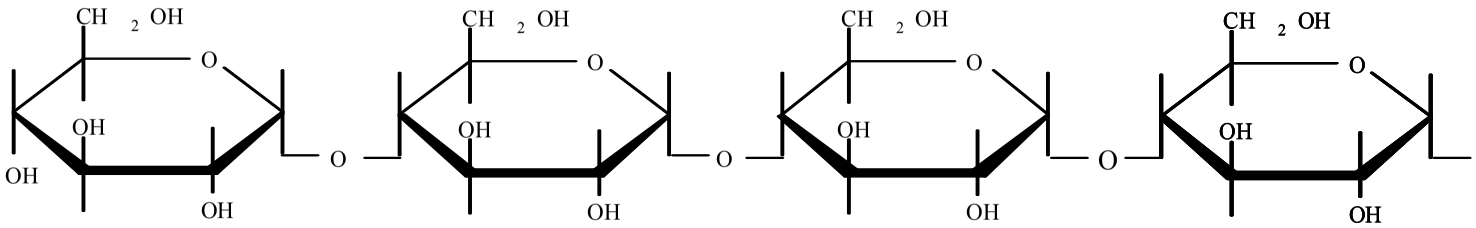
# b. Oligosaccharides:

Some sugar units (>1,<10) are condensed together forming di-, tri-, tetra-saccharides: Sacharose, lactose, raffinose, stachyose



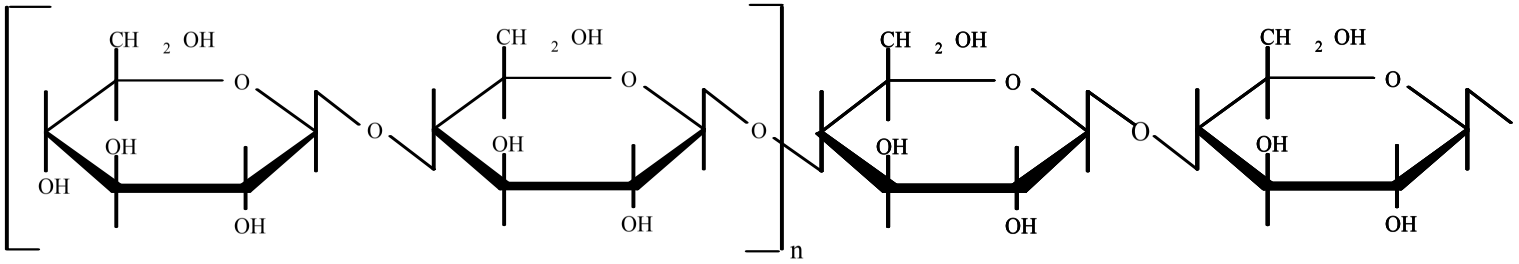
**II. Nonsugars**: Polysachharides

a. Nutrient polysacharides: They are metabolic reserves of plants and animals ,and are basically all polymers of glucose (dextrins, starch, glycogen)

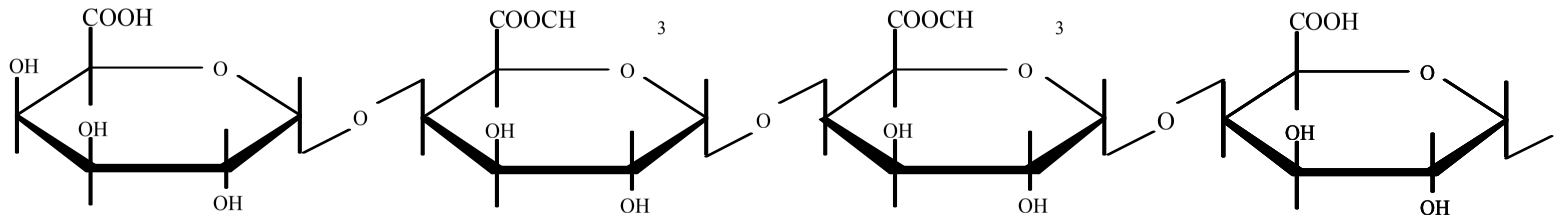


b. Structural polysacharides: They constitute the rigid mechanical cell wall structure in plants. Cellulose( polymer of b-D-Glucose b(1, 4) linkage.

Repeating cellobiose moiety)



# Pectin(galacturonic acid and methylgalacturonate polymer)




Homoglicans are polymers of same sugar
(i.e.xylan:xylose polymer)
Heteroglicans are polymers of more than one sugar group
(i.e.arabinogalactans)

# FUNCTIONAL PROPERTIES OF CHO's

-Storage material for carbon and energy in live systems

-Component of Structure :

1. Viscosity control
2. Texture control
3. Emulsifying agent
4. Stabilizer due to water binding capacity

# Carbohydrate contents of some foods

Honey: 75-82 % fructose + glucose + sacharose

Apples: 15%( fructose+ glucose+saccharose+ starch)

Dried figs: 30%fructose + 42%glucose

Dates: 50% saccharose

Seedless raisins: 70%fructose+glucose; Grapes: 16-17%

Melons, peaches, tangerines : <10% saccharose

Carrots:10%CHO

Chestnuts: 33% starch

White flour: 70 % starch , White Bread:50% CHO

Corn-patatoes: %15 starch; Corn Flakes: 86% CHO

Chicken breast: 0%

## Short-cut to CHO Quantification:

In general(in most food composition tables and for nutritional labelling), it is assumed that:

**TotalCHO(withfiber)=100-[Moisture%+Protein%+Fat%+Ash%]**

or

**CHO without crude fiber=N-free extract**

In calculating the calorific values of foods, sugars and nutrient polysaccharides of CHO's are generally assumed as having 4cal/g and structural polysaccharides (i.e.crude fiber) as having 0 cal/g. However, novel "Dietetic food ingredients"now include components which are partially metabolized and therefore do provide some calories (i.e.oligosaccharides:1.2 kcal/g; sugar alcohols: 2.4kcal/g) thus contributing to a rather new concept, "dietary fibre".

The alternative means of quantificationof calories is by use of a **BOMB Calorimeter,** which measures heat produced when sample is burnt.

# Total Carbohydrates

CHO's are destroyed by heat and acid. Continued heating in presence of acid leads to products that condense with phenolic compounds to produce colored compounds,then quantified by UV-Vis spectrophpotometric readings of absorbance at 490 nm.

Ex: Phenol-Sulfuric Acid Method(p.172-3)

## Analyses of Sugars (Water-soluble CHO's)

## SAMPLE PREPARATION:

Sample has to be homogenized before analyses.

-Removal of "Lipids and Proteins" is required before starting analyses, since they cause turbidity during analyses which interferes with polarization and refraction:

Lipids are extracted with petroleum ether, but care should be taken not to exceed 50°C, since higher temperatures might solubilize starch.

Proteins are precipitated with heavy metals using [Pb acetate] or [ZnOH or Zn ferrocyanide]. Proteins are coprecipitated in colloidal form. Carrez solution (1+1) [%15 $ZnSO_4$ + %30K ferricyanide) is an other alternative.

-Neutralizing any acidity (by $CaCO_3$), since acids may cause hydrolysis of sugars.

-Removal of pigments by adsorption an charcoal

-Inactivating enzymes [$HgCl_2$ inactivates enzymes]

# Sample preparation and extraction scheme (fig.11.1)

-Dry sample

-From dehydrated material , extract lipids with 95:5 Chloroform-methanol

-Extract residue with 80%ethanol

-Remove any interferance (i.e. by ion-exchange, Seppak cartridge etc)

Mono and disaccharides are obtained.

# I.a. Qualitative Detection Methods for sugars:

1-Total sugars ::Methods based on colour reactions of sugars.

-With strong mineral acids ($H_2SO_4$, HCl, $H_3PO_4$ etc.) EX:CHO+Phenol- $H_2SO_4$ ⟹ yellow-orange colour due to condensation of degrading sugar products : 490nm

acids+ hexoses ⟹ HMF indicator

acids+pentoses ⟹ furfurol

-With anthrone in conc. $H_2SO_4$, ⟹

characteristic blue-green colour : 620nm

2- **Specific Analyses of Individual Sugars: Methods** based on reducing properties of monosaccharides as well as reducing disaccharides like lactose and maltose(Cu++ is reduced to Cu+):

A-FEHLING Reaction(Benedict's Test, Munson Walker-Gravimetric; LaneEynon-Volumetric, Somogyi-Nelson: spectrophotometric):

Fehling A:35g$CuSO_4$ /500ml;

Fehling B:[173g $NaKC_4H_4O_6$(Rochelle salt)+50g NaOH]/500 ml

$$CuSO_4 \xrightarrow{\text{reducing sugar}} Cu_2O$$

$$Cu^{++} \rightarrow Cu^{+}$$

## B. – Reaction with Tollen's reagent:

$$[Ag[NH_3]_2]^+_{ion} \xrightarrow{\text{reducing sugars}} Ag^o$$

Silver ions is reduced to to metallic silver, which is deposited on a glass surface, forming a "mirror".

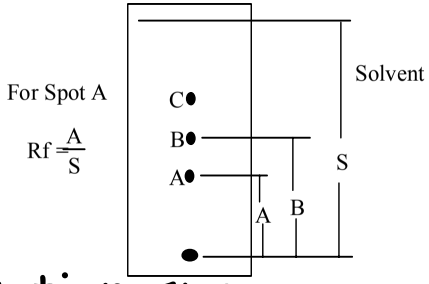
## C. Formation of "osazones":

Oxidative cleavage of hydroxyl groups of sugars and phenylhidrazine form beautiful crystalline yellow compounds, called osazones("diphenylhydrazone")

**3**. Paper Chramotography: Is by far the best and simplest qualitative method to distinguish qualitatively between various sugars in foods.

Ex: Sugar solution is spotted on Filter paper;for developing solvent system: [Phenol-$H_2O$, 80:20] or [$H_2O$:butanol:acetic acid, 5:1:4] or many other alternative solvent systems can be used. Peculiarities of different sugars lead to differences in Migration rates, resulting in different but typical $R_f$ value for different sugars in a given solvent system. Generally:

Higher Rf values indicate higher mobility, smaller MW :




Pentose>hexose>disaccharides>trisacharides>
And ketohexose>aldohexose

Then the chromatogram is sprayed with a visualisation spray-reagent (Ex. p-aninisine in n-butanol) or phloroglucinol in HCl, and after drying for 5-10 min, is put in an oven at 105°C for visualisation.

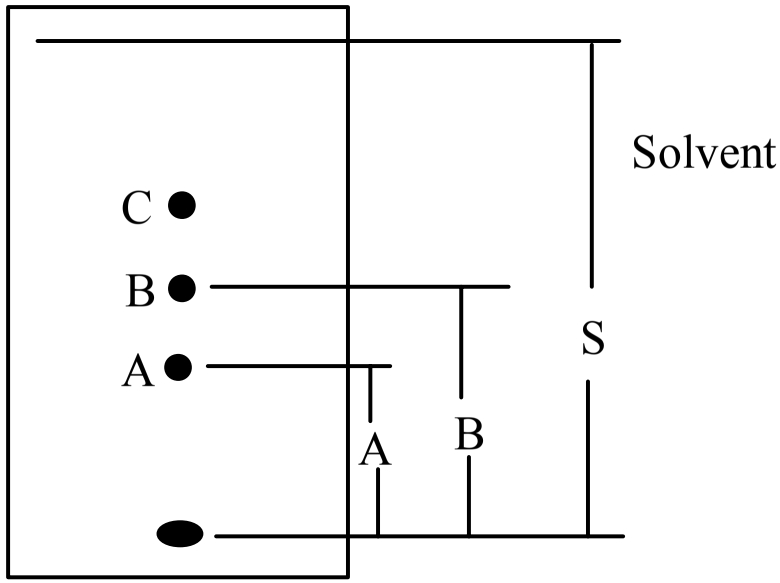
Thin Layer chromatography

Silica gel as stationary phase (250 μm)

Quantitative by densitomer (time varies with solvent, generally overnight):

For Spot A

$$Rf = \frac{A}{S}$$

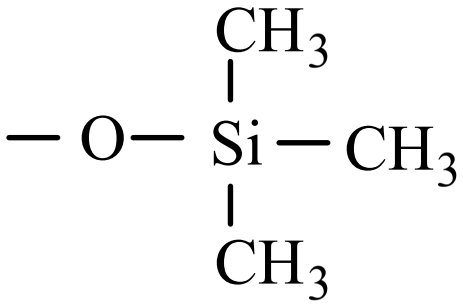


# Gas and Liquid Chromatography

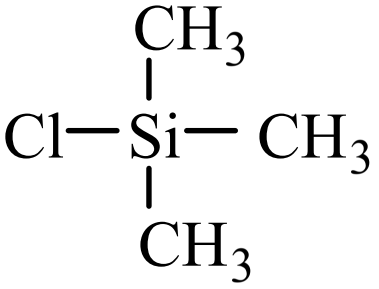
GC and HPLC: For quantification of individual sugar components, HPLC yields most satisfactory results.Also, GC following formation of volatile silyl derivatives of sugars with TMS (trimethylsilyl ether) can be used for quantitative analyses of individual sugars.

This is a requirement, since compounds must be volatile.

Sugars form volatile Trimethylsilyl Ethers:



Ether linkage

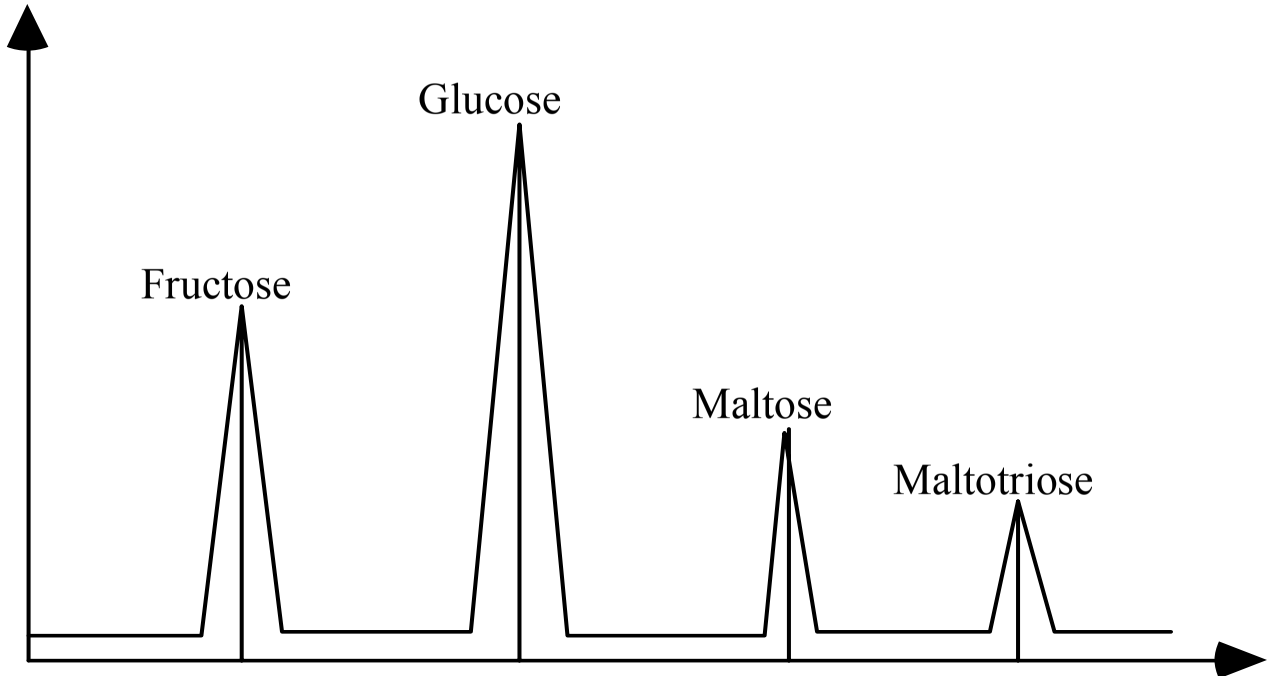


Trimethyl chlorosilane

# Liquid Chromatography

Carbohydrate Analysis (Waters Assocs. HPLC 840380 Stationary Phase)

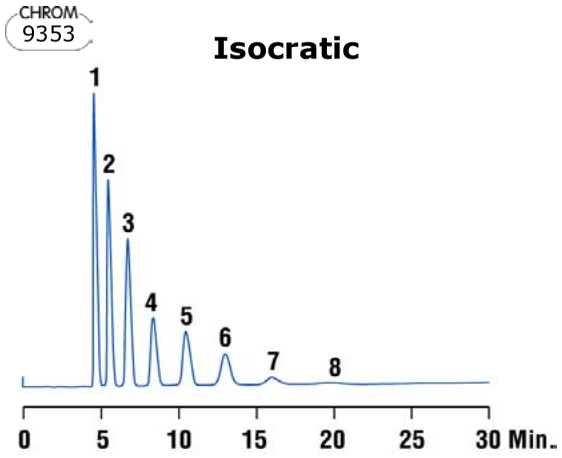
Solvent system:  H2O/CH3CN



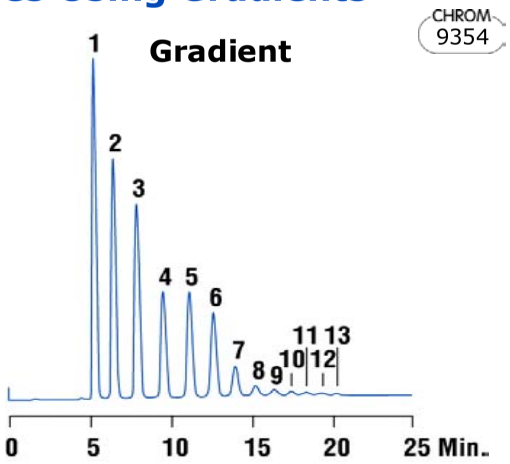
- The most commonly used detector for carbohydrate analysis is the differential refractive index (RI) detector. Because it responds to all analytes whose refractive index differs from that of the mobile phase, it functions as a universal detector. It is preferred over traditional UV detector for non-chromophoric compounds like carbohydrates.

# 42 DE Corn Syrup Maltose Oligomers

## Enhanced Sensitivity and Reduced Run Times Using Gradients




1. Glucose
2. Maltose
3. Maltotriose
4. Maltotetraose
5. Maltopentaose
6. Maltohexaose
7. Maltoheptaose
8. Maltooctaose
9. Maltononaose
10. Maltodecaose
11. Maltoundecaose
12. Maltododecaose
13. Maltotridecaose




- **Column:** Prevail™ Carbohydrate ES, 250 x 4.6mm
- **Mobile Phase:** Water:Acetonitrile (38:62)
- **Flowrate:** 1.0mL/min
- **Column Temp:** Ambient
- **Injection Volume:** 5µL

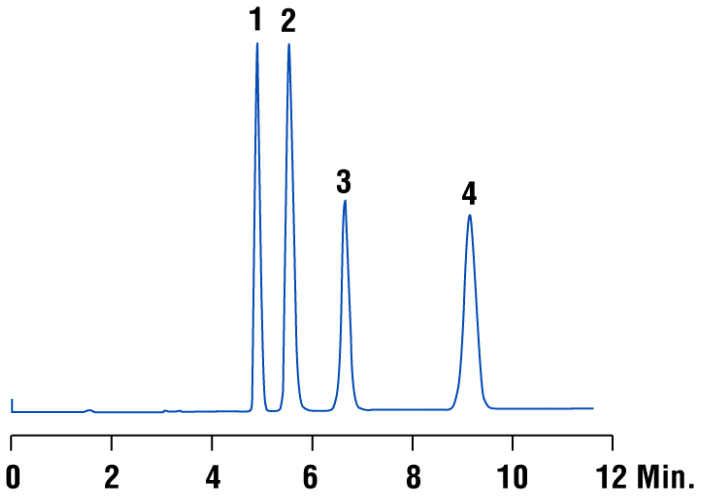
**Column:** Prevail™ Carbohydrate ES, 250 x 4.6mm
**Mobile Phase:** **A:** Acetonitrile **B:** Water
**Gradient:**

| **Time:** | 0 | 15 | 25 |
|---|---|---|---|
| **%B:** | 35 | 50 | 50 |

**Flowrate:** 1.0mL/min
**Column Temp:** Ambient
**Injection Volume:** 5µL

## Sugar Alcohols

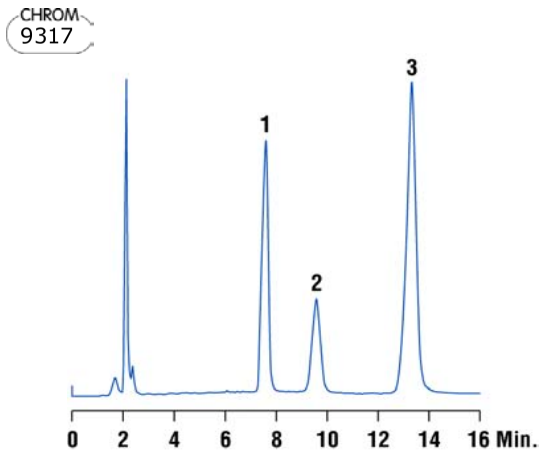
CHROM 9352




1. Iso-erythritol
2. Xylitol
3. Mannitol
4. Maltitol

- **Column:** Prevail™ Carbohydrate ES, 250 x 4.6mm
- **Mobile Phase:** Water:Acetonitrile (30:70)
- **Flowrate:** 1.0mL/min
- **Column Temp:** Ambient
- **Injection Volume:** 5µL

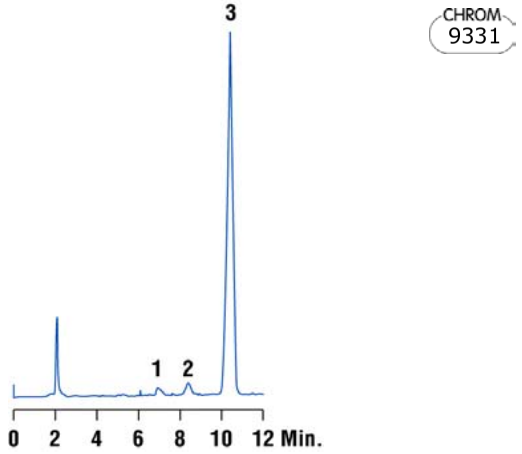
## Orange Juice




**1.** Fructose
**2.** Glucose
**3.** Sucrose

- **Column:** Prevail™ Carbohydrate ES, 250 x 4.6mm
- **Mobile Phase:** Water:Acetonitrile (25:75)
- **Flowrate:** 1.0mL/min
- **Column Temp:** Ambient
- **Injection Volume:** 20µL

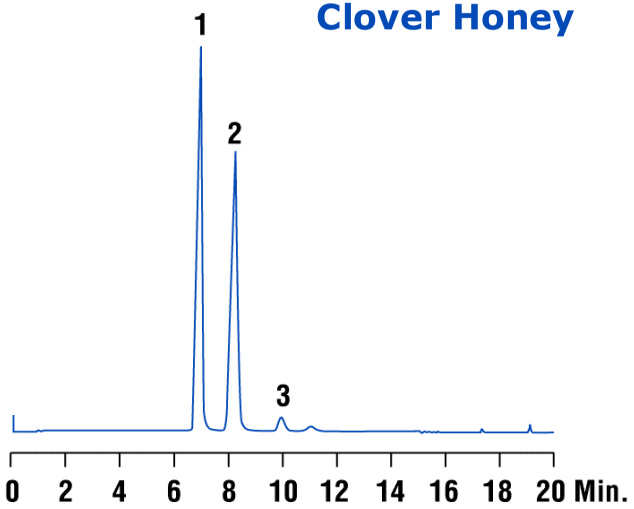
## Vegetable Juice




- **Column:** Prevail™ Carbohydrate ES, 250 x 4.6mm
- **Mobile Phase:** **A:** Acetonitrile **B:** Water
- **Gradient:** **Time:** 0 30
- **%B:** 25 50
- **Flowrate:** 1.0mL/min
- **Column Temp:** Ambient
- **Injection Volume:** 20µL

# Clover Honey

CHROM 9365




1. Fructose
2. Glucose
3. Sucrose

| | |
|---|---|
| **Column:** | Prevail™ Carbohydrate ES, 250 x 4.6mm |
| **Mobile Phase:** | **A:** Acetonitrile **B:** Water |
| **Gradient:** | **Time:** 0 \| 15<br>**%B:** 25 \| 40 |
| **Flowrate:** | 1.0mL/min |
| **Column Temp:** | Ambient |
| **Injection Volume:** | 5µL |
| **Detector:** | ELSD 2000 |

# I.b. Quantitative Sugar Analyses Methods

The following methods give total sugar content and always require calibration with standard sugar solutions.

**b.1- Chemical Methods:** Chemical methods are based on reducing properties of monosaccharides. Free aldehyde or keto group reduces alkaline solutions of metallic salts to metal oxides(CuO) or free metal(Ag).

-Gravimetric methods(Cu-Munson-Walker): Weighing precipitated metal oxide

-Titrimetric methods

*a) Lane-Eynon:*Sugar solution is added from a buret to a vigorously boiling mixture of Fehling A+B solution mixture. Near the end point, methylene blue indicator will change from blue to colorless in excess of reducing sugars.

*b) Alkaline ferricyanide method:*

$2K_3Fe(CN)_6 + 2KI_2 \longrightarrow K_4Fe(CN)_6 + I_2$

Liberated iodine is titrated with $Na_2S_2O_3$

## b.2- Physical methods-Instrumental methods(p.184)

Microscopy: especially for starch origin determination analysis

Specific gravity: hydrometers calibrated in Brix degrees (sucrose % by weight)

Spectrophotometric methods: Since in the range (0-80$\mu g$ ) the absorbance value at 490nm for reduced copper is in linear range, spectrophotometric readings can be used for quantification of total sugar content.

-Refractometry: Increasing sugar concentration increases the angle of refraction of a solution, which is also affected by concentration temperature. Pocket and Abbé types of refractometers yield ratio of soluble solids. Results are precise only for pure sugar solutions.

-Polarimetry(see 11.6.5): This is a nondestructive and rapid method depending on the fact that sugar solutions, like all compounds containing chiral carbon, have the ability to rotate a plane of polarized light through an axis parallel to its direction of propogation, and the specific angle of rotation being proportional with sugar molecular structure and concentration.

**Dextrorotatory -plane polarized light rotated clockwise (or to the right)**

**Levoratatory - plane polarized light rotated counterclockwise.**

**Invert Sugar --- when sucrose has been hydrolyzed into glucose and fructose, the rotation changes from dextrorotatory (+66.5) to levorotatory (-19.8). So, sucrose solution is called "Invert Sugar".**

ICUMSA-International Commission for Unified Methods of Sugar Analysis cites polarimetry in standard methods for sugar ındustry.

For sacharose=+66.5°

For invert sugar solution(glucose+fructose)=-20°

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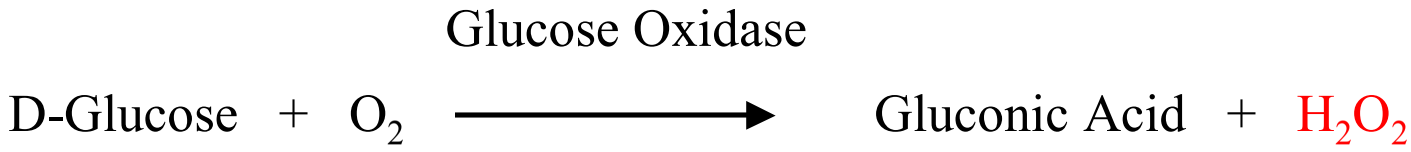
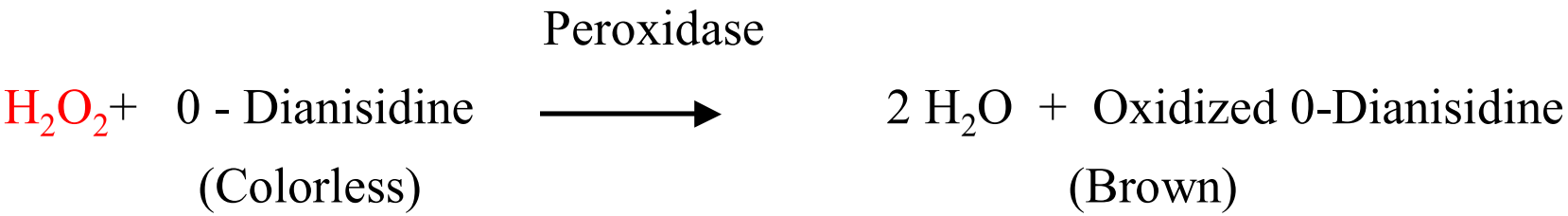
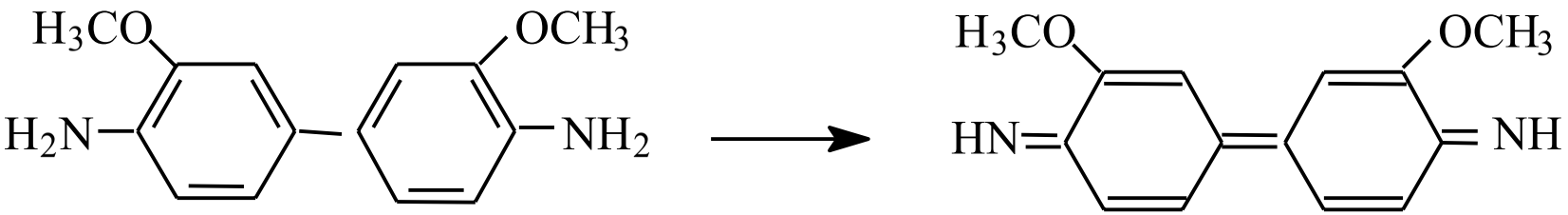
# b.3.Enzymatic Methods:Glucose Oxidase System



## II. A. Analysis of Nutrient Polisaccharides

**STARCH(11.4.1):Most abundant CHO component of foods.**
-Qualitative methods for starch determination :

-Polarizing light Microscopy: intact starch granules can be identified as to their source: like potatoe, corn, wheat since granule size , shape, iodine staining characteristics of each differ(see 11.6.1,p.184)

-Iodine test: linear straigh-chain amylose reacts with iodine t form a blue-black starch-iodide complex, whereas th branched chains of amylopectins form pale red viole complexes. Though color intensity is not spectrophotometric reaction is very sensitive(0.002g/ml) and applicable t starch,dextrins and glycogen).

## -Quantitative Methods for total Starch:

**Starch Hydrolysis:** Following Carrez Treatment*for removing interfering substances, starch is hydrolized with acids (0.4N$H_2SO_4$ ) and/or enzymes [ first with $\alpha$ and $\beta$-amylase enzymes to break amylose chains to maltose and glucose;then with amyloglucosidase(or glucoamylose) enzyme to break amylopectins] to glucose, which can then be determined colorimetrically with anthrone reagent or quantified by any other method (See Fig. 11-7,p.180.)

*CARREZ TREATMENT:First adding a solution of potasium hexacyanoferrate $K_4[Fe(CN)_6]$ then followed by first $ZnSO_4$ and then NaOH solutions, thus breaking any emulsions, precipitating proteins, then getting rid of pigments after which the clear filtrate is used directly in specific enzyme catalysed reactions for each class of CHO..

# Hydrocolloids other than Starch: Food Gums

- Structural heterogenity:linear or branched, neutral or anionic,differing solubility degrees; therefore very complex analytical schemes have been developed

  (Fig.11-8, p.182-3)

- Methods depend on extraction followed by fractionation and hydrolysis followed by specific identification tests

# Pectin

- ~80% is D-galacturonic acid.
- Saponify with NaOH, then acidify and add $Ca^{+2}$ to precipitate pectin.
- Collect Ca pectate, wash, dry and weigh.
- Degree of esterification(DE): The isolated pectin is washed with acidified alcohol to convert to acid. After washing , titration with NaOH solution gives nonesterified groups. After saponification of pectins, back titration with standardised acid solution to determine excess base gives DE.

# "Dietary Fiber"
# Food components not digestible by mammals
## (Ch.12)

- Help protect against colon cancer
- Help normalize blood lipids
- Reduce cardiovascular diseases
- Slow glucose absorbtion and insulin secretion
- Helps prevent constipation
- DRV(Dietary Reference Value): 25g/2000kcal

# Structural Polisaccharides:

**Structural Polysaccharides are components of cell walls of plants**

Plant cell wall contains

- Lignin(nonCHO)
- Celulose
- Hemicellulose

→ Crude fiber**

Dietary fiber* → Pectin, Gums, Mucilage → Solublefiber

- Proteins&Lipids

Dietary Fiber*: Sum of the nondigestible components of foods

a. Insoluble fiber : CrudeFiber**:
b.Solublefiber:Pectin+Gums+Mucilage(Hydrocolloids)

Total , soluble, insoluble dietary fiber contents of selected foods
g/100 g of Food

| Food | Soluble DF | Insoluble DF | Total DF |
|---|---|---|---|
| Apricots | 0.53 | 0.59 | 1.12 |
| Prunes | 5.07 | 4.17 | 9.29 |
| Carrots | 1.10 | 2.81 | 3.93 |
| Green Beans | 1.02 | 2.01 | 2.89 |
| Dry beans | 1.41 | 5.25 | 6.66 |
| Oat bran | 7.71 | 9.73 | 16.92 |

**Botanists classify fiber as:**

1. **Cell wall Polysaccharides**
2. **Non-cell wallpolysaccharides**

1. **Cell wall P:S.:**
   **Cellulose**(long chain molecules made up of glucose arranged in microfibrils, with parallel groups forming strong bundles)
   **Hemicellulose**(heterogeneous molecules containing a number of sugars,and tying cellulose microfibrils; soluble in dilute alkali but not in water,
   **Pectin**(uronic acid polymer soluble in hot water and forms gels)

**2. Non-cell wall P.S.:P**olysaccharides not involved in cell wall structure and are not nutritional. They include hydrocolloids such as mucilages(guar and locust beans gums ) plant exudate gums(arabic, tragacanth gums) and algal polysaccharides(alginates, agar. Carrageenan). Generally they form viscous solutions in water, but insoluble in alcohols.

Lignin is a non-CHO three dimensional polymer with ~40 phenol units covalently linked to hemicellulose.

# Dietary Fiber METHODS

- 1. Gravimetric: DigestibleCHO's, lipids, proteins are selectively solubilized by chemicals and enzymes ; undigestibles are collected by filtration and quantitated by weighing.

- 2. Chemical: Digestible CHO's are removed by enzymatic digestion, fiber components are hydrolysed by acid, and monosaccharides in the acid hydrolysate represent fiber.

**Extraction Procedures for Selected fiber components:**

**Cellulose:** Insoluble residue from extraction with 17.5%NaOH; can be solubilized by adding 72 % $H_2SO_4$

**Pectin:** Extract with hot water or aqueous ammonium oxalate (0.5%). which chelates calcium and solubilizes otherwise insoluble Ca pectate molecules. Then precipitate with 95% ethanol.

H**emicelluloses:** Extract with 25%KOH or 10% NaOH; make extract acidic by adding acetic acid. Then precipitate by adding 4 volumes of ethanol

**Lignin:**. Delignification: Treat with Cl or sulfite to break chain

## a. Gravimetric Methods:

**a.1. Crude Fiber** :: Since 1850s, it is being determined as material insoluble in dilute acid and dilute alkali under specified conditions (Weende method ). Sequential extraction of sample with 1.25 N $H_2SO_4$, 1.25NNaOH, the insoluble residue being dried weighed, ashed to correct for minerals. Tecator Inc. has automated the Weende method (FiberTec).

This analysis does not detect hemicellulose, pectins and hydrocolloids, but only cellulose and lignin. Southgate has developed the method.

### a.2.Detergent Methods:

Two methods have been developed:

1. Acid detergent fiber method : determines lignin + cellulose
2. Neutral-detergentfiber= determines acid detergentfiber+ hemicellulose

NDF = Neutral-detergent fiber (hemicellulose+cellulose+lignin)

NDF-ADF = hemicellulose

(Neither include pectins or hydrocolloids)

# Detergent Method for Dietary Fiber

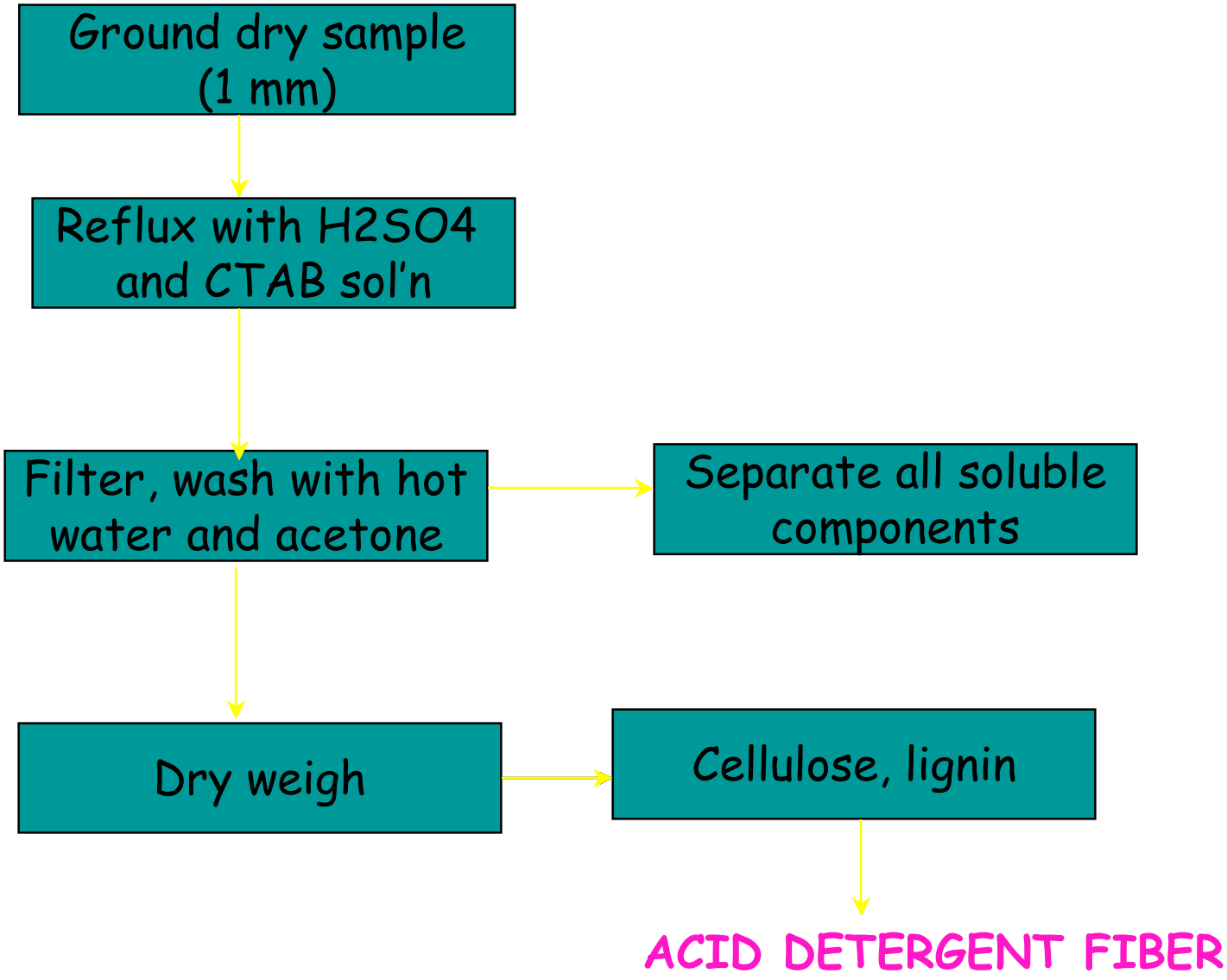



**Fig 1: The acid detergent fiber method**

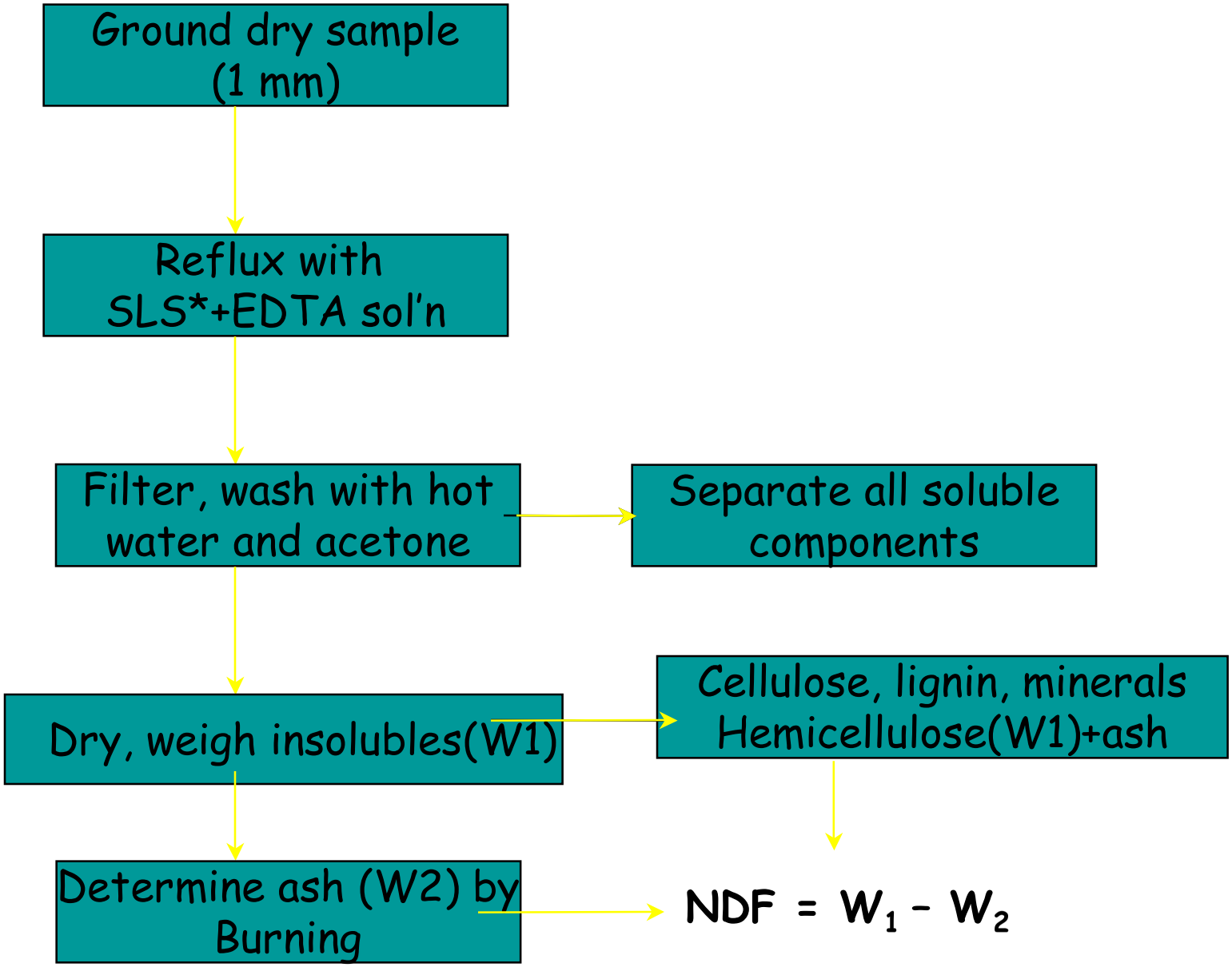



**Fig 2: The neutral detergent(*SLS:SodiumLaurylSulfate) fiber method**

# Enzymatic Method for Dietary Fiber

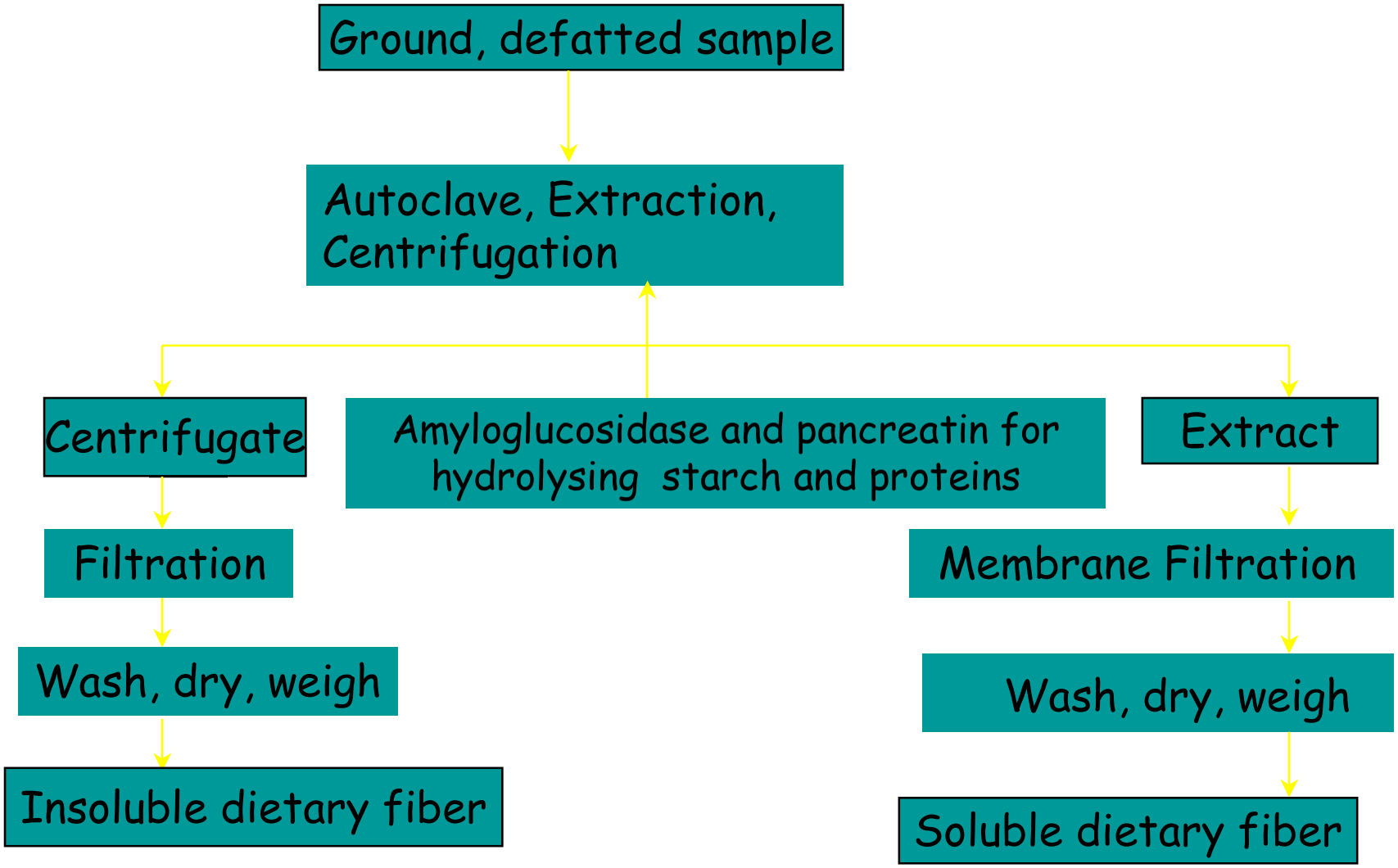



**Fig 3: Determination of dietary fiber using enzymatic(Meuser) method**

# *a.3.* The AOAC Method

- This method combines crude fiber, detergent fiber and Southgate metodologies (see fig.12.1, and Table 12-1, p.192-5 for total dietary fiber analysis).
- Principle:
  Dry fat-extracted ground food samples are enzymatically digested with $\alpha$- amylase, amyloglucosidase, and protease to remove proteins and starch. Insoluble fiber is collected by filtration. Soluble fiber is precipitated by bringing filtrate to 78 % ethanol and collected by filtration. The filtered fiber residues are washed with ethanol and acetone, oven –dried and weighed.

The fiber residue is analysed for protein and ash.

Total fiber= residue fiber weight- (wt of protein + wt of ash)

# b. Chemical Dietary Fiber Analyses ENGLYST-CUMMINGS Procedure

Definition:

Fiber= Sum of all non-starch monosaccharides + lignin

Principle of ENGLYST-CUMMINGS Procedure:

Starch is first gelatinised and then enzymatically (pullulanase and amyloglucosidase)digested; remaining non-starch polysaccharides are hydrolysed by $H_2SO_4$ to liberate free monosaccharides, which are determined either by GC or by colorimetric methods. The result is total dietary fiber.

To measure insoluble fiber, extract the water-soluble fiber by heating the digested mixture for 30 minutes in a boiling water-bath.The precipitate after centrifuging is insoluble fiber. So continue the same way with this precipitate with the hydrolysis step.

Soluble fiber= Total fiber-Insoluble fiber

Drawbacks:lignin and resistant starch are not measured by this method.

Any sugar initially present should be extracted previously, or will yield high results..

See Fig. 12-2, p. 196-7.