QUALITY WHEAT CRC PROJECT REPORT

Project 5.2.1 University based education

Short Course: Polymer Colloids in Cereal-Based Foods

Editor: Chris Fellows

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Polymer Colloids in Cereal-Based Foods

A Short Course Offered by the
Key Centre for Polymer Colloids
The University of Sydney
and the
Quality Wheat CRC Ltd

6–8 June 2000
in The University of Sydney
Polymer Colloids in Cereal-Based Foods

Key Centre for Polymer Colloids

Quality Wheat CRC Ltd.

Tuesday 6 June

Architecture Lecture Theatre 2, University of Sydney

8:30 Registration

9.30 – 9:35 Introduction to the Key Centre for Polymer Colloids

9:35 – 9:40 Introduction to the Quality Wheat CRC Ltd.

9.40 – 11.00 Introduction to polymer colloids – structure, function, synthesis, applications

(Professor Bob Gilbert, University of Sydney)

11.00 – 11.30 Break

11.30 – 12.45 Polymer colloids in cereal-based foods – chemistry and biochemistry

(Professor Bob Gilbert, University of Sydney)

12.45 – 13.30 Lunch

13.30 – 14.30 Rhcology and cereal-based foods (Professor Roger Tanner, University of Sydney)

14.30 – 15.30 Starch granule structure (Associate Professor Norm Cheetham, University of NSW)

15.30 – 16.00 Break

16.00 – 17.00 Cereal-based food extrusion - on-line NIR spectroscopy to measure quality changes

(Dr Rachel Kelly, Food Science Australia)

18:00 Mixer followed by dinner at Selera Malaysian Restaurant, 264 King St. Newtown

($25 per head)
Polymer Colloids in Cereal-Based Foods

Key Centre for Polymer Colloids

Quality Wheat CRC Ltd.

Wednesday 7 June

9.15 – 10.15  Overview of analytical methods for polymer colloids (Professor Bob Gilbert)
10.15 – 11.15 Particle size analysis (Professor Bob Gilbert and Dr Chris Fellows, University of Sydney)
11.15 – 11.45 Break
11.45 – 12.45 Characterisation of proteins in cereal grains
            (Dr Colin Wrigley, Grain Quality Research Laboratory, CSIRO Plant Industry)
12.45 – 13.30 Lunch
13.30 – 14.30 Uses of starch in foods and industrial applications (Dr Ken McNaught, Starch Australasia)
14.30 – 15.30 Field flow fractionation – what is it and what can it do?
            (Dr Ian McKinnon, Monash University)
15.30 – 16.00 Break
16.00 – 17.00 Scientific underpinnings of gene technology regulation in the food industry
            (Dr Geoff Annison, Food and Grocery Council of Australia)
Thursday 8 June

9:30-10:50  Chemistry Building
Tour of facilities at the Key Centre for Polymer Colloids
(Mrs Jelica Strauch and Dr Brian Hawkett)
Demonstration of starch debranching and Gel Permeation Chromatography of starches
(Mr Herbert Chiou)
Demonstration of Capillary Hydrodynamic Fractionation
(Mr Joshua Taylor)

11:00-12:00  Madsen Building
Tour of the Key Centre for Microscopy and Microanalysis (Prof David Mackenzie)

12:30 – 1:30  Mechanical Engineering Building
Demonstration of Rheological Techniques (Dr Mattik Keentok and Mr Marcus Newberry)

1:30 – 2:30  Staff Club
Lunch and Informal Discussion
Introduction to polymer colloids –
structure, function, synthesis, applications

Prof Bob Gilbert

Key Centre for Polymer Colloids, University of Sydney
Key Centre for Polymer Colloids

Established and supported under the Australian Research Council Key Centres Program

A new era?
- Using methods developed for artificial polymer colloids for natural ones:
- Characterization
- Mechanism of formation by enzymes
- Data can help plant breeders, GM, ...

Polymer colloid science

What are polymers?

Many molecules ("monomers") joined together like beads on a necklace:

- e.g. ethylene, polyethylene ("Gladwrap®")

Typically 10^4 monomers per polymer chain
Polymers are everywhere

"Plastic" -- poly(vinyl chloride)

Cellulose (a component of wood):

What are colloids?
- Tiny solid particles suspended in water
- Examples: clay, paint, starch
- A polymer colloid contains many polymer chains
- Stabilizers (e.g. soap) to stop particles coagulating

What they look like
- Electron micrograph of a polystyrene polymer colloid:
- Often very monodisperse (all particles have same size)
Colloidal crystals

- The colloidal suspension can form regular arrays:
  Results in opalescence (diffraction because particles align with glass walls)

Polymer colloids are everywhere

- Foods containing starch
- Alginate hydrocolloids (dental impressions)
- Paints
- Adhesives
- Soil conditioners
- Latexes for diagnostic kits
- Tyres (made by emulsion polymerization)

Highly diverse

- Considerable economic importance in Australia
- Commonality not previously unified
- Range of appropriate experts in KCPC

Artificial & natural polymer colloids

Artificial
- Made by emulsion polymerization
- Free-radical polymerization
- Discrete phase (particle) usually organic polymer, disperse phase usually water
- Paints, adhesives, paper coatings, flocculants, wet suits, tyres, ...

Natural
- Made by plants, animal's
- Made by enzymes
- Discrete phase usually water, particle phase hydrophobic or hydrophilic
- Natural rubber latex, starches (e.g. food texture), living cells, ...

The science required for polymer colloids

- Free radical polymerization
- Chemical engineering science
- Biochemistry
- Analytical chemistry
- Rheology
- Food chemistry
- Agricultural chemistry
- Adhesion science
- Colloid and surface science
- Diffusion
- Materials science
Biochemistry

- Understanding of enzymatic synthesis
- Understanding and control of species giving colloidal stability
- How properties of polymer colloid (e.g., molecular weight and degree of branching of starch) affect "end use" (food texture,...)

Analytical chemistry

- Characterizing polymer colloids by:
  - Particle size distribution
  - Molecular weight distribution
  - Colloidal stability
  - Copolymer composition
  - Morphology
  - Viscosity and mechanical properties (storage and loss modulus)

Rheology

- Flow properties of polymer colloid:
  - Viscosity of flour dough
  - Applying paper coating at high speed
  - Food processing

- Rheology of colloid or formed polymer is sometimes an important specification of commercial product

Food chemistry

- One of the things I have to find out about!
**Agricultural chemistry**

- Vast potential
  - Seed coatings
  - Mycrobacteriols
  - Controlled release

**Adhesion science**

- Polymer colloids are often used for surface coatings, including adhesives
- Need structure-property relations between molecular architecture, rheology, ..., and adhesion/cohesion

**Colloid and surface science**

- Colloidal stability of natural and artificial polymer colloids is an important property
- Industry:
  - Sometimes need high colloidal stability, e.g., paint in a can
  - Sometimes need controlled colloidal instability – e.g., coagulating latex to give “crumbs” of neoprene pellets from the latex in which polymer is formed
- Different types of stabilizers (ionic, polymeric, electrosteric – discussed later)
- Often have more than one type of stabilizer especially in natural polymer colloids

**Colloidal stability**

- Stability of colloid: what keeps particles from coagulating
- Sometimes need high stability, e.g., stopping paint or adhesive from coagulating
- Sometimes need low stability, e.g., solids from latex.
**Origin of stability**

- Electrostatic: double layer $\Rightarrow$ electrostatic repulsion

- Steric: thermodynamically unfavourable to compress chains in water phase

**Electrostatic stability**

- Basic theory: DLVO
- Improvements, e.g., Healy-Hogg
- Find potential of interaction between double layers from electrostatic repulsion of double layer held to charge density on surface of sphere (repulsion) and long-range (fluctuating dipole) attraction of dielectric inside spheres (attraction).

**Aims ...**

- What do YOU need from this course?
Polymer colloids in cereal-based foods –
chemistry and biochemistry

A/Prof Les Copeland
Department of Agricultural Chemistry and Soil Science and Key Centre for Polymer
Colloids, University of Sydney
Polymer colloids in cereal based foods - chemistry and biochemistry

Les Copeland
Key Centre for Polymer Colloids
Department of Agricultural Chemistry and Soil Science
University of Sydney

Foods
- Most foods are multi-component, polymer colloid systems
- Macro- and micro-components
- Important characteristics of components
  - molecular properties
  - physical properties
  - relationship between molecular structure and function
  - interactions between components (proteins, polysaccharides, lipids, minor constituents)
  - behaviour of components during processing, storage, digestion
  - contribution to food systems (nutrition, organoleptic properties)

Functional properties of foods
- Depend on properties of the components and interactions between them
- Proteins have good emulsifying and foaming properties
  - polyelectrolytes with molecular flexibility
  - hydrophilic and hydrophobic domains
- Polysaccharides are good stabilisers
  - have good water-holding, gelling and thickening properties
  - many texture modifying agents are polysaccharides (native and modified starches, NSPs, gums)
- Lipids act as emulsifying agents and have strong influence on taste and texture
  - Tm suited to melting and release of entrapped volatiles (flavours)

Interactions between proteins, polysaccharides and lipids
- Covalent bonds
- Ionic bonds
- H bonds
- Hydrophobic interactions

- Stability of assemblies influenced by
  - pH
  - ionic strength
  - temperature
  - water activity
Seeds

- Storage organs for nutritionally useful reserves
  - oligo- and polysaccharides
  - proteins
  - oils
  - minerals

- Low moisture content

- Toxic and anti-nutritional constituents afford protection against herbivory and pathogens

Composition of seeds

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Oil</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>12</td>
<td>3</td>
<td>76</td>
</tr>
<tr>
<td>Corn</td>
<td>10</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>Oats</td>
<td>13</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>Rice</td>
<td>8</td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>Rye</td>
<td>12</td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>Wheat</td>
<td>12</td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>Legumes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broad bean</td>
<td>23</td>
<td>1</td>
<td>56</td>
</tr>
<tr>
<td>Chickpea</td>
<td>23</td>
<td>6</td>
<td>66</td>
</tr>
<tr>
<td>Garden pea</td>
<td>25</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>Lentil</td>
<td>29</td>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>Peanut</td>
<td>31</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>Soybean</td>
<td>37</td>
<td>17</td>
<td>26</td>
</tr>
<tr>
<td>Oilsseeds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton</td>
<td>50</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Oil palm</td>
<td>9</td>
<td>46</td>
<td>28</td>
</tr>
<tr>
<td>Rape</td>
<td>21</td>
<td>48</td>
<td>19</td>
</tr>
<tr>
<td>Sunflower</td>
<td>30</td>
<td>45</td>
<td>22</td>
</tr>
</tbody>
</table>

* % dry matter

Proteins

- Macromolecular polyelectrolytes with highly flexible structures
- Greats diversity of size, structure, physical and chemical properties, functions

- Amino acid sequence determines structure and function
  - synthesised from 20 α-amino acids
  - sequence is genetically prescribed, with post-translational modifications

- Roles include
  - contribution to cellular and subcellular structure
  - energy transduction and mechanical work
  - catalysis
  - sensory, regulatory, signal perception and transduction
  - transport

Protein structure

Four levels of structure produce a flexible molecular architecture

Primary sequence of amino acids in the polypeptide

Secondary arrangement of polypeptide into helical and sheet-like configurations

Tertiary folding of helical and sheet regions

Quaternary interaction between subunits in multisubunit proteins
**Disulphide bridges**

![Chemical structure of disulphide bridge]

**Protein structure**

- **Primary structure**
  - Ala-Glu-Val-Thr-Asp-Pro-Gly-

- **Secondary structure**
  - [Helix structure]

- **Tertiary structure**
  - [Folded protein structure]

- **Quaternary structure**
  - [Protein complex structure]

**SEED STORAGE PROTEINS**

- Storage of N and S
- Complex mixtures of individual proteins
  - may be linked by intermolecular disulphide bonds, ionic bonds, H-bonds, and hydrophobic bonds
- Polymorphic
  - multiple forms with minor differences
    - products of multigene families
    - post-translational modifications
- Deposited in subcellular organelles (protein bodies)
- Determine the nutritional value and suitability of seeds for end uses

**Osborne classification of proteins based on solubility**

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Solubility Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumins</td>
<td>soluble in water and dilute buffers at neutral pH</td>
</tr>
<tr>
<td>Globulins</td>
<td>insoluble in water but soluble in salt solutions</td>
</tr>
<tr>
<td>Prolamins</td>
<td>soluble in 60-70% aqueous ethanol</td>
</tr>
<tr>
<td>Glutelins</td>
<td>soluble in dilute acid or alkali solutions</td>
</tr>
</tbody>
</table>
Prolamins

- Highly polymorphic mixtures of proteins of MW 30,000 - 90,000
- Classified into S-rich, S-poor and high molecular weight groups
  - S-rich group is the most important quantitatively
- Account for approximately half of the total grain N in wheat, barley and rye
- Have high gln and pro content
- Suggested to have evolved from a single ancestral protein

Gluten

- Soft, extensible proteinaceous material remaining after wheat flour dough is washed free of starch
- Gliadins
  - more readily extractable protein fraction of gluten
  - mixture of individual proteins with MW <100,000
  - high in glu and pro; low in lys, thr
  - extensible, but have relatively low elasticity
- Glutenins
  - least soluble fraction of gluten
  - mixture of high MW polymeric proteins
  - polypeptides joined by disulphide bonds
  - high elasticity but relatively low extensibility

Lipids

- Fatty acids based
  - glycerolipids
    - phosphoglycerides (membranes)
    - triacylglycerols (storage)
  - eicosanoids
  - cuticular waxes

- Polyisoprenoids
  - sterols
  - terpenes

Fatty acids

- α-Linolenate C18:3 (ω 3)
- γ-Linolenate C18:3 (ω 6)
- Olate C18:1
- Linoleate C18:2
- Stearate C18
Lipids

- Triacylglycerol
- Phospholipid (Phosphatidyl choline)
- Digalactosyl diacylglycerol

Solubility of polysaccharides

- Solubility in H_2O is related to size and how well polymer molecules fit together
- Large linear polymers with regular structures tend to be more stable in solid (insoluble) state; form semi-crystalline structures
  - solubility is increased by branching and substituents

Random coils  Semi-crystalline  Crystalline

Viscosity of polysaccharide solutions

- Physical interactions between polysaccharide molecules cause them to become entangled in solution
- Degree of entanglement of polymer molecules determines viscosity of solutions

Gels

- Form when soluble and insoluble macromolecules establish a network in which there are localised regions of ordered structure held together by weak intermolecular forces (e.g., H bonds)
- Macromolecules entrap water in which they are dissolved; solutes may be entrapped in the network

Gel network

Gel network of κ-carrageenan
Polysaccharide composition of cereal grains

<table>
<thead>
<tr>
<th></th>
<th>Wheat</th>
<th>Rye</th>
<th>Corn</th>
<th>Barley</th>
<th>Oats</th>
<th>Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>59</td>
<td>52</td>
<td>63</td>
<td>52</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>NSP</td>
<td>10</td>
<td>17</td>
<td>8</td>
<td>20</td>
<td>23</td>
<td>5</td>
</tr>
</tbody>
</table>

Average % content based on 12-13% moisture

Starch
- Homopolymer of α-D-glucose
- Main reserve polysaccharide in plants
  - occurs in most plant tissues
  - has a negligible osmotic effect
  - breakdown releases energy and C substrates
- Deposited in insoluble granules
- Main yield component of cereal crops
  - major source of dietary energy intake of animals (50-70%)
- Consists of amylose and amylopectin

Amylose
- An essentially unbranched α[1 → 4] glucan
  - 0.2 - 0.5% of glucose units in α[1→6] links
- DP between 1000 and 20,000; MW 130,000 - 3.5 x 10⁵
- Unbranched α[1 → 4] glucan chains form helices
  - can interact with small organic molecules
- Large AM molecules tend to have low solubility in water
- AM has good film-forming properties

Amylopectin
- α(1 → 4) glucan with α[1→6] linked branches
  - 4 - 5% of glucose units in α[1→6] links
- DP may be > 10⁶
- Most starches have 65-90% amylopectin
- Complex structure
  - A chains - carry no branches
  - B chains - have A chains attached and are attached to other B chains
  - C chain - the chain that has the reducing end
- AP has good gelling and pasting properties
Differentiation of starches

- Properties and suitability of starches for particular end uses are determined by
  - AM:AP ratio
  - size of AM and AP molecules
  - degree of branching of AP
  - spatial distribution and average chain length of branches in AP

Gelatinisation

- Insoluble starch granules take up water and swell on heating
  - viscosity increases initially as granules swell and AM and AP lose their crystalline structures
  - with further heating, viscosity falls as granules collapse and AM molecules are leached out
  - on cooling, H-bonds form between glucan chains, resulting in a gel
- Gelatinisation temperature for most starches is between 60°C and 80°C
  - measured in a Rapid Visco Analyser (RVA) or in a differential scanning calorimeter (DSC)
- Gelatinisation of starch is an important property in food processing and digestion

Retrogradation

- Reversion of starch from a gelatinised to semi-crystalline state
- AM is more susceptible to retrogradation than AP
- Important in forming crumb structure of bread and cakes, and in staling
- Influences shelf life of processed foods
- Fat content can influence gelatinisation and retrogradation of starches

Analysis of starch

- Starch must be extracted quantitatively as unchanged as possible from its in situ form
- AM and AP separated by
  - preferentially leaching AM at slightly above the gelatinising temperature (not quantitative)
  - complete dispersion of starch (autoclaving, strong alkaline solution, DMSO) and selective precipitation of AM with I₂, n-BuOH or thymol; AP can be recovered from supernatant after centrifugation
- AM and AP molecules can be quantified by I₂ binding, size-exclusion chromatography
- Structure of AP examined after enzymic degradation (β-amylase, isoamylase)
Enzymes of starch synthesis
- ADP-Glc pyrophosphorylase
  - leaves: located in chloroplasts; activity highly regulated by 3-PGA/Pi
  - non-photosynthetic tissues: plastid and cytosolic location in some species; less sensitive to 3-PGA/Pi
- Starch synthases
  - soluble and granule bound starch synthases with different specificities
- Starch branching enzymes
  - different specificities
- Debranching enzymes

Enzymes of starch degradation

Resistant starch
- Starch was considered to be completely digested in upper gut of monogastrics
- Approximately 10% of starch may be available for fermentation in the large intestine
- Most recovered RS is retrograded amylose
- Resistant starch is a good substrate for butyrate production by microflora of lower gut

Resistant starch
- Types
  - Physically inaccessible: Partly milled grains; large food particles; encapsulated with protein; associated with lipid
  - Resistant granules: Raw potato, green banana, high amylose starches, some legume starches
  - Retrograded: Cooked and cooled foods (eg, potato, bread, breakfast cereals)
  - Chemically modified: Etherised, esterified or cross-linked starches

Examples of occurrence
Cellulose
- Main structural component of plant cell walls; most abundant form of C in nature; content varies from <2% of cereal endosperm to ~98% of the secondary walls of cotton seed hairs
- Unbranched homopolymer of $\beta[1\rightarrow4]$-linked D-glucose units; DP ranges from 2,000 to 20,000
  - $\beta[1\rightarrow4]$ linkages create an extended flat ribbon-like structure in which every second glucose residue is rotated 180°; cellobiose is the repeating unit
  - Ribbons amenable to close packing into fibres

Non-cellulosic polysaccharides
- Xylans, arabinoxylans, arabinogalactans, glucans, mannans, glucomannans, galactomannans and glucuronomannans, pectins
- Solubility varies according to structure
- Mainly structural in function, but some are mobilisable carbon reserves in seed
- Affect germination behaviour, milling and malting characteristics of grains, processing of raw products, and nutritional value of foods and feeds

$\beta$-Glucans
- $\beta$-Glc(1$\rightarrow$4)$\cdot$ $\beta$-Glc(1$\rightarrow$4)$\cdot$ $\beta$-Glc(1$\rightarrow$4)$\cdot$ $\beta$-Glc
  1
  ↓
  3
  $\beta$-Glc(1$\rightarrow$4)$\cdot$ $\beta$-Glc(1$\rightarrow$4)
- Linear glucans linked by $\beta$(1$\rightarrow$3) and (1$\rightarrow$4) glycosidic links
- More than 90% of glucose units are in cellotriose and cellotetraose units joined by (1$\rightarrow$3) $\beta$ linkages
- DP may be as high as 1250 - 1850 (MW 200,000 - 300,000)
- Important constituents of endosperm cell walls of Gramineae
- Mobilized during germination by $\beta$-glucan hydrolases
- $\beta$(1$\rightarrow$3) links increase solubility, but extended and irregular conformation gives very viscous aqueous solutions

Xylans
- $\beta$-Xyl(1$\rightarrow$4)$\cdot$ $\beta$-Xyl(1$\rightarrow$4)$\cdot$ $\beta$-Xyl(1$\rightarrow$4)$\cdot$ $\beta$-Xyl(1$\rightarrow$4)$\cdot$ $\beta$-Xyl(1$\rightarrow$4)$\cdot$ $\beta$-Xyl(1$\rightarrow$4)$\cdot$ $\beta$-Xyl(1$\rightarrow$4)$\cdot$ $\beta$-Xyl(1$\rightarrow$4)$\cdot$
  2
  $\uparrow$
  3
  $\uparrow$
  1
  $\alpha$-Ara
  4-O-MeGlcU
- Have a $\beta$ (1$\rightarrow$4) linked D-Xyl backbone substituted with $\alpha$-linked D-arabinose and D-glucurionate residues and acetyl groups substituted at 2-O or 3-O positions of some xylloses
- Pentosans (highly branched arabinoxylans without uronic acids) are abundant in cereal grains (2 - 3% of wheat flour, 6 - 8% of rye flour)
- Very hydrophilic - form extremely viscous aqueous solutions; important in water-binding of wheat flour doughs
Rheology and cereal-based foods

Prof Roger Tanner
Department of Mechanical and Mechatronic Engineering and Key Centre for Polymer Colloids, University of Sydney
DOUGH RHEOLOGY PROGRAM

Prof R.I. Tanner
Dr M. Keentok
Dr S. Uthayakumaran
Marcus Newberry
T. Hubraq
Prof N. Phan-Thien

Department of Mechanical and Mechatronic Engineering

The University of Sydney

The University of Sydney  Rheology Group
Rheology

- What is it?

- Πᾶντα ρεῖ

  - Heraclitus ~500 BCE)

  - ρεῖ = rhei → Rheology (1929)

Rheology is the study of the deformation and flow of matter

(E. C. Bingham 1929)
Simple Shearing

- Fluid is contained between two infinite parallel plates separated by a distance $d$. The top plate moves with a constant velocity $U$ in the $x$ direction. The shear rate is $\gamma = \frac{U}{d}$. 
Shear Stress $\tau$ vs. Shear Rate $\gamma$ (1)

- Bingham body. $\tau_y$ is the yield stress and the slope $\beta$ gives the incremental viscosity.
Shear Stress $\tau$ vs. Shear Rate $\gamma$ (2)

- Pseudoplastic or shear-thinning fluid. Dashed line is Newtonian behaviour.
Shear Stress $\tau$ vs. Shear Rate $\gamma$ (3)

- Dilatant or shear-thickening fluid. Dashed line is Newtonian behaviour.
Barnes and Walters's results (1985) showing the problems of defining $\tau_y$ for a PVA latex sample. As the shear rate is progressively reduced, the yield stress reduces, and finally vanishes for this sample.
Fig. 4. Shear stress as a function of time, at different shear rates, for a wheat flour dough. The shear rates $\dot{\gamma} (s^{-1})$ are noted next to the curves.
**Figure 1.** (a) Magnified section of a typical solid surface showing scale of asperities ($l_1$) and the projection of possible streamlines for viscous flow from left to right along the surface.

(b) Mean velocity profile for flow over a plane, where the scale of the asperities is now negligible compared with the length scale ($l_0$) over which the velocity profile is essentially one of simple shear.

**Fig 13.**
Three Scales

- $L =$ Size of Apparatus
- $I_r =$ Scale of Roughness
- $I_p =$ Size of Microstructure

- Pearson and Petrie (1966)
  - $I_p << I_r$ No slip appears to occur
  - $I_p >> I_r$ Slip occurs
  - $I_p \sim I_r$ Other factors determine slip
Three Scales

$L = \text{size of apparatus}$

$\ell_r = \text{scale of roughness}$

$\ell_p = \text{size of microstructure}$
Relaxation and Dynamic Response.

\[ G(t) \]

\[ \text{Step Strain} \]

\[ \text{dynamic response} \]

\[ \text{strain} = \gamma = \hat{\gamma} \exp(i\omega t) \]

(sinusoidal)
Sinusoidal Signal Testing

Input strain = $\gamma_0 \cos \omega t$
Stress leads strain by angle $\delta$. If $\delta = 0^o$ ——— Elastic response

$\delta = 90^o$ ——— Viscous response

$0^o < \delta < 90^o$ ——— Viscoelastic

$\tau = \text{Stress} = \tau_0 \cos (\omega t + \delta)$

$\tau = \tau_0 \cos \omega t \cos \delta - \tau_0 \sin \omega t \sin \delta$

In phase

Out of phase

or elastic part

Viscous / lossy part

Dividing $\tau$ by magnitude of strain ($\gamma_o$) gives two moduli ———

$\tau / \gamma_o = G' \cos \omega t - G'' \sin \omega t$

$\tan \delta = G'' / G'$
Fig. 22(b) \[ G'(\omega) \] for polyvinyl-chloride sample of Fig. 2.9(a) — Computed from \[ G(t) \], where \[ G'(\omega) = \int_{0}^{\infty} \omega G(s) \sin \omega s \, ds \]. × Direct measurement of \( G' \).
Shearing - Stress pattern

\[ N_1 = \sigma_{xx} - \sigma_{yy} \]
\[ N_1 = \sigma_{yy} - \sigma_{zz} \]
\[ \tau = \sigma_{xy} \]

Elongation rate \( \varepsilon \)
Fig 2.98 Extensional viscosity measurements on the M1 fluid. The lines and envelopes represent data by the various M1 investigators, as published in the special M1 issue of the Journal of non-Newtonian Fluid Mechanics. (From James and Walters 1993.)
Example: Low-Density Polyethylene

- Integral model fits well, but is complex.
- Needs several relaxation times (1-2 per decade of frequency)
Effects of flour components on dough rheology

AIM:

• Determine viscoelastic properties of gluten dough and compare the properties with those of the wheat flour. Confirm that basic rheological tests can detect very significant differences in the gluten and flours studied.
• Investigate gluten-starch interaction
• Determine the effect of various HMW-GS on rheological properties
• Investigate the role of water in dough rheology
Stress sweep experiment on starch-gluten mixtures

![Graph showing the storage modulus (G') Pa against strain for different starch-gluten mixtures. The graph includes markers for starch 100%, gluten 100%, gluten 80%, gluten 60%, gluten 40%, gluten 20%, and starch 100%. The x-axis represents strain, and the y-axis represents storage modulus. Different mixtures are distinguished by different symbols and shapes.]
Linear Strain Limit

Linear Viscoelastic Strain Limit (%)

Gluten %

0  20  40  60  80  100

0.01  0.1  1  10
<table>
<thead>
<tr>
<th>Sample Olympic line</th>
<th>Glu1-A</th>
<th>Glu1-B</th>
<th>Glu1-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

High molecular weight glutenin subunits (HMW-GS) are coded by the genes present in chromosome 1 in wheat. A, B and D denote loci in which the genes are present. + indicates the presence of HMW-GS coded by that loci (A, B or D). - indicates the absence of HMW-GS coded by that loci (A, B or D).
Effect of HMW-GS on elongational properties

Elongational viscosity Pa.s

Strain
A plot of the shear stress versus shear strain (in a simple shear flow) reveals three regions: 1. a strain-rate independent region, which includes the linear viscoelasticity region at very small strain, 2. a strain-softening region, and 3. a fracturing region (Phan-Thien et al [19]).
2.1 Oscillatory shear flow

In the case of an oscillatory flow with shear rate $\dot{\gamma} = \delta \omega \cos \omega t = \dot{\gamma}_0 \cos \omega t$, the particle trajectories are given by

$$X(t') = x + \delta y (\sin \omega t' - \sin \omega t), \quad Y(t') = y, \quad Z(t') = z,$$

where \{x, y, z\} are the Cartesian coordinates of the particle at time $t$, which at time $t'$ is located at \{X, Y, Z\}. This leads to the deformation gradient

$$[F(t')] = \begin{bmatrix} 1 & \delta (\sin \omega t' - \sin \omega t) & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}.$$
Figure 4: The normalised shear stress as a function of time at amplitude 0.05, and at various frequencies.

indication of non-linearity. It is clear that we have a departure from linearity at amplitude as low as 0.01. At high amplitudes, the peak stresses occur at strain of about zero. The data strongly suggest a discontinuity in the slope of the stress versus the strain there. This departure from linear behaviour at low strain levels is in contrast to Bercland and Launey [5] who used a cone-and-plate system and found an equivalently low viscoelastic limit ($\delta \approx 0.22\%$) but found only a mild departure from linear theory up to strains of 10-20%. No gluing or attachment regime was discussed in their paper, however.

Before examining the quality of fit to the large amplitude data, we first look at the transient response of eq (8). Plotted in Fig. 4 are the predicted normalised stress versus $\omega t$ at a moderate amplitude of 0.05 and at different $\omega$ (different levels of strain rates). There are two main features: first, the non-linearity sets in after half a cycle, relatively independent of the strain rate; second, the steady-state response is reached after about one cycle, again, relatively independent of the strain rate.

The prediction of (8) at steady state is now compared with experimental data in Fig. 5. It is pleasing to find that the agreement is quite good, both in terms of the trend and the magnitude of the stress. In fact, both the location of the stress gradient discontinuity and its magnitude are well predicted at large amplitudes.

For quality control purposes, it may be better to plot the maximum shear stress against the strain
Figure 5: A comparison between the theory and the experimental data at different amplitudes and $\omega = 1$ Hz. The symbols are experiments, and the solid lines are the predictions using shear flow parameters, $\Gamma_1 = 300$, $\Gamma_2 = 1/14$, $m = 0.55$, $\alpha = 0$, and the discrete relaxation spectrum in Table 1.
**SUMMARY**

- Water plays an important role in dough rheology.
- Dough is a major contributor to strength and stability.
- Increase in water content in dough results in decreased elongational viscosity, \( \eta^* \) and \( n^* \) values.
- Increase in starch in a gluten-starch complex results in a lower linear visco-elastic limit.

1. Linear visco-elastic limit for flour is below a strain of 0.1%.
2. Elongational viscosity of gluten < flour for gluten > 3%.
   - Gluten is different from flour in...
Some Rheological References

- Rheological Phenomena in Focus: D.V. Boger and K. Walters – Elsevier, Amsterdam, 1993
Starch Granule Structure

A/Prof Norm Cheetham
Department of Chemistry, University of New South Wales

The structure of starch falls into two broad categories: the structure of the granule, and the molecular structure of the carbohydrate components consisting of the linear amylose and the branched amylopectin, both of which are macromolecules composed of glucose monomers. To define the structure of starch at each of these levels has been the aim of scientists for well over a hundred years. The fact that current scientists are still trying pays tribute to the complexity of the task, and indicates the importance of the endeavour to industry.

Increasingly, the "natural" starches are being modified, chemically, biochemically, and genetically, to yield an even wider range of functionalities.

This discussion will concentrate on the structure of native starches in the two categories. Mentioned above. The range of physical, chemical and biochemical techniques which have been brought to bear on the problem will be discussed, but the main emphasis will be to illustrate the relationship between underlying molecular structure and industrially useful properties.
Cereal-based food extrusion –
on-line NIR spectroscopy to measure quality changes

Dr Rachel Kelly
Food Science Australia
Cereal based food extrusion: Using on-line NIR spectroscopy to measure quality changes

presentation by Rachel Kelly
on work by Sonya Huang

- Background to the study
  - the project team
  - the project concept
- Starch in the extruder
- NIR experimental setup
- Further applications & correlations
- Conclusions

- FSA, Sydney  C. Chessari, A. Evans,
  S. Huang, J. Sellahewa
  J. Murray (sensory)
- CSIRO, CMIS - J. Best, M. O’Sullivan
- BRI Australia - B. Osborne, Z. Kotwal
- CSIRO Plant Industries - I. Wesley
- FOSS NIR Systems - P. Brimmer, L. Saunders
What happens to starch in an extruder???

\[ \eta_{\text{app}} = K \gamma^{(n-1)} \]

<table>
<thead>
<tr>
<th>Product</th>
<th>K</th>
<th>n</th>
<th>SV</th>
<th>etc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw material</td>
<td>1.0</td>
<td>0.6</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Fluid A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid B</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid C</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4: Graph of viscosity parameters $\eta_0$ and $\eta_\infty$ versus DC ratios.
- Viscosity - Rapid visco analyser
- Bulk density
- Specific volume - by displacement of glass beads
- Radial expansion index - use of Vernier calipers
- Longitudinal expansion index - derived from formula
- Texture (strength, hardness, crispiness) - by TAST2
- Colour - Minolta Meter

- NIR Systems Process Analytics Analyser with paired 0.5" transmission fibre optic probes
- 32 scans, 400 to 2500nm
- APV Baker MPF 40 twin screw extruder
- Modified die block

Path Length = 1.63 mm
- Data analysed using NIRS3 v.4 (NIR Systems Inc) software
- Raw log 1/R data corrected using SNV and transformed into second derivative \( \frac{d^2 \log 1/R}{d\lambda^2} \) using a 16nm gap 16 nm smoothing function
- All subsequent analysis performed on second derivative data

- Screw speed: typically 200-400rpm
- Moisture: typically 17 - 25%
- Temperatures: typically 120 - 170°C
- Analysis used:
  - Partial Least Squares
    - Correlate signals directly to extrudate characteristics: K, n, REI, LEI, SV
  - 3 PLS factors were used to develop the calibration matrix

\[ R^2 = 0.91 \]
• These results indicate that NIR signals CAN be used to predict the rheology (and shape) of the simple wheat flour extrudate characteristics

• Use of a complex formulation containing the following ingredients:
  • Wheat flour/ Rice flour / Maize flour
  • Sugar
  • Salt
  • Baking powder
  • at defined conditions (speed, T, moisture)

A. Complexity of formulation - what happens when we move from a simple system to a multi-component system?

B. Shear effect - how does the presence of the probes affect the product?

C. Probe path-length - what happens when we increase the probe path length (as is more practical to industry)?

D. Correlations - do any exist between on-line NIR and, for example, sensory evaluation?

E. Sensitivity to detect changes - can online NIR detect changes in formulation or raw material?

R² values of PLS analysis correlate NIR signals to instrumental measurements from 3x3x3 experiment

<table>
<thead>
<tr>
<th>Spectra</th>
<th>Curri-Med</th>
<th>Bulk density</th>
<th>Bulk strength</th>
<th>Texture hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>0.97</td>
<td>0.96</td>
<td>0.96</td>
<td>0.85</td>
</tr>
<tr>
<td>2D</td>
<td>0.99</td>
<td>0.98</td>
<td>0.98</td>
<td>0.97</td>
</tr>
</tbody>
</table>
• Comparison made between product with no probes, 1.7mm and 2.5mm probe path-lengths at standard conditions

\[
\begin{array}{|c|c|c|}
\hline
\text{Parameter} & 1.7\text{mm} & 2.5\text{mm} \\
\hline
K & 0.11 & 0.28 \\
\eta & 0.01 & 0.16 \\
bulk density & 0.75 & 0.78 \\
strength & 0.04 & 0.12 \\
hardness & 0.38 & 0.67 \\
crispiness & 0.10 & 0.41 \\
\hline
\end{array}
\]

Results from a paired t-test - 95% confidence interval

• Longer path-lengths are more practical to industrial applications
• 4 different path-lengths tested 1.7, 2.5, 3.5 and 5mm at defined conditions
<table>
<thead>
<tr>
<th>Parameter</th>
<th>R² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>0.95</td>
</tr>
<tr>
<td>Bulk density</td>
<td>0.93</td>
</tr>
<tr>
<td>Browning index</td>
<td>0.87</td>
</tr>
<tr>
<td>K-value</td>
<td>0.94</td>
</tr>
<tr>
<td>n-value</td>
<td>0.95</td>
</tr>
<tr>
<td>force</td>
<td>0.95</td>
</tr>
<tr>
<td>gradient</td>
<td>0.90</td>
</tr>
<tr>
<td>mean</td>
<td>0.90</td>
</tr>
<tr>
<td>area</td>
<td>0.90</td>
</tr>
<tr>
<td>count</td>
<td>0.91</td>
</tr>
</tbody>
</table>

R² values for PLS analysis (NIR spectra and instrumental parameters) at defined conditions

Star symbol: Can NIR spectra at 3.5mm path-length be correlated to sensory assessment?

- Trained sensory panel of 15 people.
- Screened for sensory acuity.
- Assessed these 18 products (from the 3x3x2 experimental design for screw speed, moisture and T) by descriptive sensory analysis.
- 23 sensory attributes

<table>
<thead>
<tr>
<th>Classification</th>
<th>Attribute</th>
<th>R² value</th>
<th>(1- pij)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handfeel</td>
<td>Rough</td>
<td>0.52</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Hard</td>
<td>0.92</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Grainy</td>
<td>0.96</td>
<td>1.00</td>
</tr>
<tr>
<td>Appearance</td>
<td>Colour intensity</td>
<td>0.97</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Uniformity of shape</td>
<td>0.70</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Degree of aerated</td>
<td>0.91</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Roughness of surface</td>
<td>0.52</td>
<td>0.96</td>
</tr>
<tr>
<td>Aroma</td>
<td>Overall intensity</td>
<td>0.80</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Cereal aroma</td>
<td>0.79</td>
<td>1.00</td>
</tr>
<tr>
<td>Flavour</td>
<td>Overall intensity</td>
<td>0.58</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Cardboard</td>
<td>0.17</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Cereal</td>
<td>0.52</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Sweet</td>
<td>0.55</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Classification</th>
<th>Attribute</th>
<th>R² value</th>
<th>(1- pij)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td>Crispy</td>
<td>0.80</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Crunchy</td>
<td>0.94</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Hardness</td>
<td>0.94</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Brittle</td>
<td>0.89</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Melt in mouth</td>
<td>0.95</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Sticky mouthcoating</td>
<td>0.89</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Grainy</td>
<td>0.93</td>
<td>1.00</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>Toothpacketing</td>
<td>0.53</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Aftertaste duration</td>
<td>0.51</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Dehydration</td>
<td>0.20</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Conclusions this far:

- NIR spectra can be correlated with 13 sensory attributes

Use NIR spectroscopy to detect:

1. Changes in quality of a raw material (e.g. change batch of corn)
2. Changes in formulation (e.g. increase wheat content)
   at defined conditions

- The spectra in each of the changed formulations was subtracted from the original spectra, with the same extrusion conditions.
- A 1-sample t test was done on this difference, with the hypotheses that the difference = 0.
- This hypothesis was rejected in all the cases, indicating that there were differences in spectra of the changed formulations.

Conclusion:

- Differences in quality of raw materials
- Formulations
  can be detected by the NIR spectra
- On-line NIR analysis can also be applied to a more complex formulation
- The NIR probes were found to have a slight effect at 1.7mm but no effect at 2.5mm
- The NIR probe path-length of 3.5mm is the most practical - potential to increase further?

- NIR spectra can be successfully correlated to certain instrumental and sensory parameters
- NIR has an advantage over other on-line measurements in measuring product quality
- NIR spectra can detect changes in formulation of product and quality of raw materials

6 variables that NIR can correlate > 0.95:
- Moisture
- n-value
- Strength
- Sensory colour
- Sensory grainy handfeel
- Sensory melt in mouth

- NIR is the best at detecting changes in product quality
- NIR can detect changes in the quality of the raw material which other online systems cannot
- NIR can detect changes in formulation which other online systems cannot
Overview of analytical methods

Prof Bob Gilbert and Dr Chris Fellows

Key Centre for Polymer Colloids, University of Sydney
Key Centre for Polymer Colloids

Established and supported under the Australian Research Council Key Centres Program.

Analytical methods

Effects of particle size

- PSD’s of latex affect rheological properties: flow, shear stability, how well paint sticks to surface, ...
- Simplest: Einstein derivation of increase in viscosity with volume fraction $\phi$ of (solid) latex particles
  \[ \eta/\eta_0 = 1 + \frac{3}{2} \phi \]
- Viscosity increases when colloid present
Reason for viscosity increase

- Flow in a pipe containing colloidal spheres
- Presence of spheres causes additional energy dissipation
- Actual systems only obey Einstein result qualitatively

Viscosity

- Latexes often non-Newtonian at higher shear:
- Shear thickening or shear thinning
- Various reasons
- E.g., shear thickening when aqueous structure of associative thickener, which is water-soluble polymer held together by hydrophobic bits, is broken with shear

More PSD effects

- Polydisperse latex flocculates more readily than monodisperse one of same solids content: easier to squeeze particles together to exclude water if polydisperse
- Effect increased by lower double-layer barrier between small and large particle
- Polydisperse latex: film structure disrupted → poor film formation
- PSD's can also give mechanistic information

Particle size and concentration

- Kinetics calculates \( N_p = \frac{\text{total mass monomer}}{\frac{4}{3} \pi r_{\text{momen}}^3 \rho_p} \)
- Average size and particle number related:
- Note: PSD and MWD are controlled by some of the same kinetic events, but PARTICLE SIZE DOES NOT DIRECTLY INFLUENCE MOLECULAR WEIGHT!
Understanding PSDs

Count number of particles from electron micrograph in a given radius range, put into bins.

Cumulative distribution

- As well as number distribution, sometimes report cumulative distribution.

Definitions

- Particle size distribution \( n(r) \) = number of particles with radii between \( r \) and \( r+dr \)

- Cumulative distribution
  = number of particles with radius \( \leq r \)
  = \( \int_0^r n(r') \, dr' \)

Averages of PSD

- Number average \( <r> = \frac{\int_0^\infty r \, n(r) \, dr}{\int_0^\infty n(r) \, dr} \)

- \( t \)th moment \( <r^t> = \frac{\int_0^\infty r^t \, n(r) \, dr}{\int_0^\infty n(r) \, dr} \)

- "Average" depends on which moment is used
- \( r \) = mean-squared radius ≠ mean radius
  (unless PSD is monodisperse)
Polydispersity

\[ \gamma = \frac{\int r^4 n(r) \, dr}{\int r^2 n(r) \, dr} \]
\[ = \frac{\int r^2 n(r) \, dr}{\int n(r) \, dr} \]

(= 1 if monodisperse, >> 1 if polydisperse)

- Much more information in full distribution function, not just average

Measurement

- Size separation techniques
  - Physically separates particles of different sizes
  - E.g. CHDF, transmission electron microscopy
  - Gives true distribution function
  - Often laborious
  - Usually need calibration

- Size average techniques
  - E.g. light scattering
  - Often quick and absolute (often do not need calibration)
  - But only give an AVERAGE of two averages

- Although software often produces a distribution (assumed functional form) – beware!

CHDF

- Capillary hydrodynamic fractionation

- Gives PSD in ~ 10 minutes
- 30–700 nm diameter
- Care needed with small, broad PSDs

How CHDF works

- Eluent moves through capillary
- Higher velocity in middle

- Large particles excluded from edge, hence elute faster
**CHDF results**

- Normal PSD

- Broad (clearly seen on electron micrograph)

**Flow techniques**

- Examples:
  - DCP (disk centrifugation with photosedimentation detection)
  - Analytical ultracentrifuge (not commercial)
  - Field-flow fractionation ($F^3$)
  - Asymmetric-flow $F^3$
- All work on separation in flow field
- Once particles separated by size:
  - Light scattering can be used to give absolute size

**TEM**

- Transmission electron microscopy: direct measure of PSD
- Requires counting ~1000 particles to obtain reasonable distribution, $> 100$ for average
- Essential to have standard, e.g. add monodisperse particle with accurately known size
- Modern image analysis hardware and software can reduce effort
- Soft particles deform under beam, require hardening
- Some particles not highly visible, need staining

**Light scattering**

- For light of given wavelength, angle it is scattered off single particle $\propto$ size:
  - Absolute measure of size (no standard required)
- Can also use photon correlation: "twinkle" of scattered light varies in time because of Brownian motion
- Correlation function of twinkle (intensity) $= \langle d(0)/d(t) \rangle$
- This depends on diffusion coefficient
- Stokes-Einstein relation between diffusion coefficient and size:
  $D = \frac{k_B T}{6\pi\eta a}$ (radius)
- Hence finding correlation function $\propto$ size
- Unique only if sample is monodisperse!
Light scattering cannot give distribution

- Without physical separation of particle size, actual signal has contributions from all sizes
- Example: \( F(t) = \int_0^1 R(t,r) \, dr \)
- To obtain exact distribution, need to solve this integral equation pointwise for each radius "box" to obtain \( n(t) \) in each "box"
- Unless infinite range of infinitely precise data, this inversion (equivalent to finding inverse of very large matrix) is highly sensitive to small errors, ...

Light scattering "distributions"

\[
\text{PCS: measures } mcs = \sqrt{\frac{1}{\int_0^1 r^2 n(r) \, dr}}
\]

- More sophisticated light-scattering devices often show distribution
- Unless they use actual size separation (as in CHDF or F3), then this distribution is obtained from assumed functional form (e.g. log-normal for cumulative distribution)
  - cannot trust light-scattering distributions!

AcoustoSizer

- Works on electroacoustic effect
- Apply sound to sample, charges on colloid then oscillate
- This gives electrical signal
- Relate to size through Poisson-Boltzmann equation
- Works for concentrated dispersions
- Assumed functional form for cumulative distribution for polydisperse samples
- Also gives \( \zeta \) potential

Measurement of molecular size distributions
MWDs

- Not all chains have the same molecular weight. Must consider molecular weight distribution.
- \( P(M) \) = no. chains with molecular weight \( M \)

Molecular weight distribution

- \( P(M) \) = number distribution = number of chains with mol. wt. \( M \)
- \( \omega(\log M) = P(M) \log M^2 \)
  - reason explained later
- \( \omega(\log M) \) as a function of \( \log M \)
  (small corrections for non-linearity of calibration necessary)

Averages

- Number average molecular weight: \( \bar{M}_n = \frac{\int M P(M) \, dM}{\int P(M) \, dM} \)
- Weight average molecular weight: \( \bar{M}_w = \frac{\int M^2 P(M) \, dM}{\int M P(M) \, dM} \)
  - Typically: emulsion: \( \bar{M}_n = 5 \times 10^6 \), bulk \( \bar{M}_n = 5 \times 10^4 \)
- Viscosity average: \( \bar{M}_v^a = \frac{\int P(M) M^a \, dM}{\int P(M) \, dM} \)
  - \( a = \text{Mark-Houwink parameter} \) (0.5 ≤ \( a \) ≤ 0.8)

Average molecular weights

- Number average molecular weight:
  \[ \bar{M}_n = \frac{\int M P(M) \, dM}{\int P(M) \, dM} \]
- Weight average molecular weight:
  \[ \bar{M}_w = \int M^2 P(M) \, dM \]
  \[ \bar{M}_v^a = \int P(M) M^a \, dM \]

GPC distribution

\( \omega(\log M) \) as a function of \( \log M \)
Polycdispersity

- Polydispersity index $\gamma = \bar{M}_w / \bar{M}_n$
- Monodisperse: $\gamma = 1$ (all chains have same molecular weight)
  Never seen in artificial emulsion polymerization
  Perhaps in natural polymer colloids?
- More common: $\gamma = 2 - 4$

Osmometry

- Gives absolute number average molecular weight
  (no need for standards)
- Measure osmotic pressure $\pi$ as function of concentration of polymer $c$ in solvent
  $\pi = \frac{1}{RTc} + Bc + Cc^2 + \ldots$
  2nd, 3rd virial coefficients
- Hence plot $\frac{\pi}{RTc}$ against concentration, intercept gives $\bar{M}_n$

Light scattering

- Absolute measure of number average molecular weight
- Scattering intensity as function of angle $\theta$:
  
  $2n_{ov} \frac{d\alpha}{d\theta} (1 + \cos^2 \theta) d\bar{M}_n$

  Derivative of refractive index of solution
  wavelength

  Refractive index of solute

Solution viscosity & mol wt

- Knowing Mark-Houwink parameters $\eta$ measuring viscosity of polymer solution = "viscosity average" molecular weight

  $\bar{M}^\eta = \int P(M) M^{1+\eta} dM / \int P(M) M dM$

  Mark-Houwink parameter

  $\bar{M}_w = \frac{\int P(M) M^2 dM}{\int P(M) M dM}$ = weight-average mol. wt.
**GPC**

- Gel-permeation chromatography (or size exclusion chromatography)
- Measures full molecular weight distribution
- Column: crosslinked polystyrene, contains nooks and crannies
- Small chains become lost in tortuous paths, thus elute last

**GPC calibration**

- GPC requires calibration (with every run!)
- Use monodisperse standards (e.g., from anionic polymerization or RAFT) with known molecular weight
- Calibration: elution volume $V$ at which given molecular weight elutes:
  - Approximately linear
- Use Mark-Houwink parameters if no monodisperse standards

**Molecular weight distribution**

- $P(M) = \text{number distribution} = \text{number of chains with mol. wt. } M$
- GPC distribution $= w(\log M) = P(M)/M^2$
  - reason explained later
- GPC trace $\sim w(\log M)$ as a function of $\log M$

**Reason for $M^2$**

- Number distribution $P(M) = \text{GPC signal } / M$
- Signal $\propto$ mass of polymer $M \Rightarrow \text{one the two } M$'s
- GPC calibration curve: elution volume $V$ as function of known molecular weight $M$ is approximately linear:
  - $\ln M \sim V$, or $M \sim (\text{cln}M)/V$
- Increment of elution volume $\sim$ increment of mol. wt $M$

These together give $M^2$
MALDI

- Matrix-assisted laser-desorption ionization (time-of-flight) mass spectrometry
- Put sample onto matrix (care needed for selection - e.g. Na benzoate, but varies with polymer)
- Energize with laser; polymer goes into vacuum

Electrospray Mass Spectrometry

(This material prepared by Dr. Hank de Bruyn)

- Electrospray - "soft" ionization process
- Sample in volatile solvent (H₂O/MeOH, DCM)
- Sprayed through high voltage nozzle (~4 kV) → charged droplets
- Evaporation → increased charge density → expels ions → mass detector → m/z

MALDI data

Poly - N-isopropyl acrylamide

Signal

Molecular weight

M = 10^6

Electrospray Mass Spectrometry

- Vinyl acetate oligomer - 36 dalton repeat unit

Intensity

m/z
Branched polymers

- Major problem to obtain MWD if there is branching
- GPC separates by hydrodynamic volume:
  branched and unbranched chain with same
  hydrodynamic volume will have different
  molecular weights
- Obtaining information about branched chains requires detection
  which is ABSOLUTE in molecular weight:
  Osmotic pressure, viscosity, light scattering
- If used with size-exclusion chromatography (GPC) AFTER size
  separation, these will give (incomplete) information on
  branching
- Requires multiple detector
- Examples: triple-detector SEC, A-F^4
NMR Spectroscopy of Polysaccharides

Chris Fellows
June 2000 Short Course
Polymer colloids in cereal-based foods

What is NMR Spectroscopy?

- Nuclear Magnetic Resonance
- Each atomic Nucleus is like a tiny Magnet
- Can exist in different Spin States (I = 0, 1/2, 1, 3/2 etc.) in a magnetic field B, the nuclei will have distinct energy levels

\[
\Delta E = 2\mu B
\]
\[
\mu = \text{constant for a particular nucleus}
\]

For I = 1/2

What is NMR Spectroscopy?

- At a particular Resonance frequency \( v = \gamma B \), nuclei can absorb energy to pass from one Energy level to another
- \( v \sim \text{MHz range} \)
- Some nuclei:

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Unpaired p</th>
<th>Unpaired n</th>
<th>Net spin</th>
<th>( \gamma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1\text{H})</td>
<td>1</td>
<td>0</td>
<td>1/2</td>
<td>42.56</td>
</tr>
<tr>
<td>(^2\text{H})</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6.53</td>
</tr>
<tr>
<td>(^{13}\text{C})</td>
<td>0</td>
<td>1</td>
<td>1/2</td>
<td>10.71</td>
</tr>
<tr>
<td>(^{31}\text{P})</td>
<td>0</td>
<td>1</td>
<td>1/2</td>
<td>17.23</td>
</tr>
</tbody>
</table>

- The higher \( \gamma \), the more sensitive the nucleus

Chemical Shift

- Exact magnetic field a nucleus experiences, \( B' \), will not be the same as \( B \)
- Surrounded by moving electrons...
- Each chemical environment in the sample will have a different resonance frequency \( v' \)
- Expressed in "ppm" (\( \delta \)) shift from resonance frequency \( v \)

0–12 for \(^1\text{H}\)
0–200 for \(^{13}\text{C}\)
**1H NMR**

- Strongest signal (γ high, almost all hydrogen is 1H)
- MRI - Magnetic Resonance Imaging
  - "Even the Archbishop of Canterbury is 70% water"
- Image based on:
  - (a) the amount of water present in a particular tissue, or
  - (b) the relaxation times, T1 and T2, of the water nuclei.
  - T1 = time to reduce magnetisation by factor of e
  - T2 = time to 'dephase' by factor of e

**1H NMR**

- Too much information:

![NMR Spectrum](image)

- arabinopyranonosecarbohydrate (Hoffmann et al., Carbohydrate Res. 223:19-44)

**1H NMR**

- Bound and Unbound Water
- Broad peak = water stuck in one chemical environment
- Sharp peak = water that sees an average of environments

- 1H NMR Spectra for waxy corn starch at various water contents (Li, Dickinson, Chinachoti, J. Agric. Food. Chem. 1996, 45, 62-71)

---

**Some Synthetic Polymers**

- Random
- Alternating
Solid State 13C NMR

Retроградация

Solid State 13C NMR
Characterisation of proteins in cereal grains

Dr Colin Wrigley
Grain Quality Research Laboratory, CSIRO Plant Industry
**What is a protein?**

- Examples of the diversity of proteins
  - Silk & Sinew
  - Hair & Horn
  - Wool & Web
  - Feathers & Flagella
  - Enzymes & Epidermis
  - Gluten & Gore

- Functions of proteins
  - Structure, contraction, enzymic activity,
  - Hormonal control, antigenic activity,
  - Oxygen transport, toxicity, antibiotic action,
  - Storage of amino acids & nitrogen

---

**What is a protein?**

- Amino acids (20 of them) - the primary source of the diversity

- Primary structure - a string of amino acids

- the peptide bond

  \[-\text{COOH} + \text{H}_2\text{N} \rightarrow -\text{CO-NH}^- + \text{H}_2\text{O}\]

---

**What is a protein?**

- Secondary and tertiary structures - coiling and folding
  - alpha-helix, beta-pleated sheets, beta-helix (pro)
  - stabilised by covalent SS bonds and non-covalent bonds
  - ionic, hydrophobic (phe, i-leu), Van der Waals (ser), hydrogen bonds

- unfolding (denaturation) with urea or SDS
What is a protein?

- Quaternary structure - subunits joining together
- Homo-proteins versus hetero-proteins

The classes of grain proteins

- Cytoplasmic proteins versus gluten-forming storage proteins
- Wheat gluten
  - 35% glutamine (amide), 20% proline (molar basis)
  - significant levels of hydrophobic amino acids
  - (to save protein washing out during germination, or to make gluten insoluble for bread-making?)

The classes of grain proteins

- The Osborne system: albumins, globulins, prolamins, glutelins
  - Wheat: prolamin = gliadin, glutelin = glutenin (a prolamin - Shewry)
  - Barley: prolamin = hordein
  - Rye: prolamin = secalin
  - Oats: prolamin = avenin
    (18-23 kDa, only 10% of oat protein; mainly globulins)
  - Maize (corn): prolamin = zein (10-28 kDa)
- Grain legumes - legumin-type globulins
- Enzymes - mainly water-soluble, globular proteins

Nomenclature - using the right names for wheat proteins

"Nomenclature: establishing a common gluten language."

- ‘Gluten’
  - function in dough versus in coeliac disease versus in commerce
- Glutenin
  - a polymer of polymers (polypeptide subunits)
- Grain proteins and gene locations on chromosomes
- Enzymes
1. Gluten proteins

- "Gluten proteins" - proteins that impart the unique viscoelastic properties to dough made from wheat flour (the mass remaining when dough is thoroughly washed under running water).
- "Gluten" may be (mis)used in relation to other crop species (e.g., "corn-gluten"), so use the term "wheat gluten" in such cases.
- The nutritional concept of "gluten" refers to the range of proteins that cause various food intolerances, especially coeliac disease. In this case, the term may also cover prolamins and glutelins from the grain of rye, triticale, barley and possibly oats.

2. Gliadin and Glutenin

- Gliadin proteins are monomeric polypeptides, with many disulfide bonds being intra-polypeptide.
- Glutenin proteins are polymeric, having disulfide bonds joining between the polypeptides.
- The genes coding for the gliadin and glutenin polypeptides have specific locations in the wheat genome.
- In practice, gliadins have molecular sizes smaller than glutenin proteins.
  - The dividing line between the two groups of proteins being an "apparent molecular weight" of about 100,000 daltons, using gel filtration, SDS-gel electrophoresis, SE-HPLC, FFF, and newer physical methods.
  - "Glutenin" should always be used for this group of gluten proteins before the rupture of disulfide bonds.

3. Gliadin polypeptides

- Genes for gliadin polypeptides occur in groups or "blocks" (sets of tightly linked genes) located on the short arms of Group 1 and 6 chromosomes (referred to as the Glh-1 and Glh-2 loci, respectively).
- Specific alleles at these loci are referred to as Gli-A1a, Gli-A1b, etc.
- A further conceptual level of nomenclature is emerging, based on amino-acid (or nucleotide) sequence.
- The omega-gliadins present a special case, their identification being facilitated by their presence in the "D"-zone of an SDS-electrophoresis gel, and their potential for cross-reference to the LMW subunits of glutenin.

4. HMW subunits (or polypeptides) of glutenin

- They have larger apparent sizes (after reduction of disulfide bonds) as indicated by SDS gel electrophoresis.
- The genes are located on the long arm of Group 1 chromosomes (at the Glu-1 loci).
- The naming of specific HMW glutenin subunits depends on mobility in an SDS gel on electrophoresis, using numbers for the bands - see Payne and Lawrence: "Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat." Cereal Research Communications 11: 29-35, 1983)
- Numbered bands are linked to specific alleles - e.g., Glu-A1a.
- Each locus comprises two tightly-linked genes - an x-type, that encodes for the subunit of higher molecular weight, and a y-type, that encodes for the smaller subunit.
5. LMW subunits (or polypeptides) of gluten

- They have smaller apparent size on SDS-gel electrophoresis
- The alleles (Glu-3) according to the respective loci on the short arms of Group 1 chromosomes (e.g. as Glu-A3a).
- There are rather more individual LMW subunits compared to the HMW ones, so greater reliance must be placed on the designation of the genetic allele, in the way recommended for the gliadin polypeptides. A basic reference to this is by Gupta and Shepherd (“Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin: I. Variation and genetic control of the subunits in hexaploid wheats” Theor. Appl. Genet. 80: 65-75, 1990).
- The alleles can also be specified by indicating the allele for the tightly linked gliadin proteins on appropriate Gli-1 loci, as indicated by Gupta RB, Metzkovsky EV and Wrigley CW (1993) “The relationship between LMW-glutenin-subunit and gliadin alleles in Australian wheat cultivars”. Fifth International Workshop on Gluten Proteins, Darmstadt, Germany, pp. 589-597) and extended by Jackson et al. (1996) cited in “Gluten ‘96.
- Nomenclature is best based on amino-acid and nucleotide sequences e.g., equivalence between sub-groupings of the subunits compared to gliadin polypeptides
  - SHPGLEK/R... METSC/HIPG..., VRVPUPQL... potentially valuable “handles” on nomenclature.

Analysis of protein content

- Total N determination, by Kjeldahl, by Dumas (10-50 mg protein)
  - Conversion factors: N x 5.7 for wheat; N x 6.25 for other grains
- Alkaline distillation → amide-nitrogen content
- Gluten washing of wheat flour
- Colorimetric methods
  - Biuret (Cu²⁺ chelation in alkali; 0.2-2 mg protein)
  - Lowry (20-400 µg protein)
  - Bradford (Coomassie blue; 10-200 µg protein)
  - Absorbance at 280 or 214 nm (DNA at 260 nm)
  - Near infrared reflectance and transmittance (NIR, NIT)

Analysis of protein composition

- Amino-acid composition
  - Analysis of amino-acid sequence
  - N-terminal sequence (Edman degradation)
  - C-terminal sequence
  - Derived sequence from cDNA clone (complementary to mRNA)
  - Derived sequence from genomic DNA
- Content of sulfur, phosphorus, ... ‘other’ elements
- Composition before and after breaking disulfide bonds
- ELISA (enzyme-linked immuno-sorbent assay)
Protein fractionation methods

- Centrifugation → ‘dough liquor’
- Gluten washing
- Fractional extraction (especially Osborne solvents)
- Fractional precipitation (with salts or solvents)

Protein fractionation methods

- Gel electrophoresis (fractionation based on charge &/or size)
  - at pH 3, cathodic (especially for gliadins)
  - in SDS, anodic, + SS rupture (+ mercaptoethanol for HMW subunits)
  - two-dimensional ... the proteome (the full set of proteins of an organism)
  - multi-stacking SDS gel (for the size distribution of unreduced glutenin)
  - capillary zone (CZE: electrophoresis with HPLC advantages)
  - Western blotting (for antibody detection of zones)

Protein fractionation methods

- Isoelectric focusing (fractionation based on isoelectric point)
- HPLC (high-performance liquid chromatography)
- Reversed phase (fractionation based on hydrophobicity)
- Size exclusion (fractionation based on molecular size)
- FFF (field-flow fractionation)
- Gradiflow (preparative fractionation based on size &/or charge &/or pl)
- Affinity chromatography (fractionation based on antibody reaction)

Nutritional aspects of grain proteins

- Food and feed value
- Anti-nutritional factors
- Bakers’ asthma
- Grain-dust inhalation
- Pollen allergies
- Ingestion allergies
- Schizophrenia
- Coeliac disease (celiac, US spelling)
- Taxonomic relationships and predicted cross-reactions
Protein-quality testing equipment

John Meers (N.B. Loves, 1985): “Here we are in the 1980s, using dough-testing equipment developed in the 1930s. We need to devise new methods, based on 1990s technology.”

- Farinograph
- Extensograph
- Mixograph
  - 35-gram and 10-gram mechanical recording
  - 2-gram direct-drive, computerised, auto-interpretation
- Baking, noodles, pasta
- Falling/Stirring Number
- Micro-malting
- Rapid Visco Analyser

Quality attributes preferred in wheats for specific products

<table>
<thead>
<tr>
<th>Product</th>
<th>Protein content</th>
<th>Grain hardness</th>
<th>Dough strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>BREADS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pan bread</td>
<td>&gt;13%</td>
<td>Hard</td>
<td>Strong</td>
</tr>
<tr>
<td>Flat bread</td>
<td>11-13%</td>
<td>Hard</td>
<td>Medium</td>
</tr>
<tr>
<td>Steamed</td>
<td>11-13%</td>
<td>Hard</td>
<td>Medium/Strong</td>
</tr>
<tr>
<td>Nihon China</td>
<td>10-12%</td>
<td>Soft/Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Steamed/Sita China</td>
<td>10-12%</td>
<td>Soft/Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>NOODLES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline</td>
<td>11-13%</td>
<td>Hard</td>
<td>Medium</td>
</tr>
<tr>
<td>White</td>
<td>10-12%</td>
<td>Medium/Soft</td>
<td>Medium</td>
</tr>
<tr>
<td>Instant</td>
<td>11-12%</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>BISCUIT/CAKE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8-10%</td>
<td>Very soft</td>
<td>Weak</td>
</tr>
<tr>
<td>STARCH/GLUTEN</td>
<td>&gt;13%</td>
<td>Hard</td>
<td>Strong</td>
</tr>
</tbody>
</table>

Functional proteins in relation to grain uses

- Grain hardness (Grain Softness Protein)
- Starch granule proteins
  - Starch synthases (soluble & granule-bound)
  - Branching enzymes
- Enzymes of germination; rain damage
- Malting; changes on storage
- Enzymes of insect attack e.g. of Nysius hutoni (NZ “bug”)
- Feed quality
- Dry gluten use in baking, foods and industry
  - Modification of wheat gluten e.g. enzymic or alkali solubilisation

- Homo-proteins versus hetero-proteins

Protein function in dough

- Protein content versus composition and wheat dough
- Albumins & globulins, HMW-albumins (β-amyloses), triticiins (legumins of wheat)
- Gladiins (single polypeptide chains, 30-50 kD)
  - Sulfur-rich alpha-, beta- and gamma-gladiins (ProGlnGluPro repeats)
  - Sulfur-poor omega-gladiins
- Glutenin polymer (200-20,000+ kD) (the largest proteins in nature?)
Protein function in dough

- Glutenin subunits (15-150 kd)
  - A-zone on SDS gel, HMW polypeptides (80-120 kd)
  - B-, C-zones on SDS gel, LMW polypeptides (30-60 kd)
  - Repetitive domain (ProGlyGlnGlyGlnGln or GlyTyrTyrProThrSerProGlnGln)
  - N- and C-terminal domains

Protein function in dough

- Gluten composition and dough properties
  - Glutenin : gliadin ratio
  - Gliadin composition: Gli-1, Gli-2
  - HMW glutenin subunits: Glu-1 (Payne score)
  - LMW glutenin subunits: Glu-3
  - HMW:LMW subunit ratio
  - Molecular-weight distribution
  - Over-expression of specific gluten genes

HMW glutenin subunits and dough quality

Glu-1 dough quality scores assigned to HMW glutenin subunits and corresponding alleles, according to Payne (1987). A high score (maximum of 10) indicates a prediction of strong dough properties.

<table>
<thead>
<tr>
<th>Score</th>
<th>Gli-A1</th>
<th>Gli-B1</th>
<th>Gli-D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>a</td>
<td>1</td>
<td>17+18</td>
</tr>
<tr>
<td>3</td>
<td>b</td>
<td>2</td>
<td>7+8</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>13+16</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>2+12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>b</td>
<td>3+12</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>c</td>
<td>Null</td>
<td>c</td>
</tr>
<tr>
<td>1</td>
<td>d</td>
<td>5+10</td>
<td></td>
</tr>
</tbody>
</table>

The 8 multi-null alleles for the 'waxy' (Wx) loci of bread wheat

Presence of genes for granule-bound starch synthase (GBSS)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Gene location</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wx-A1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wx-D1</td>
<td>7DS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wx-B1</td>
<td>4AL (form 78)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Angle</td>
<td>20 to 25%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>40% normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For HMW 65/7, D genome is most important for strength, present in Triticum turgidum.
Protein engineering to make ‘better’ gluten

- Chemical modification of size distribution
- Conferring gluten function on S-free barley C-hordein
  - How many cysteines are best?
- Chain length and gluten function
- Super-strong gluten
- Super-extensible gluten
- Resistance to genetically engineered foods

History of grain-protein research

- Beccari
- Origins of the name ‘protein’
- Osborne
- Guthrie
- Aussies

History of grain-protein research in Australia

- Dry gluten - Aussie, NZ or US?
- Protein function in ‘exotic’ products
- Wheat segregation on protein content
- Very-small-scale testing of protein function
- Genetic control of wheat- and barley-protein synthesis
- Growth environment and gluten quality

Simplified testing of functional properties using proteins

- Requirements in different situations
  - Standard methods
  - Breeding (early/late), as seed
  - At harvest, or mill
  - During processing
- Determination of protein content by NIR
  - Use of NIR to determine the wider range of quality aspects
    - Malted quality
    - Wheat-grain colour (red-white)
- Image analysis for detection of defects and contaminants
Simplified testing of functional properties using proteins

- Identification of variety (quality type)
  - Traditional methods
  - Pre-cast gels for electrophoresis
  - RP-HPLC
  - PatMatch program to provide ‘automatic’ identification
  - Wheat program to summarise methods and results
- Antibody-based kits for detecting key proteins
  - Rapid test kits versus high through-put
  - Determination of gluten content, dough strength
  - 1B/1R and 1A/1R lines
  - Pesticide residues
  - WheatRite test for amylase

PREDICTIVE (diagnostic) TESTS FOR QUALITY

- Safestore: prediction of ‘safe’ storage period for malting barley
- Antibody-based testing for gluten content
- Pre-cast gels for electrophoretic identification of varieties
- WheatRite antibody-based test for rain damage
  - Grind grain
  - Shake with buffer
  - Place 2 drops onto card, plus buffer
  - Close card, and read colour after 5 min

Breeding for protein quality

- Strategies for modifying dough properties
  - Glutenin subunits: Gla-1, Gla-3, Glb-1, Glb-2
  - Choice of parents and segregation based on Gla alleles
  - HMW:LMW subunit ratio
  - Molecular-weight distribution
  - Over-expression of specific gluten genes
- Heritability of gluten attributes versus gluten genes
- Efficient testing methods, including antibody-based kits
- Molecular markers
- Breeding for genetic potential vs tolerance to (a)biotic stresses
- Genotype X Environment interactions

Growth conditions and protein quantity/quality

- The interaction of genotype with environment
- Environmental factors modifying protein content and quality
  - Soil nutrition (nitrogen, sulfur, micronutrients)
  - Temperature (modest, heat stress)
  - Drought
**Growth conditions and protein quantity/quality**

- Sulfur deficiency
  - S deficiency and loss of extensibility, loaf volume
  - Grain N:S ratio of 17:1 as critical limit
  - Colour test for S deficiency in grain

- Modest temperature variation
  - Dough strengthening with rising temperatures (to 30°C)

- Heat stress
  - Dough weakening with stress temperatures (>35°C)
  - Genotypic tolerance
  - A basis for breeding for tolerance to heat stress

**OVERVIEW**

A molecular jigsaw puzzle of proteins and products

- Customer satisfaction
- Product quality
- Native protein composition
- Polypeptide composition
- Identification of genes
Uses of Starch in Foods and Industrial Applications

Ken McNaught
Starch Australasia Ltd.

Starch is one of the most common renewable resources used for both food and industrial applications. It is often used in its native state; however, many applications require it to undergo some form of chemical or physical modification. It is this latter class of starches which have received the greatest attention, though there has been a consumer push over the past few years to use natural starches in foods.

Starch is used extensively in the dairy, confectionary, canned and frozen and convenience food markets as a texturizer to control the organoleptic and visual appeal of foods.

It is also extensively used in industrial applications in the fibreglass, mining, building products, paper and surface coating industries. Starch is encountered in most of the products used in everyday survival in our modern society.
Field flow fractionation – what is it and what can it do?

Dr Ian McKinnon
Monash University
FIELD FLOW FRACTIONATION

PARTICLE CHARACTERIZATION IN CEREAL FOODS

IAN MCKINNON
i.r.mckinnon@sci.monash.edu.au

FIELD FLOW FRACTIONATION

WHAT IS IT FOR?

Separation and Characterization of particles
0.001 - 50 µm
- clays/sediments
- bacteria
- casein micelles
- starch granules
- humic substances
- viruses
- proteins

Fractions collected for subsequent analysis
- microscopy
- light scattering
- elemental analysis (AAS)

FIELD FLOW FRACTIONATION

WHAT IS IT?

• Family of Separation techniques
• Based on the differential elution of particles from a thin channel
• The differential elution is caused by the response of the particles to a field applied across the channel, the parabolic nature of the rate of flow of the eluting fluid through the channel.

FIELD FLOW FRACTIONATION

HOW IT WORKS

Injection of sample

CENTRIFUGAL FFF

• Ribbon-like channel is wound round a centrifuge drum.
• A dilute dispersion of the particles is injected into the beginning of the channel.
FIELD FLOW FRACTIONATION
HOW IT WORKS

CENTRIFUGAL

- The field is turned on.
- The centrifugal force thrusts the particles against the wall.
- The Particle distribution depends on the field, particle mass, and density

Distribution of particles across the Channel
Silica in water 3000 rpm (450 g)
density = 2.2 g/cm³

- Particle concentration decreases exponentially with increase in height
- Applied force drives the particles towards the wall.
- Back diffusion leads to the observed distribution

Distribution of particles across the Channel
Silica in water 3000 rpm (450 g)
density = 2.2 g/cm³

- In centrifugation equilibrium is reached.
- The distribution is determined thermodynamically.

- distribution depends on particle mass
- on particle density
- not particle shape
Distribution of particles across the Channel Flow Field Flow Fractionation

Force applied to particles
- Particles are driven to the channel wall by flowing medium across the channel.
- The walls of the channel are porous. The particles are retained by lining the outlet wall with a semipermeable membrane.

Basis of separation
- Particle diffusion coefficient $D$. Higher diffusivity particles are more widely distributed over the height of the channel and are eluted first. $D$ is related to the frictional force between a particle and the medium. For spherical particles $D$ is inversely proportional to the particle diameter.

FIELD FLOW FRACTIONATION CLASSIFICATION

Field | Basis of Separation
--- | ---
centrifugation | buoyant mass (mass and density)
cross flow | diffusion coefficient
electric potential | charge, diffusion coefficient (size)
temperature gradient | thermal diffusion coefficient

FIELD FLOW FRACTIONATION HOW IT WORKS

CENTRIFUGAL FFF normal mode
- Flow of the carrier fluid is started
- Parabolic flow gives differential elution. Central (smaller) particles elute first
- Continual redistribution of particles across the channel
FIELD FLOW FRACTIONATION
HOW IT WORKS

Particle Measurement
- Eluted particles are measured and/or collected
- dynamic light scattering
turbidity
static light scattering
absorbance uv/visible/nir
AAS, AES
refractive index
mass spectroscopy
electron microscopy

Fractogram
Graph of detector response against elution time

FIELD FLOW FRACTIONATION
INTERPRETATION OF FRACTOGRAM

1. Time axis
   - The time axis is related to the total volume of carrier eluted
     \[ V = \text{flow rate} \times \text{time} \]
   - the value of the retention ratio \( R = \frac{V_0}{V} = t^0/t \) at which a particle elutes \( (V_0 = \text{volume of the channel}) \) defines its value of the property that controls the distribution of the particle in the applied field.
   - The relationship between retention ratio and the properties of the particles eluted at that retention volume is either calculated from theory or determined by calibration using standard samples.

RELATIONSHIP BETWEEN RETENTION VOLUME AND PROPERTIES OF PARTICLES

- Particles within the sample with the same value of separation parameter \( \lambda \).
- Concentration of particles with same \( \lambda \) decreases exponentially with distance from the channel wall.
  \[ C(x) = c(0) \exp(-x/\lambda w) \]
- Retention volume \( V_r \) for particles with a particular value of \( \lambda \) is given by
  \[ V_0/V_r = 6\lambda[\coth(1/2\lambda)-2\lambda] \]
  \[ = 6\lambda \text{ (for large values of } V_0/V_r) \]

RELATIONSHIP BETWEEN RETENTION VOLUME AND PROPERTIES OF PARTICLES

Centrifugation
The separation parameter \( \lambda \) is related to \( m_b \) the buoyant mass of the particle and hence to the particle mass and density \( m_p \) and \( \rho_p \)

\[ \lambda = \frac{6kT}{m_b\omega^2 r w} \]
\[ m_b = m_p \left(1 - \frac{\rho_m}{\rho_p}\right) \]

For spherical particles the particle diameter is related to \( \lambda \)
\[ d = \left[\frac{6kT}{\lambda(\rho_p - \rho_m)\pi w^2 r^2}\right]^{1/3} \]
RELATIONSHIP BETWEEN RETENTION VOLUME AND PROPERTIES OF PARTICLES

Flow
The separation parameter $\lambda$ is related to $D$ the particle diffusion coefficient and indirectly to the particle size

$$\lambda = \frac{D V_0}{\dot{V}_{\text{cross}} w^2}$$

where $\dot{V}_{\text{cross}} = \text{cross channel flow rate}.$

For spherical particles the particle diameter is related to $D$

$$d = \left[ \frac{kT}{3mD} \right]$$

and $\eta = \text{viscosity of the medium}$

FIELD FLOW FRACTIONATION

INTERPRETATION OF FRACTOGRAM

2. Detector Response
- proportional to particle concentration
- response function must be known
  - absorbance where the detector response is proportional to the mass concentration of particulate matter
  - particle scattering intensity is, in principle a complex function of particle size and shape
- samples with particles that differ in composition are particularly complex

FIELD FLOW FRACTIONATION EXAMPLES

SdFFF Skim Milk

Fractogram

Particle size distribution

FIELD FLOW FRACTIONATION EXAMPLES

Flow FFF Skim Milk (carrier water)

Fractogram

Particle size distribution

10mM EDTA
50 mM EDTA
milk

10mM EDTA
50 mM EDTA
milk
FIELD FLOW FRACTIONATION

STERIC MODE

- Large particles (1-50) μm accumulate at the channel wall.
- Particles protrude into the eluting medium by virtue of their size.
- Larger particles elute first: **STERIC FFF**
- At higher flow rates hydrodynamic forces lift the particles away from the wall: **HYPERLAYER FFF**

\[
\frac{v_r}{V_0} = \frac{w}{3\gamma d} \quad \gamma \text{ is an empirical correction factor}
\]

FIELD FLOW FRACTIONATION STERIC MODE

Particle-Wall Repulsion

\[
\frac{v_r}{V_0} = \frac{w}{3\gamma d} \quad \text{Steric hyperlayer} \quad \gamma > 2
\]

FIELD FLOW FRACTIONATION STERIC MODE

- Applicable to: Centrifugation, Flow, Gravitational sedimentation
- Fast
- May require calibration
- Calibration depends on elution rate
- Theory applies to spherical particles
- What does the detector measure?

\[
\frac{v_r}{V_0} = \frac{w}{3\gamma d} \quad \gamma \text{ is an empirical correction factor}
\]

> 2 - steric hyperlayer

< 2 - simple steric behaviour
FIELD FLOW FRACTIONATION CLASSIFICATION

**Type**
- **normal mode**: sub-micron particles, small particles elute first
- **steric mode**: larger particles, low flow rate differentiation by degree of protrusion from the wall, large particles elute first
- **steric-hyperlayer Mode**: large particles + high flow rate, particles raised from the wall, reduces elution time.

GRAVITATIONAL FIELD FLOW FRACTIONATION

- **Sample outlet**
- **Valve**
- **Lucite**
- **Spacer**
- **Glass wall**

$50

**Dimensions**
- Length: 30-90 cm
- Breadth: 1-2 cm
- Thickness: 70-200 μm

**Materials**
- Lucite
- Glass or Polycarbonate
- Mylar or Teflon

GRAVITATIONAL FIELD FLOW BARLEY STARCH

- **OM: 12.9 ± 3.8 μm**
- **OM: 3.6 ± 1.4 μm**

(time(min))

[Graph showing data points and peaks labeled A and B]
**GrFFF PSAD**

- **Barley starch**
  - OM: 17.1 ± 7.8 μm
  - A: 15.0 μm
  - OM: 12.9 ± 3.8 μm
  - B: 4.5 μm
  - OM: 3.6 ± 1.4 μm

- **Wheat starch**
  - OM: 6.3 ± 5.6 μm
  - A: 15.5 μm
  - dₙ = 14.6 μm
  - OM: 17.1 ± 7.8 μm
  - B: 6.3 μm
  - dₙ = 5.6 μm
  - OM: 6.3 ± 5.6 μm

**Hyp/FIFFF PSAD**

- **Barley starch**
  - OM: 12.9 ± 3.8 μm
  - A: 11.7 μm
  - OM: 3.6 ± 1.4 μm
  - B: 3.5 μm

- **Wheat starch**
  - dₙ = 5.0 μm
  - OM: 6.1 ± 5.6 μm
  - A: 16.2 μm
  - B: 6.3 μm

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**FIELD FLOW FRACTIONATION**

**Useful Webb Sites**

For a bibliography of FFF publications
www.Rohmhaas.com/FFF/

For an excellent general site with conference papers on applications of FFF to cereals
DNS.UNIFE.IT/~rsk/talk
DNS.UNIFE.IT/rsk/mypapers

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Scientific underpinnings of gene technology regulation in the food industry

Public Lecture

Dr Geoff Annison
Food and Grocery Council of Australia