Polysaccharides

Definition and characteristics

- Polysaccharides are polymers of monosaccharides
- May be linear or branched
- Degree of polymerization (DP) in the range 200-3,000
  - Some are even larger (DP 7,000-15,000)
- Nomenclature: glycans
  - an ending denotes polysaccharide

Polysaccharides by source

- Seaweed extracts
  - Agars, algins, carrageenans
Polysaccharides by source

- Higher plants
  - Insoluble
    - Cellulose
  - Extract
  - Pectins
  - Seeds
    - Starch, guar

- Higher plants
  - Tubers and roots
    - Potato starch
  - Exudates
    - Gum arabic, gum tragacanth

- Microorganisms (fermentation gums)
  - Xanthan, gellan
**Polysaccharides by source**

- Derived
  - Cellulose
    - CMC, methylcellulose
  - Starch
    - Starch acetates, starch phosphates,
  - Synthetic
    - Polydextrose

**Polysaccharides by structure**

- By shape
  - Linear
    - Algins, amylose, cellulose, pectins
  - Branched
    - Short branches
      - Arabinans, galactomannans, xanthan, xylan
    - Branch-on-branch
      - Amylopectin, gum arabic, gum tragacanth

- By monomeric units
  - Homoglycans
    - Amylose, arabinans, cellulose
  - Diheteroglycans
    - Algins, carrageenans, galactomannans
  - Triheteroglycans
    - Arabinoxylans, gellan, xanthan
**Diheteroglycans**

Generally structure is one of the following:

- \( \text{AAAAAAAAABBBBBBBBBAAAAAAAAA} \)
- \( \text{BBBBBBBBBBBBBBBBBBBBBBBBBBBBBB} \)

**Polysaccharides by structure**

- Tetrahexaglycans
  - Gum arabic, psyllium seed gum
- Pentaheteroglycan
  - Gum ghatti, tragacanthin

**Polysaccharides by structure**

- By charge
  - Neutral
    - Amylose, arabinans, cellulose, galactomannans
  - Anionic
    - Algins, carrageenans, gellan, gum arabic, pectic acids, xanthan
Polydispersity

- All polysaccharide preparations are polydisperse
- This means they contain molecules covering a range of DPs

```
GGGGGGGGGG
GGGGGGG
GGGGGGGGGGGGGG
```

This collection of molecules is polydisperse

Polymolecularity

- The chemical structures of most plant polysaccharides vary in linkage types and/or proportions of monosaccharide components from molecule to molecule
- These are said to be polymolecular

```
AAABBB
AAAAAB
AABB
```

These molecules are polymolecular

Most food gums are both polymolecular and polydisperse

Polysaccharide preparation

- Plant tissue is extracted to remove interfering substances such as lipids and lignin
- Most often polysaccharide is extracted with an alkaline solution
- Polysaccharide is isolate by precipitation with ethanol (lab) or 2-propanol (commercial)
Polysaccharide preparation

Sample (wet)

Dry (freeze dry)

Dry sample

Fat extraction, 95:5 CHCl3/MeOH

Soluble sugars and other low MW compounds

70-80% aqueous EtOH, boiling

Fat free sample

Polysaccharide preparation

Fat free sample

Proteolytic digestion

Protein free, fat free sample

EtOH

Insoluble polysaccharides

Polysaccharide preparation

Insoluble polysaccharides

Amylase treatment

Starch free polysaccharide

Resuspend, centrifuge

Soluble polysaccharides

EtOH

Insoluble, dietary fiber (cellulose, hemicellulose)

EtOH insoluble polysaccharides
Fractional precipitation

- A well defined polysaccharide
- A polymolecular, polydisperse polysaccharide

Chromatography

- Size exclusion: Doesn’t work too well due to the polydispersity of most polysaccharides
- Ion exchange
- Affinity

Size exclusion separation problem

- Pure A
- Impure A and B
- Pure B
**Structural analysis**

- Determination of
  - Monosaccharide composition
  - Linkage types
  - Anomeric configuration
  - Presence and location of substituent groups
  - Degree of polymerization/Molecular weight

**Structural characterization**

- **Monoaccharide composition**
  - Acid-catalyzed hydrolysis
  - HPLC or
    - GLC after conversion to alditol acetates

- **Linkage determination**
  - Polysaccharide is methylated with methyl iodide and strong base in DMSO
  - Hydrolysis
  - Reduction to partially methylated alditols
  - Acetylation to methylated alditol acetates
  - Determination by GLC/MS

  See W&B, Figure 4.2, page 69
Shorthand notation

- Glycosyl units are represented by the first 3 letters of their names, except for glucose which is GLC
- D is omitted; if it is an L sugar, the L is put in the designation
- Ring size: p or f
- Uronic acids are indicated by a suffix A
- α and β are designated where appropriate

Examples

- Cellobiose = βGlc(p1→4)Glc or βGlc1,4Glc
- Lactose = βGal(p1→4)Glc or βGal1,4Glc
- Maltose = αGlc(p1→4)Glc or αGlc1,4Glc

Sequence characterization

- Partial depolymerization is carried out with acid or enzymes
- Determine structures
- Compare possible structures of polysaccharides to those of the hydrolytic oligomers
- Determine which one of the possible structures could produce the oligomers
### Anomeric configuration
- Done from intact polysaccharides or from oligosaccharides
- Done by
  - Enzymatic analysis—enzyme is specific for a particular type of linkage, e.g., β-galactosidase
  - Nuclear magnetic resonance (NMR)—measurement of coupling constants

### Substituents
- Possible substituents
  - Acetate ester
  - Sulfate half-ester
  - Phosphate esters
  - Pyruvyl cyclic acetal groups

### Molecular weight (DP)
- Determined by physiochemical methods including size exclusion chromatography
- Can use number average molecular weight, $M_n$, or weight average molecular weight, $M_w$
Number average MW

- Determined by osmotic pressure
- \( M_n = \frac{\sum N_i M_i}{\sum N_i} \)
- Where \( N_i \) = number of molecules and \( M_i \) = molecular weight of the \( i \)th molecule
- This determination of MW is more weighted by the small molecules in the molecular population

Weight average MW

- Determined by light scattering
- \( M_w = \frac{\sum N_i (M_i)^2}{\sum N_i M_i} \)
- This determination is more heavily weighted by the large molecules

Sample calculations

- Suppose we have a collection of molecules with molecular weights of 1,000 and 100,000
- If we have equal numbers of molecules, then
- \( M_n = 50,500 \)
- \( M_w = 99,020 \)
Sample calculations

- The ratio $M_w/M_n$ is a measure of polydispersity and in this case is equal to 1.96.
- On the other hand, if we have equal weights of molecules then:
  - $M_n = 1980$
  - $M_w = 50,500$
  - $M_w/M_n = 25.5$

$M_w/M_n$

- The interpretation of this ratio is that as its value increases there is a larger difference between the sizes of the largest molecules and the smallest molecules.

Water absorption

- Polysaccharides modify and control the mobility of water in foods.
- Thus, they have a profound effect on many food physical properties, especially texture.
Moisture sorption isotherms

<table>
<thead>
<tr>
<th>Zone I</th>
<th>Zone II</th>
<th>Zone III</th>
</tr>
</thead>
<tbody>
<tr>
<td>monolayer water, very strongly bound</td>
<td>multilayer water, less strongly bound</td>
<td>bulk phase water, not bound</td>
</tr>
</tbody>
</table>

Water zones analogy

Zone I = water in your skin (very tightly bound)
Zone II = water in your swimming suit (less tightly bound)
Zone III = water in the pool (not bound)

Effect of water on polysaccharides

- Very strongly bound zone I and zone II water does not freeze
- Exhibits a plasticizing effect on the hydrated polysaccharide
Solubility and solution characteristics

- Highly linear structures are quite water insoluble
  - They prefer their own company
  - These polysaccharides consist of both crystalline and amorphous regions
- Examples
  - Cellulose
  - Mannan
  - Chitin

Solubility and solution characteristics

- Polysaccharides become more water soluble in proportion to the degree of chain irregularity or branching

Dissolution methods

- These finely powdered, highly hydrophilic molecules (gums or hydrocolloids) are not the easiest to get into water dispersion
- Particles tend to hydrate too quickly, forming what are known as fish eyes
To avoid fish eyes

- Sift the gum slowly into a vortex of rapidly stirred water (high shear)
- Disperse with another hydrophilic (but lower molecular weight) material, e.g., sucrose, glycerol, propylene glycol
- Use an agglomerated gum which hydrates more slowly
- Raise the temperature after initial dissolution

Properties of gum dispersions

- Thicken or gel water dispersions at 0.25-0.5%
- Viscosity is a function of molecular shape in dispersion (assuming similar molecular weights)
  - Linear: higher viscosity
  - Branched: lower viscosity

Effect of molecular shape on viscosity
**Molecular shape in dispersion**

- Influenced by
  - Location of anomeric bonds
  - Alpha or beta configuration

**Effect of DP on viscosity**

- Cleavage of a single molecule in the middle gives two molecules of half the original size
- However, this reduces the viscosity to one-fourth of the original value
- Thus, it doesn’t take many cleavages to **significantly** reduce the viscosity

**Anomeric linkage and shape**

- **Cellulose**
  - Lots of hydrogen bonding--rigid, flat, ribbon-like, highly extended molecule
- **Starch**
  - Less hydrogen bonding--random coil
Non-uniformity of shape

- Non-uniformity along the polymer chain decreases intermolecular interaction and allows greater chain folding.

Effect of charge

- Ionic polysaccharides (in their charged state) adopt a fully extended shape (due to Coulombic repulsion) and thus impart great viscosity to their solutions.

Colligative properties

- Polysaccharides have little effect on osmotic pressure or freezing point depression because they are used at such low concentrations and are of such high molecular weight.
Junction zones will naturally increase in size over time (fringed micelle).
This may adversely affect the texture of the food in which the polysaccharide is found.
This increase in size may be accelerated by cooling.
In starch, this junction zone growth accompanies retrogradation.
Junction zones

- Tendency to form junction zones is highest among linear glycans
- This tendency is reduced if the glycan is derivatized, has short branches, or is charged

Gelation
Notice areas of association. These are called junction zones.

This picture is not yet complete as we haven’t accounted for the water in the system.

This is a gel.
The texture gets very ugly when this happens.
Polysaccharide modification

- Usually these modifications involve:
  - Derivatization
  - Depolymerization
  - Oxidation
  - Transglycosylation

Derivatization

- Hydroxyl groups can be etherified or esterified
- These are characterized by the average number of hydroxyl groups per glycosyl unit (degree of substitution, DS)
- For most polysaccharides, the DS_max is 3

Molar substitution

- When hydroxyethyl or hydroxypropyl groups are introduced, the resulting substituent group contains a hydroxyl group that can undergo further reaction
- Here we use molar substitution (MS) to denote the average number of moles of substituent added to a glycosyl moiety
**Characterization**

- Modified polysaccharides are characterized by their
  - Molecular weight
  - Physical properties
  - DS or MS
- Distribution of substituents along the length of the chain will vary with reaction conditions, reagent type, and extent of substitution

**Depolymerization**

- Hydrolysis
- Oxidation-elimination

**Hydrolysis**

- Catalyzed by
  - Acid (H⁺)
  - Enzymes
**Factors affecting depolymerization**

- **pH**: Lower the pH, the more rapid the hydrolysis
- **Time and temperature used in the process**
- **Nature of the glycosidic linkages**
  - Monosaccharide units
  - Anomeric configurations
  - Linkage positions

**Sugar structure and hydrolysis**

- Furanosides are more susceptible to hydrolysis than pyranosides
- Deoxy sugars are more acid labile than non-deoxy sugars
- Uronic acids are much less susceptible to hydrolysis than normal monosaccharides
Importance in foods

- These reactions may occur during food processing and decrease viscosity or gel strength
- Can possibly be overcome by using
  - More gum
  - A higher viscosity grade gum
  - A more acid stable gum

Enzyme-catalyzed hydrolysis

- Rates of reaction and end products are under control of
  - Enzyme specificity
    - Depends on anomeric configuration, linkage position, nature of the neighboring units
  - pH
  - Time/temperature
  - Other environmental factors
    - Salt concentration

Microbial degradation

- Gums may be susceptible to microbial (enzymatic) hydrolysis
- Commercial gums may not be sterile
- They should be sterilized or preserved
- Preservatives
  - Sodium benzoate (0.1%)
  - Methyl p-hydroxybenzoate (0.17%)
  - Propyl p-hydroxybenzoate (0.03%)
  - Sorbic acid (0.7%)
Oxidation-elimination

- Converts a hydroxyl group into a carbonyl group
- Usually catalyzed by a transition metal ion
- These reactions may produce off-flavors and aromas
- Several mechanisms have been proposed

β-Elimination 1

β-Elimination 2