Why study “starch fine structure”? 

- To differentiate starches (and starch derivatives) at molecular levels
- To define and document unique starches or starch derivatives (e.g. maltodextrins) in both research reports and patents
- To monitor product profiles and maintain product consistency
- To control important properties (digestibility, retrogradation, solubility, water adsorption, viscosity, gel strength, stability) of starches and starch derivatives
- To define goals for starch modifications
- To guide new product development
Starch fine structure is usually characterized by chain length distribution using cluster model.

Chains are non-randomly clustered.

Chain Length Distribution

- Chain length distribution of following materials have been characterized:
  - Starch (containing both amylose and amylopectin)
  - Isolated amylopectin
  - Beta-limit dextrin of amylopectin
  - Other starch derivatives

- Then, but not always, the data of chain length distribution are used to calculate structural parameters of starch or AP clusters
These figures (by HPSEC) are called “chain length distribution (profile)”

MW, MN, and polydispersity index can be calculated from the curve

Usually, the chain length profiles are compared among different starches, to differentiate starches at molecular level

Starch molecules need to be debranched to release chains
A procedure to prepare debranched starch materials

Starch extraction

Dispersed in DMSO

Adding buffer + isoamylase

Incubation

Moisture evaporated

Debranched starch in DMSO

Waxy starches do not need AP isolation

Amylopectin isolated

Adding buffer + beta-amylase

Incubation

Moisture evaporated

Debranched AP in DMSO

Adding buffer + isoamylase

Incubation

Beta-limit dextrin (BLD) extraction

Re-dispersion in DMSO

Adding buffer + isoamylase

Incubation

Debranched BLD in DMSO

Pullulanase needed to remove short stubs (DP2)

Adding pullulanase

Incubation

Moisture evaporated

Incubation
Analysis of chain length distribution

- Three types of debranched starch material are usually prepared
  - Debranched starch
  - Debranched amylopectin
  - Debranched beta-limit dextrin
- Three types of separation methods are often used to describe the chain length distribution of these materials
  - Size exclusion chromatography (SEC or HPSEC)
  - Fluorophore-assisted carbohydrate electrophoresis (FACE)
  - Anion exchange chromatography (HPAEC)
Size exclusion chromatography (SEC or HPSEC)

By Dr. Shulamit Levin, http://www.forumsci.co.il/HPLC/modes/modes14.htm
Chain Length Distribution

- Eluent from HPSEC columns passes through a refractive index (RI) detector for quantifying the mass of carbohydrate molecules
- Molecular standards are used to calibrate columns and determine the molecular weight of samples
- Eluent may pass through a multi-angle laser light scattering (MALLS) detector for molecular weight determinations
Chain Length Distribution

Calibration curve and MW distribution of SEC

By Dr. Shulamit Levin, http://www.forumsci.co.il/HPLC/modes/modes15.htm,
Fluorophore-assisted carbohydrate electrophoresis (FACE): labeling of oligosaccharide using 1-aminopyrene-3,6,8-trisulfonate (APTS)

Oligosaccharide + APTS $\xrightarrow{\text{NaBH}_3\text{CN}}$ APTS adducts

Excitation 488 nm
Emission 520 nm

Courtesy of Beckman-Coulter
APTS adducts of carbohydrate molecules have 2 properties

- The APTS adducts are negatively charged, so may migrate in an electric field of electrophoresis
- The APTS adducts may release detectable fluorescent emission with laser excitation at 488 nm
**Chain Length Distribution**

Molecular weight is determined by migration time
Number of molecules is determined by fluorescent signal

Longer migration time due to larger CHO molecule
Weaker fluorescent signal due to fewer molecules

Shorter migration time due to smaller CHO molecule
Stronger fluorescent signal due to more molecules
FACE conducted using capillary electrophoresis with laser-induced fluorescence

Courtesy of Beckman-Coulter
Electrophoreogram of FACE

Courtesy of Beckman-Coulter
Comparison between HPSEC and FACE

**HPSEC**

**FACE**
High performance anion-exchange chromatography equipped with pulsed amperometric detector (HPAEC-PAD)

Sample molecules, different types of charge or neutral

Stationary phase, positively charged

Mobile phase, negatively charged

Negatively charged sample molecules adsorbed by stationary phase may be desorbed by mobile phase

Courtesy of Amersham Biosciences
The adsorption/desorption equilibrium is governed by:

- The amount of negative charge of sample molecules
- The ionic strength of mobile phase

The greater the net charge, the stronger the adsorption.

The greater the net charge, the higher the salt concentration required for desorption.

Courtesy of Amersham Biosciences
The retention time of a molecule is determined by

- Its net negative charge, which may be affected by pH
- Elution power, which is controlled by gradient elution

*Courtesy of Amersham Biosciences*
Carbohydrates can be separated via anion-exchange

- In basic solution (high pH), carbohydrates are negatively charged.
- Thus, there exists an adsorption/desorption equilibrium of carbohydrate molecules with stationary phase.
- The higher DP of carbohydrate molecule, the greater net negative charge it possesses, the higher ionic strength (salt concentration) needed to desorb the molecule.
- Using gradient eluent, carbohydrate molecules with different DP are eluted at different retention time and separated.
PAD catalyzes the electrooxidation of carbohydrate molecules in high pH solutions, and gives signals proportional to the amount of molecules.

Typical waveform for PAD in alkaline solution at a gold working electrode (Courtesy of Dionex Corporation)
HPAEC-PAD gives a chromatogram similar to FACE

Chain length distribution of debranched amylopectin of sorghum starch by HPAEC-PAD
Pros and cons of HPSEC, FACE, and HPAEC

- HPSEC provides information of a broad range of molecular weight (>DP10,000) but with relatively low resolution >DP5
- FACE provides baseline resolution up to DP100, but unable to (with current techniques) give a chain length profile with broader DP range
- HPAEC is similar to FACE
Chemically Modified Starches

- Converted starches
  - Acid conversions
  - Oxidized starches
  - Pyroconversions or dextrinizations
- Cross-linked starches
  - Distarch phosphate
  - Distarch adipate
- Stabilized starches
  - Starch acetate
  - Starch phosphate
  - Starch sodium octenyl succinate
  - Hydroxypropylated starch
## Chemically Modified Starches

### Properties and applications of regular modified starches

<table>
<thead>
<tr>
<th>Process</th>
<th>Function/property</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid conversion</td>
<td>Viscosity lowering</td>
<td>Gum candies, formulated liquid foods</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Adhesion, gelling</td>
<td>Formulated foods, batters, gum confectionery</td>
</tr>
<tr>
<td>Dextrins</td>
<td>Binding, coating, encapsulation, high solubility</td>
<td>Confectionery, baking (gloss), flavorings, spices, oils</td>
</tr>
<tr>
<td>Cross-linking</td>
<td>Thickening, stabilizing, suspension, texturizing</td>
<td>Pie filling, breads, bakeries, puddings, infant foods, soups, salad dressings</td>
</tr>
<tr>
<td>Esterification/Etherification</td>
<td>Stabilization, low temperature storage</td>
<td>Emulsions, soups, frozen foods</td>
</tr>
<tr>
<td>Pregelatinization</td>
<td>Cold water swelling</td>
<td>Premix</td>
</tr>
<tr>
<td>Dual modifications</td>
<td>Combinations of properties</td>
<td>As you can imagine...</td>
</tr>
</tbody>
</table>
Converted starches

- To reduce the viscosity and swelling power and increase the concentration in the dispersions

- Acid-thinned starch:
  - Starch suspension is treated with dilute acid at a temperature below the gelatinization point
  - Granular form of the starch is maintained and the reaction is ended by neutralization, filtration, and drying once the desired degree of conversion is reached
  - This results in a reduction in the average molecular size of the starch polymers. Acid-thinned starches tend to have a much lower hot viscosity than native starch and a strong tendency to gel when cooled.

- Oxidized starch
  - Starch suspension is usually treated with sodium hypochlorite
  - Commercial oxidized starch is granular and is insoluble in cold water
  - It is characterized by a whiter color than native starch, increased paste clarity and a low, stable viscosity on storage of the paste

- Dextrin
  - Starch hydrolysis products obtained in a dry roasting process either using starch alone or with trace levels of acid catalyst
  - Dextrins are characterized by good solubility in water to give stable viscosities. Four types exist: White, Yellow, British Gums and Solution-stable Dextrins
Cross-linked starch

- Cross-linked (bonded) starch
  - Starch is treated with bi-functional reagents so that a small number of the starch polymer chains are chemically linked by the cross linking reagent
  - Cross-linking inhibits granule swelling on gelatinization and gives increased stability to acid, heat treatment, and shear forces
  - Cross-linking is widely used to prepare chemically-modified starches for the processed food industry

- Distarch phosphate
  - A starch cross-linked with a phosphate linkage, e.g. from reagents such as sodium trimetaphosphate or phosphorus oxychloride

- Distarch adipate
  - A starch cross-linked with an adipate linkage, from adipic acid
  - Most cross-linked food starches contain less than one crosslink per 1000 glucopyranosyl units
Starch stabilization

- “stabilization” is sometimes used to indicate the presence of a monofunctional chemical substituent which has the effect of stabilizing paste viscosity

- Stabilized modified starches may be hydroxypropyl or carboxymethyl starch ethers

- The monofunctional substituents also can be phosphate or acetyl ester groups

- Generally the D.S. (degree of substitution) of these starches is between 0.01 and 0.2. The substituent groups have the effect of providing steric hindrance to chain association which stabilizes viscosity by preventing possible retrogradation.
Chemically Modified Starches

Starch alkenyl succinate

- **Starch alkenyl succinate**
  - A chemically modified starch produced by treating starch with alkenyl succinic anhydride under controlled pH conditions
  - Commercial alkenyl succinic anhydride available for use in food is the octenyl form
  - These starches have lipophilic ("oil-loving") properties and are used in emulsions and encapsulation

- **Starch octenyl succinate**
  - Common name given to Starch n-Octenyl succinate
  - Made by treating starch with n-Octenyl succinic anhydride at pH 8-8.5
  - Anionic due to a carboxyl group and hydrophobic due to the C8 unsaturated alkene chain
  - Food uses include encapsulation of flavors and emulsion stabilization
Pregelatinized Starch

Pregelatinized starch

- A type of starch which has been gelatinized and dried by the manufacturer before sale to the customer in a powdered form

- Pregelatinized starch can be made by drum drying, spray drying, or extrusion from either native or modified starch

- Pregelatinized starch develop viscosity when dispersed in cold or warm water without the need for further heating

- Pre-gelatinized starch is also known as precooked starch, pregelled starch, instant starch, cold water soluble starch, or cold water swelling starch (CWS)

- The degree of granular integrity and particle size have a major influence on their properties, e.g. dispersion and texture
Starch digestion

- Starch is digested in the human body
  - Firstly by $\alpha$-amylase (salivary and pancreatic)
  - Yields maltose, maltotriose, and $\alpha$-dextrins
  - Maltose and maltotriose are hydrolyzed to glucose by maltase
  - $\alpha$-dextrin by $\alpha$-dextrinase (intestinal brush border enzymes)
  - $\alpha$-Dextrinase (glucoamylase) is found at the brush border of the small intestines. It hydrolyzes $\alpha$-D-(1,4) and $\alpha$-D-(1,6)-linkages to produce glucose for absorption

- Other carbohydrate–digesting enzymes
  - Sucrase & Lactase

Adapted from Dr. Hamaker’s lecture in Starch Short Course
Starch digestion

- Starch, maltodextrin, glucose, sucrose, lactose make up “net carbs”
- Fiber constituents are not digested by the body’s enzymes
- Starch undigested by host enzymes, or “resistant starch” is usually readily digested in the proximal colon by bacterial amylases, and is about 20% utilized as energy for the body

Adapted from Dr. Hamaker’s lecture in Starch Short Course
Resistant starch

- **Resistant starch (RS)**
  - Starch that is resistant to digestion by $\alpha$-amylase
  - Defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals."
  - While RS escapes digestion in the small intestine, it may be fermented in the large intestine by colonic microflora

- **RS has been classified in four different categories**
  - Type I, resulting from physical inaccessibility in intact tissues or other large particulate materials
  - Type II, resulting from the physical structure of the uncooked, native starch granules
  - Type III, resulting from the physical structure of retrograded starch molecules after the starch granules are gelatinized
  - Type IV, resulting from chemical modification (e.g. cross-linking) that interferes with the enzyme digestion.