“The Development of Novel Functional Ingredients Using Milk and Soy Protein Formulations”

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OBJECTIVES

1. Formation of modified protein-carbohydrate (Maillard) & protein-protein (TGase) conjugates

2. Biochemical characterization of modified adducts

3. Rheological/functional characterization of protein-carbohydrate & polymeric protein complexes

Grande Cheese Company

http://www.bocafoods.com/product.html

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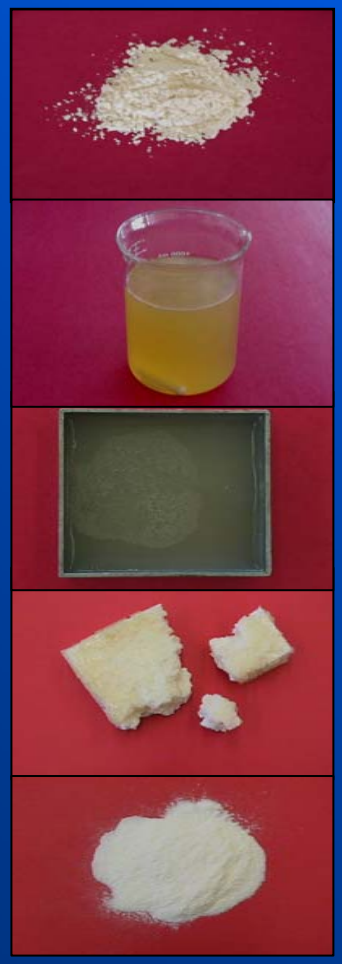
Preparing Modified Whey Protein



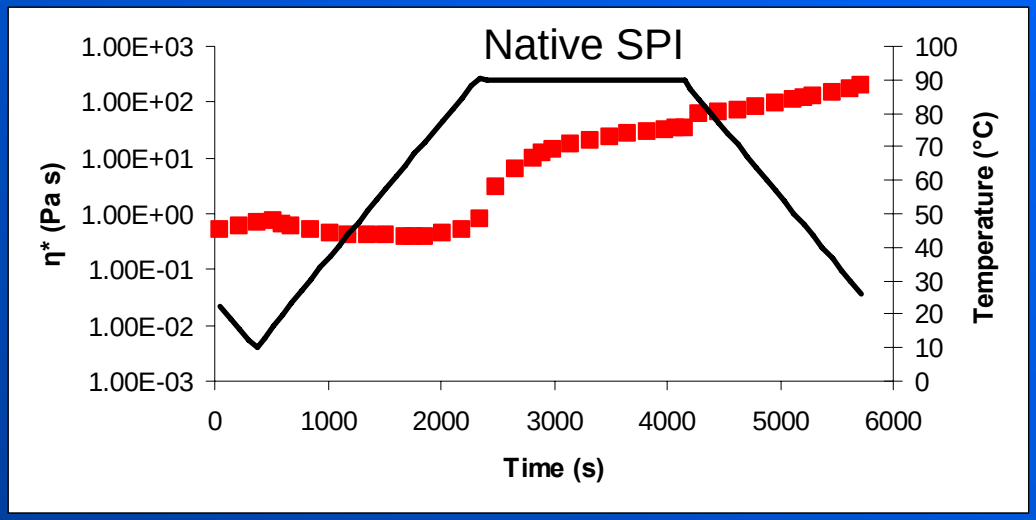
- Commercial Whey Protein Isolate
- Hydrate protein in water
- Adjust pH to <3.5, HCl
- Heat at 80 ºC, 3 h
- Freeze dry gel
- Grind into fine powder- mWPI

Same Approaches with SPI

Gelation requirements for SPI:

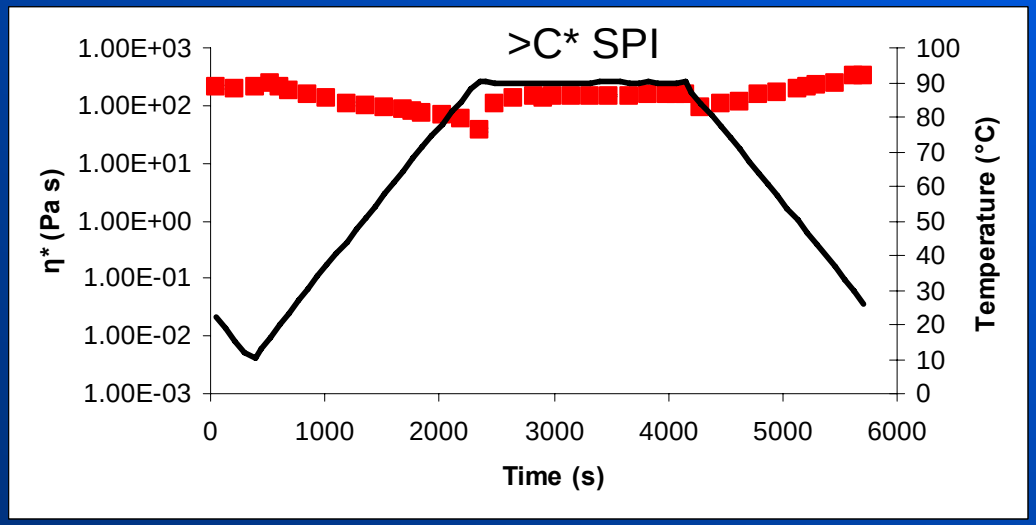
- **Heat treatment (90-95°C), 3 hours**
- **Denature both 7S, 11S subunits**

Molecular Interactions of Native and 8% SPI



In water

Temp



- **CRITICAL Protein Concentration (C* 7-8%) required for gelation**
- **Heated mSPI INGREDIENT was developed**

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Unique Properties of mWP/mSP Ingredients

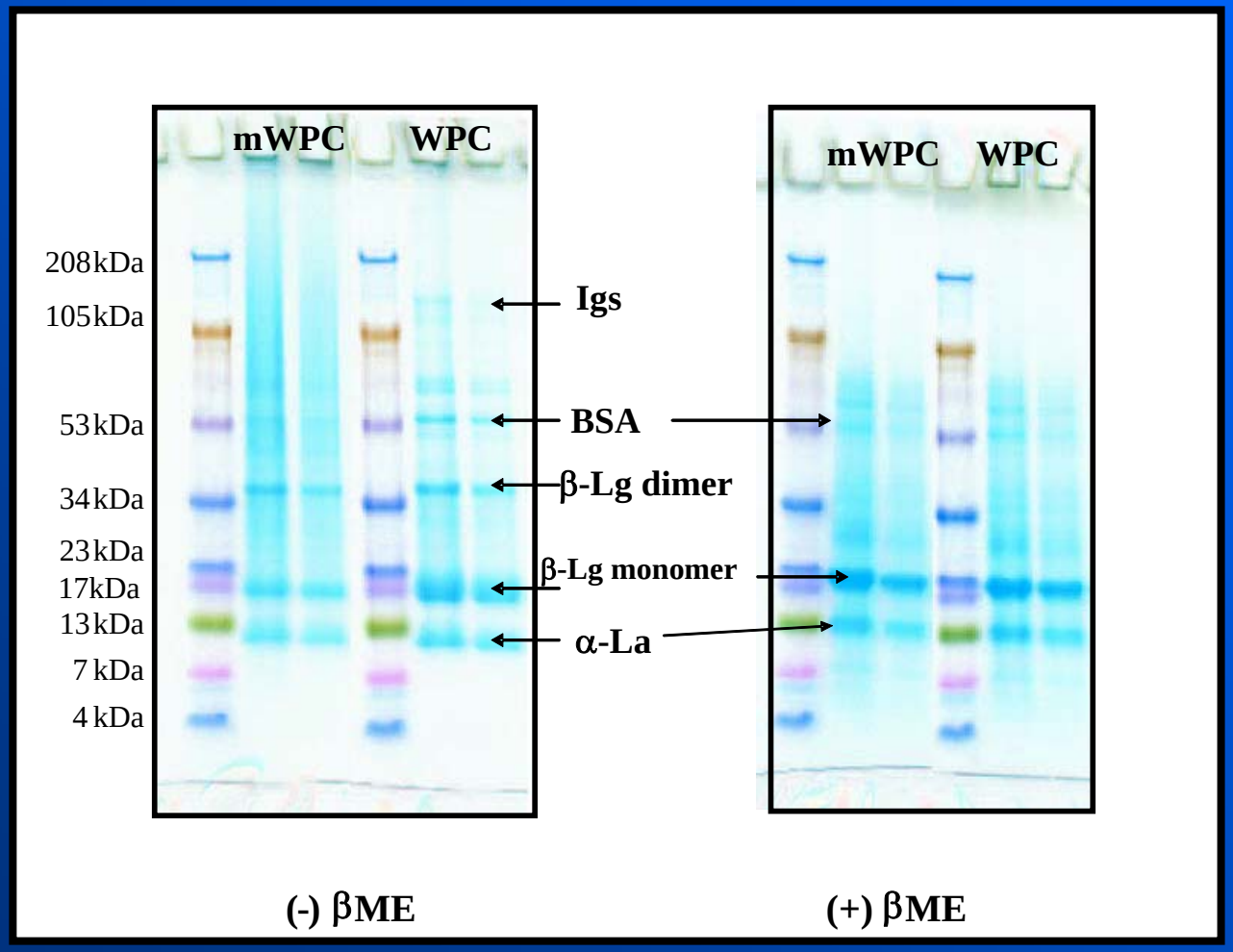
- ↑ viscosity
- ↑ water holding capacity
- ↑ nutritive value over starch and hydrocolloids
- Form weak gels without heating or the addition of salt

APPROACH

- Conjugate mannose and two sized dextran materials (35-45 kDa & 100-200 kDa) to mWPC/SPI via the Maillard reaction
  - Dry Heating at 100°C for 2 hours

- Characterize the effect of prior heat treatment (denaturation) and carbohydrate size on complex formation and ingredient functionality

SDS-PAGE results of mWPC, pH 3.5, vs. WPC, pH 6.5



- Heat treatment resulted in disulfide bond formation
- Almost a total loss of Igs, BSA bands
- Diminution of α-La and β-Lg monomer

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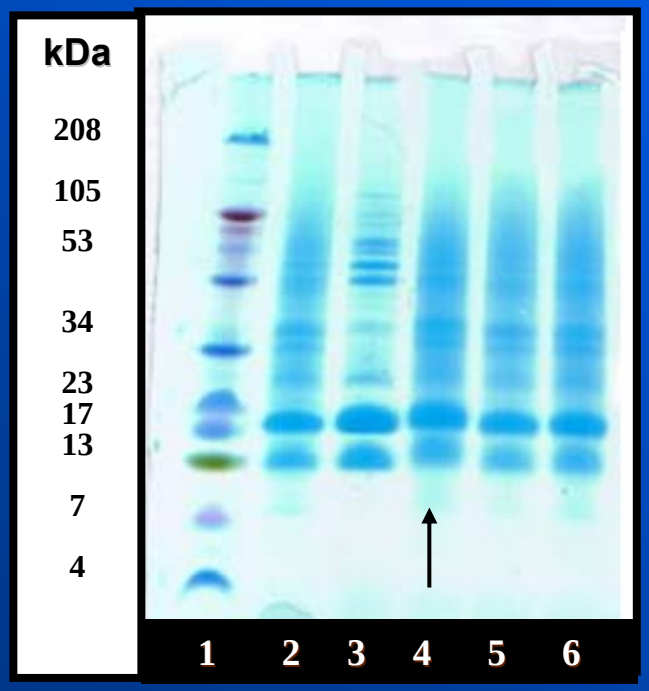
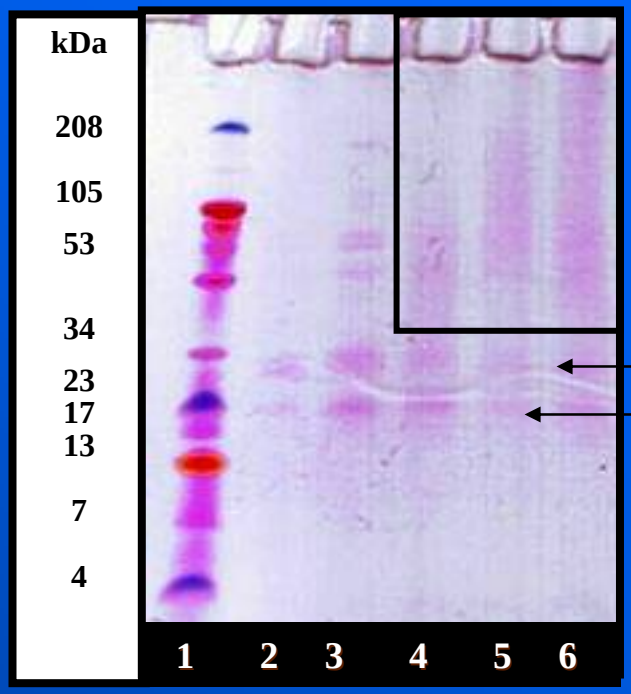
mWPC (+) Dextran: Maillard Results

Protein Stain



β- Lg
α-La

Glycoprotein Stain



GLYCO-CONJUGATE

β-Lg dimer
monomer

Lactosylation of BOTH α-La and β-Lg

1. Mw markers
2. mWPC, pH 3.5
3. Commercial WPC, pH 6.5
4. mWPC + Heat
5. mWPC + DX 35 + Heat
6. mWPC + DX 200 + Heat

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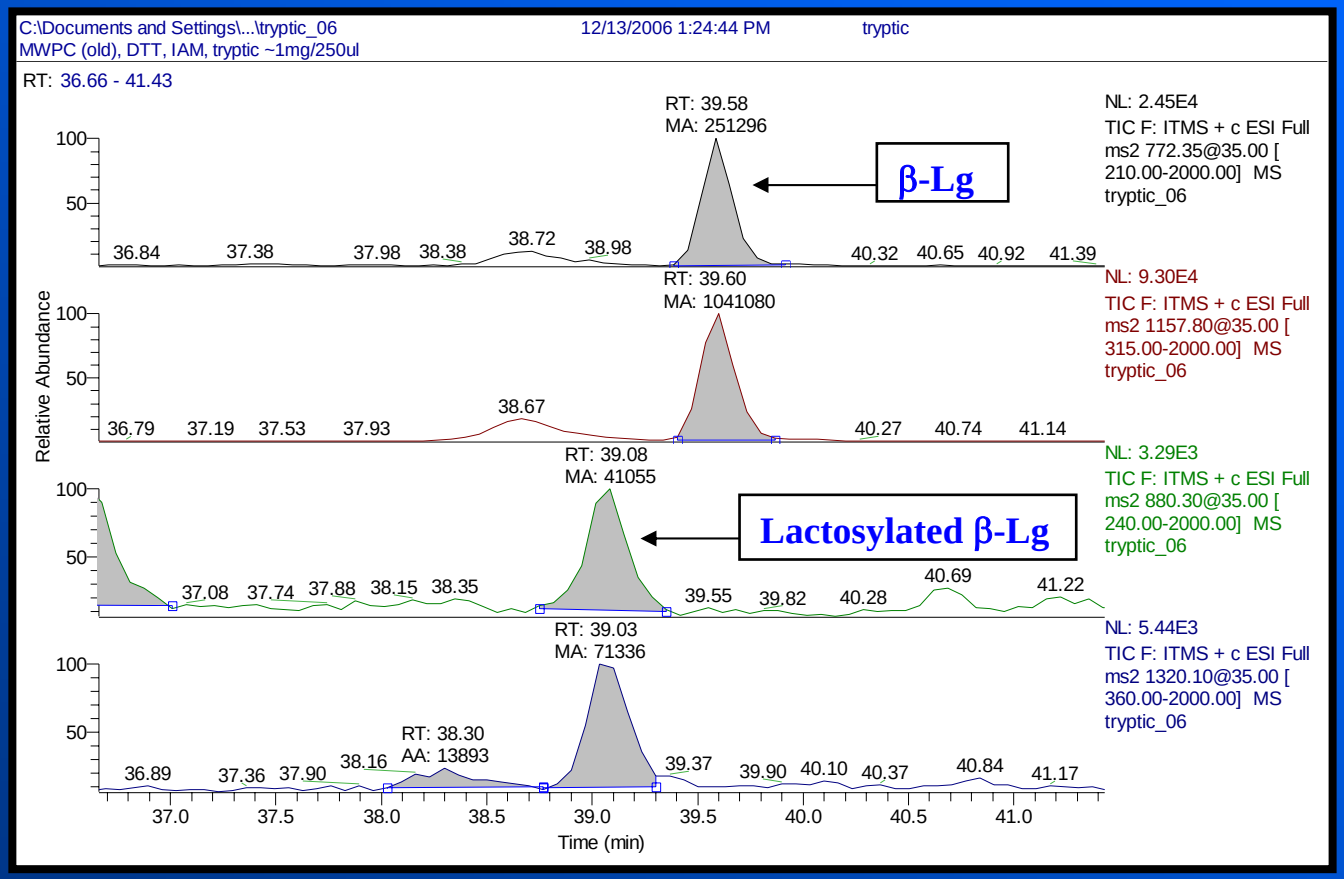
Mass Spec Confirmation of Lactosylation



mWPC, pH 3.5 - 8.5% lactosylation

WPC, pH 6.5 - 21.4% lactosylation

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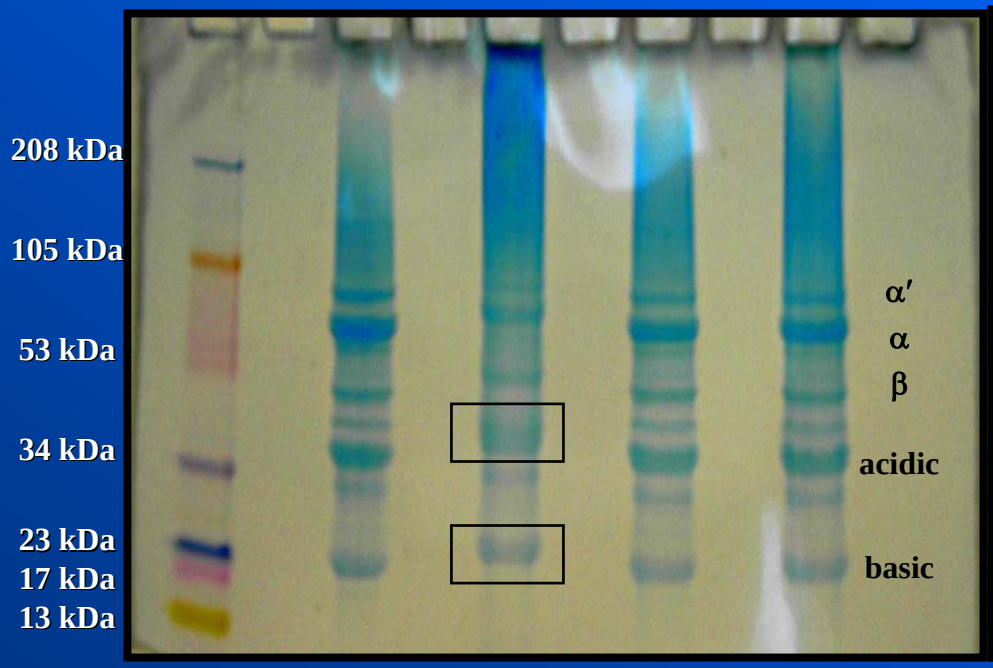
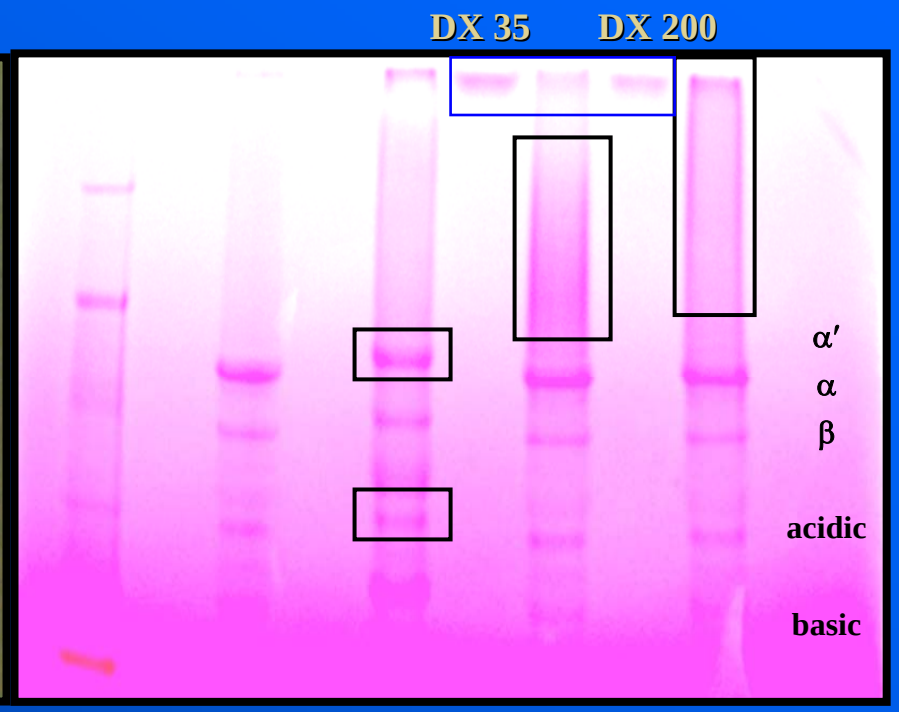
Fundamental Differences Between WPCs, pH 6.5 *vs.* mWPCs, pH 3.5

- mWPC, itself, already exists as a glycoprotein product
- Upon heating, WPCs, pH 6.5, were lactosylated to a greater extent than mWPC, pH 3.5, due to more favorable pH conditions for the Maillard reaction
- Maillard reactivity was observed at pH 3.5

SPI + Carbohydrate Conjugation, pH 6.5, Dry Heat





Glycosylation of SPI proteins was also achieved, pH 6.5

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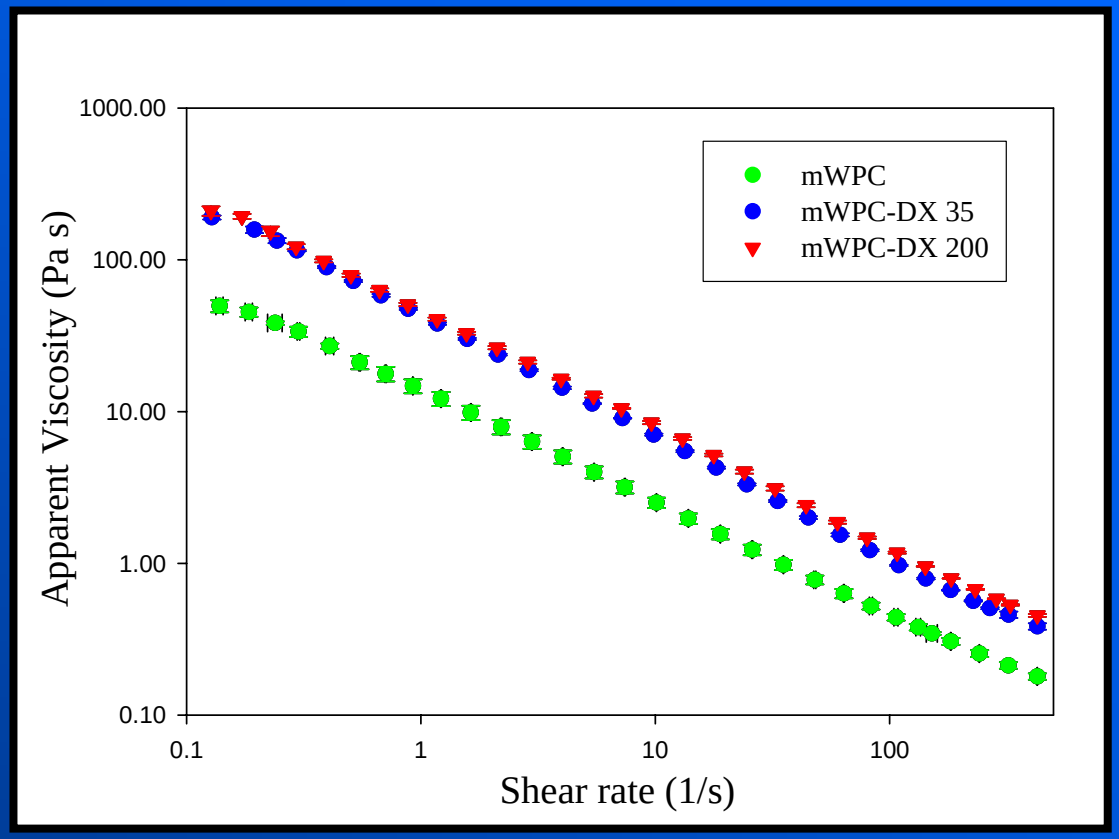
Changes in *FUNCTIONALITY* attributed to GLYCOSYLATION

- VISCOSITY
- GEL STRENGTH
- EMULSION STABILITY
  - Creaming Index
  - Emulsion Viscosity

Apparent Viscosity (7% protein, mWPC dispersions)
Prior to Dry Heat Treatment

Sample	η at 50 (1/s)
mWPC	0.78 Pa s
DX 35	2.00 Pa s
DX 200	2.42 Pa s



Inclusion of dextran ↑ the viscosity over that of mWPC dispersions, alone (~ 3X)

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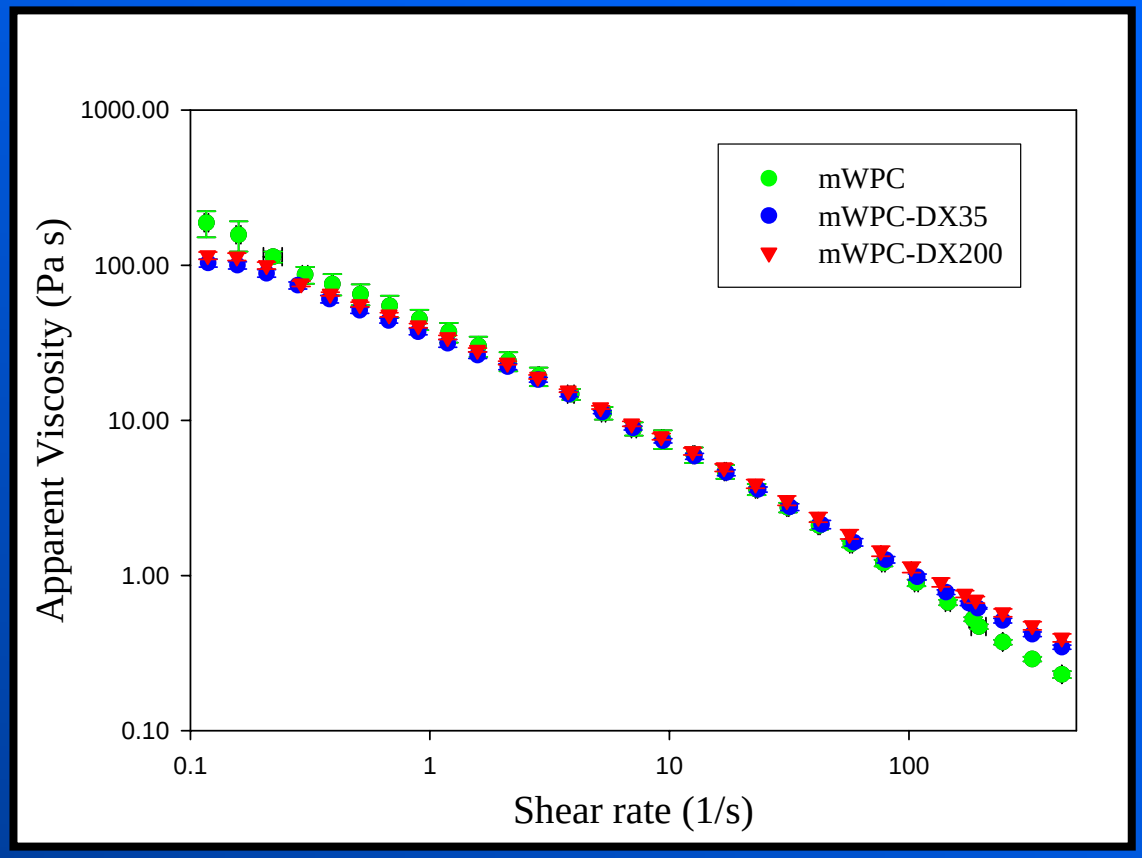
Apparent Viscosity (7% protein, mWPC dispersions)
After Dry Heat Treatment

Sample	η at 50 (1/s)
mWPC	2.09 Pa s
DX 35	2.13 Pa s
DX 200	2.37 Pa s



- ↑ viscosity mWPC was attributed to further lactosylation during dry heat treatment
- viscosity of mWPC: dextrans remained unchanged

mWPC, pH 3.5 - 8.5% lactosylation

mWPC (+) dry heat, pH 3.5 - 38.4% lactosylation

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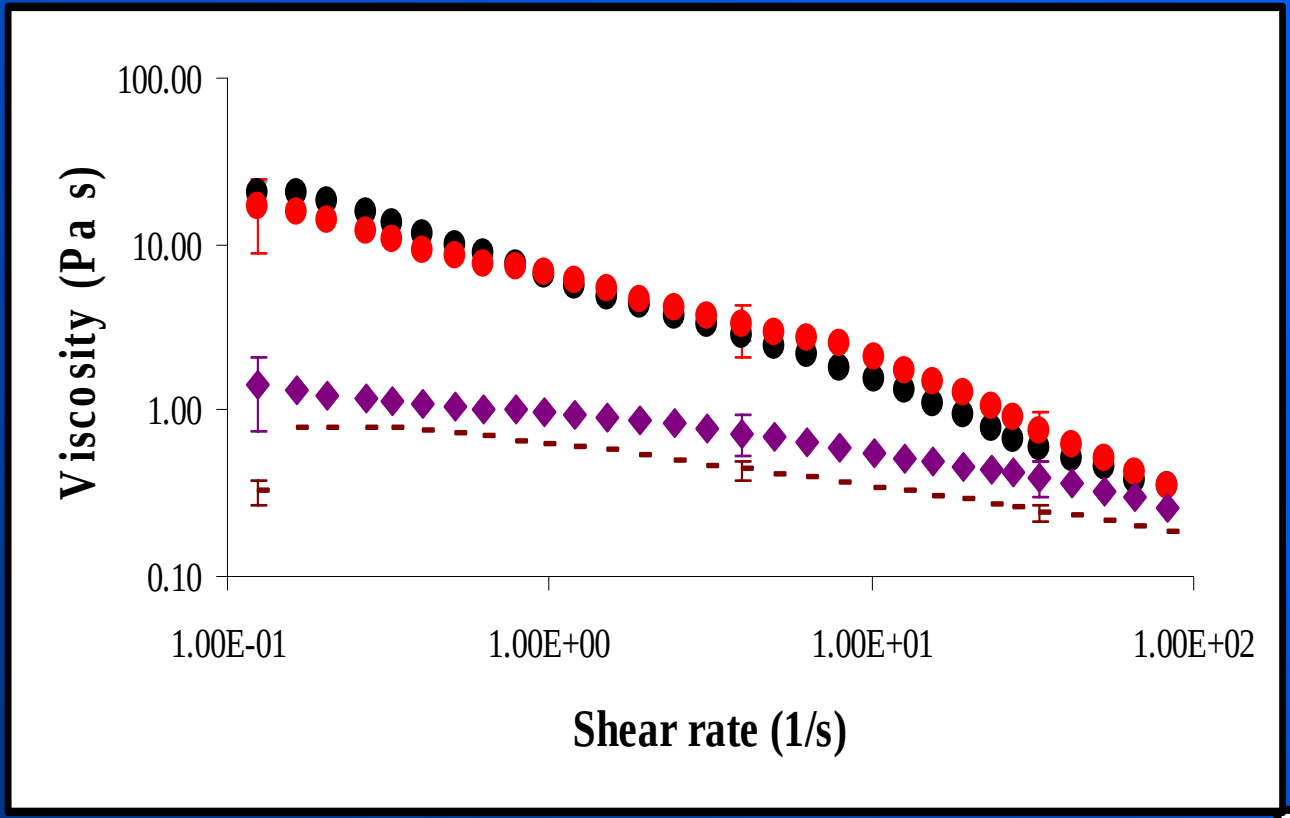
Soy Protein Viscosity Measurements - 8% protein



Additional heating steps

↓ VISCOSITY of SPI:Dextran conjugates

mSPI: 95°C, 3h	●
SPI: dry heat	●
mSPI + Dry Heat	♦
mSPI:Dextran-100/200 kDa	▬

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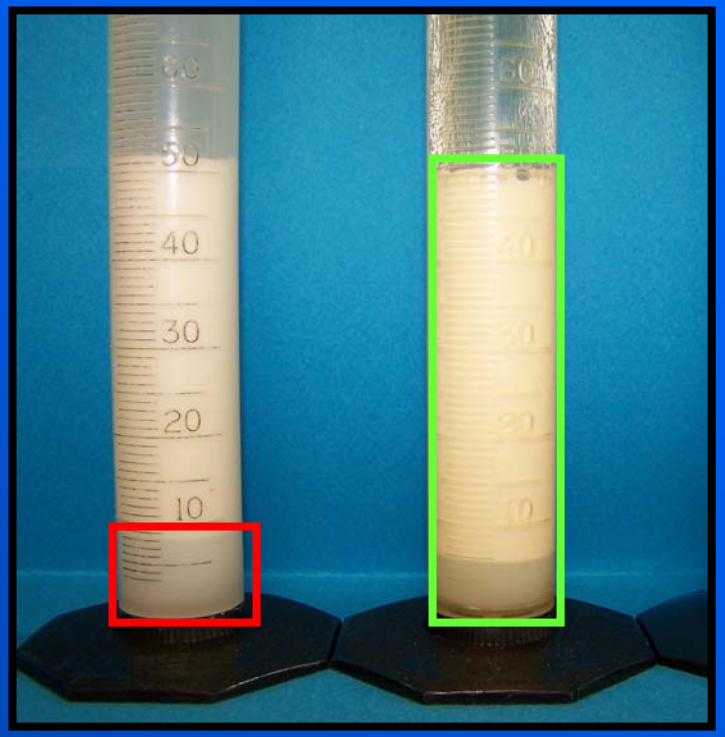
Emulsion Stability

- Based on Creaming Index values

$$\left[\frac{\text{Height of Droplet Depleted Layer}}{\text{Height of the Emulsion}}\right] \times 100$$



- Average modal diameter of mWPC particles ~3.8 µm
- Average modal diameter of SPI particles ~ 8.5 µm

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Emulsion Stability of mWPC & mWPC:Dextran Dispersions

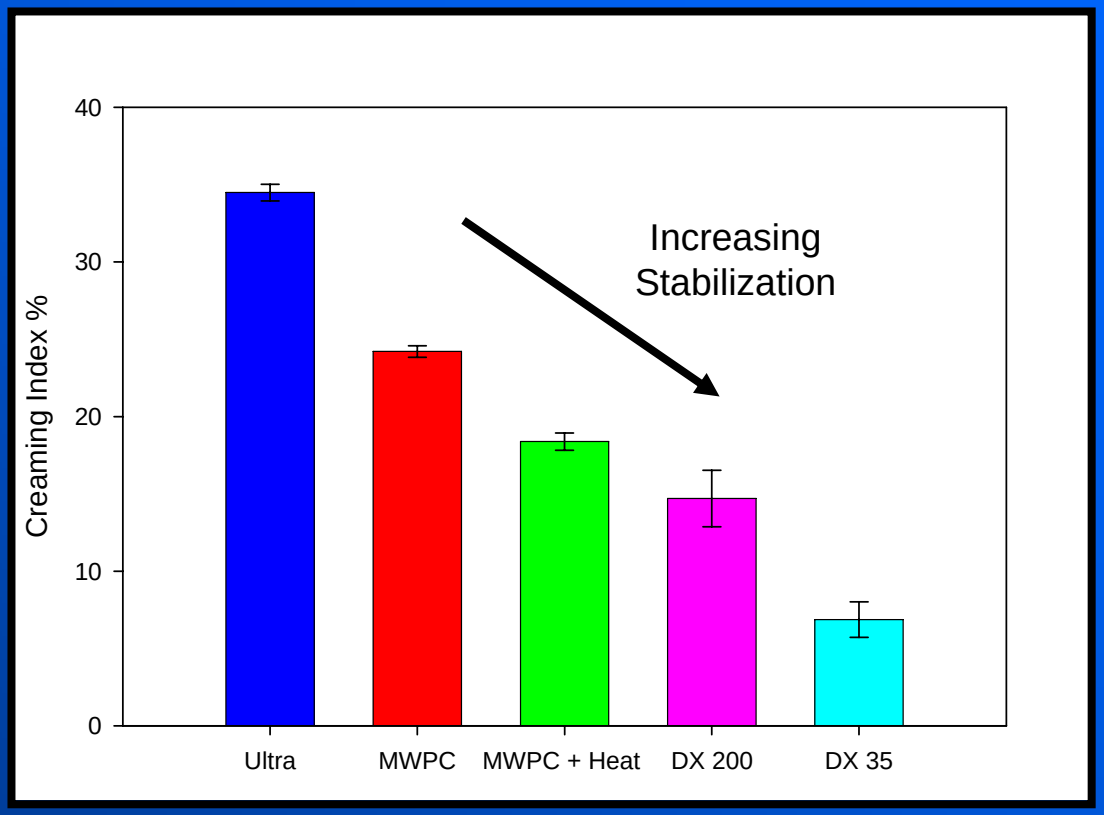
- Based on Creaming Index values

  Creaming Index = 100 x (HD/HE)

- Average modal diameter of particles approximately 3.8 µm

Conjugation increased emulsion stability ~2-3X

mWPC *vs.* mWPC:Dextran



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Modification of protein functionality using transglutaminase

$$R\text{-}Gln\text{-}(CH_2)_2\text{-}\overset{\overset{\displaystyle O}{||}}{C}\text{-}NH_2 \;+\; NH_2\text{-}(CH_2)_4\text{-}Lys\text{-}R$$

Glutaminyl residue **Lysyl residue**

- Intra- & Inter-chain crosslinking of proteins
- New functional ingredients

Tgase

$$R\text{-}Gln\text{-}(CH_2)_2\text{-}\overset{\overset{\displaystyle O}{||}}{C}\text{-}NH\text{-}(CH_2)_4\text{-}Lys\text{-}R \;+\; NH_3$$

ε-(γ-Glutamyl)Lysine

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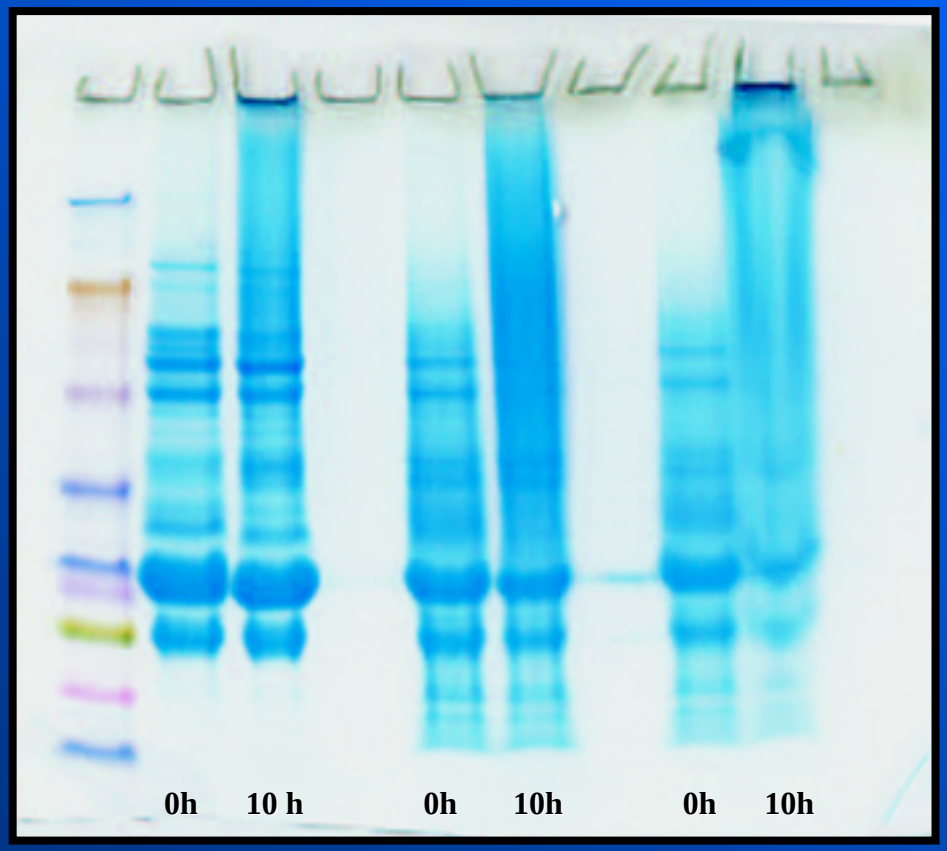
TGase Catalysis of WPC substrates



Ultra WP pH 6.5 | mWPC (-) DTT | mWPC (+) DTT

CONCLUSION:

TGase catalysis of mWPC was observed in the absence of DTT, pH 8.0

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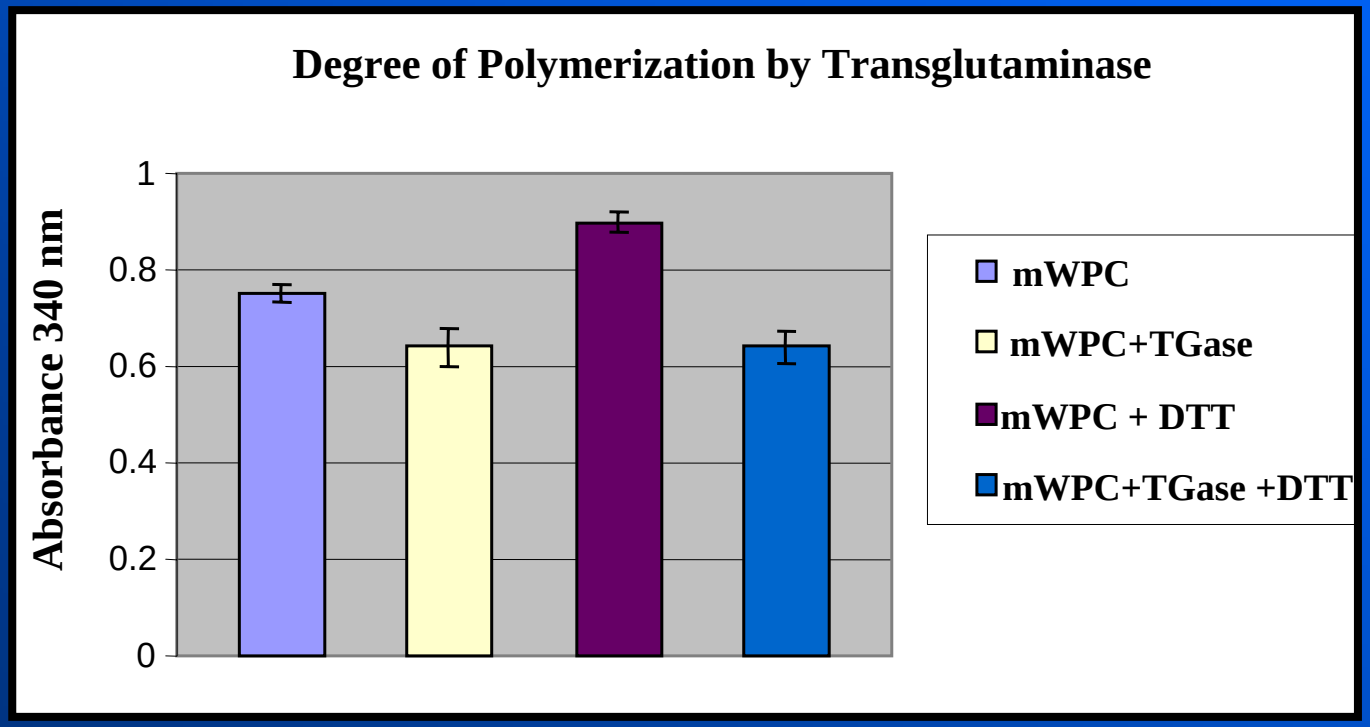
OPA assay of Transglutaminase Crosslinking Reactions



- Enzyme catalysis was observed to a greater degree upon inclusion of DTT

- 15% *vs.* 29%

  2 hours, 37°C

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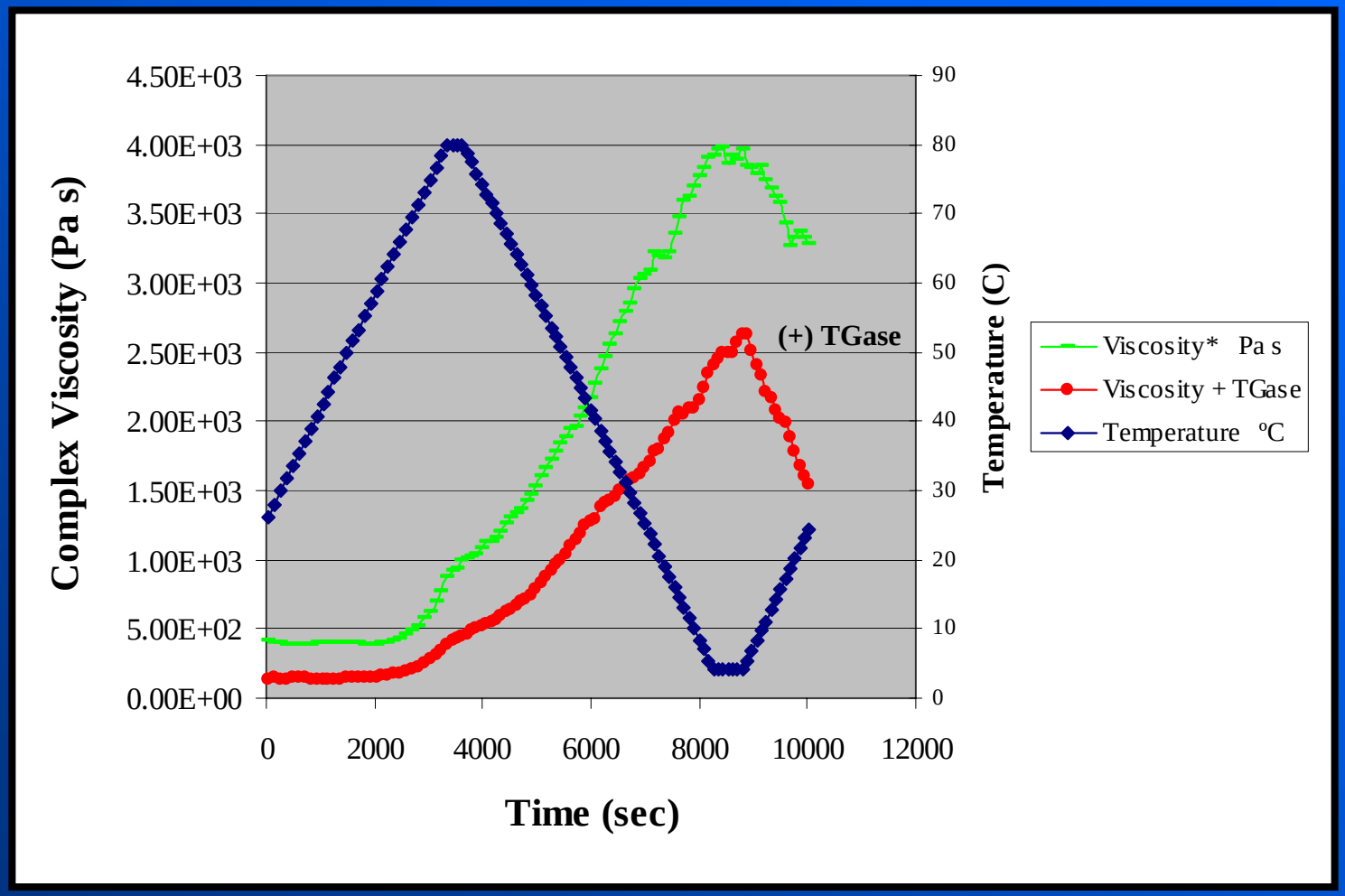
Effect of TGase Crosslinking on Complex Viscosity: 10% mWPC - 15h, pH 8.0



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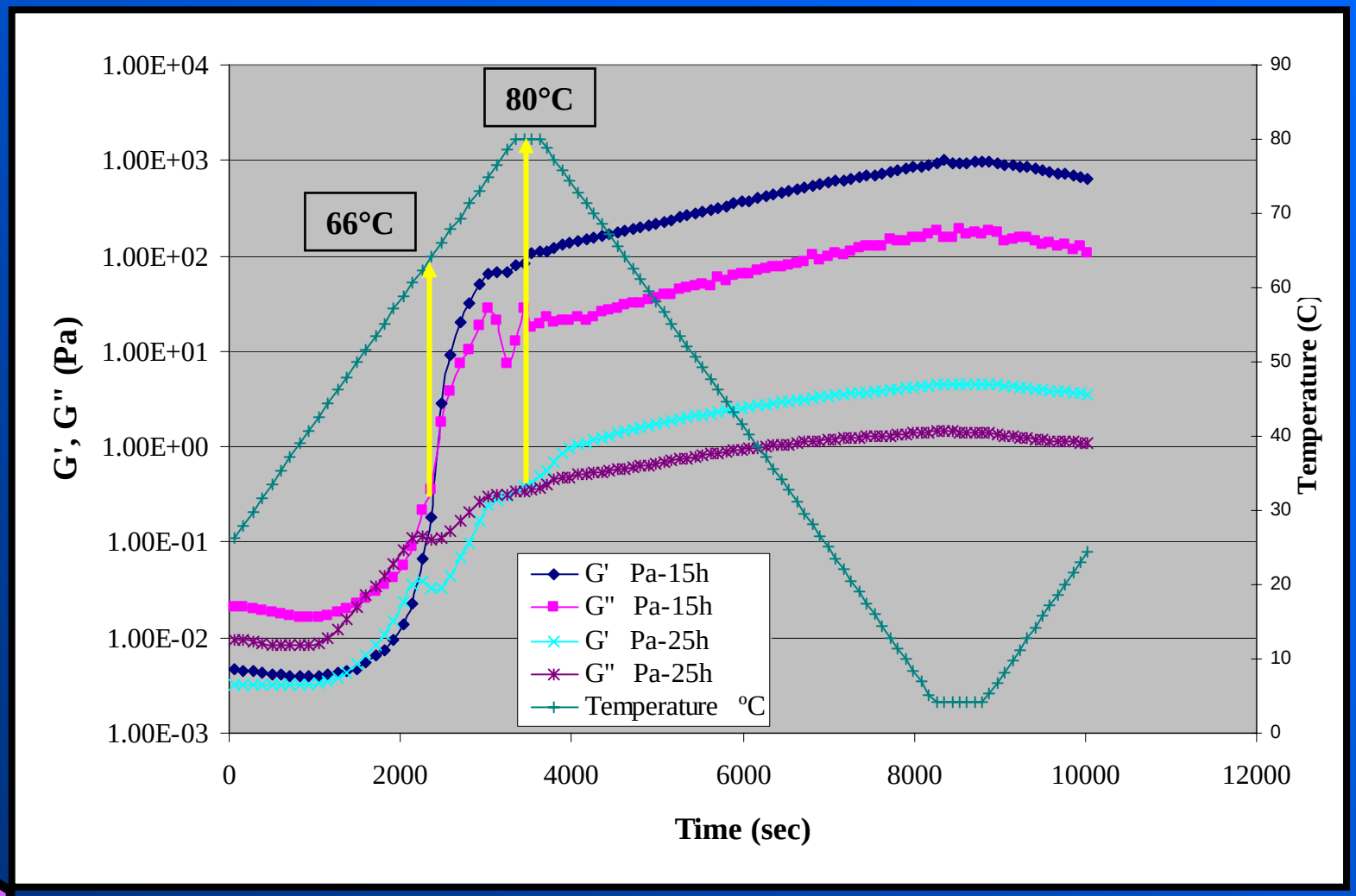
Complex viscosity was LOWERED (~1.5X) after treatment with TGase

Effect of TGase Crosslinking on GELLING TEMPERATURE 15h vs. 25h



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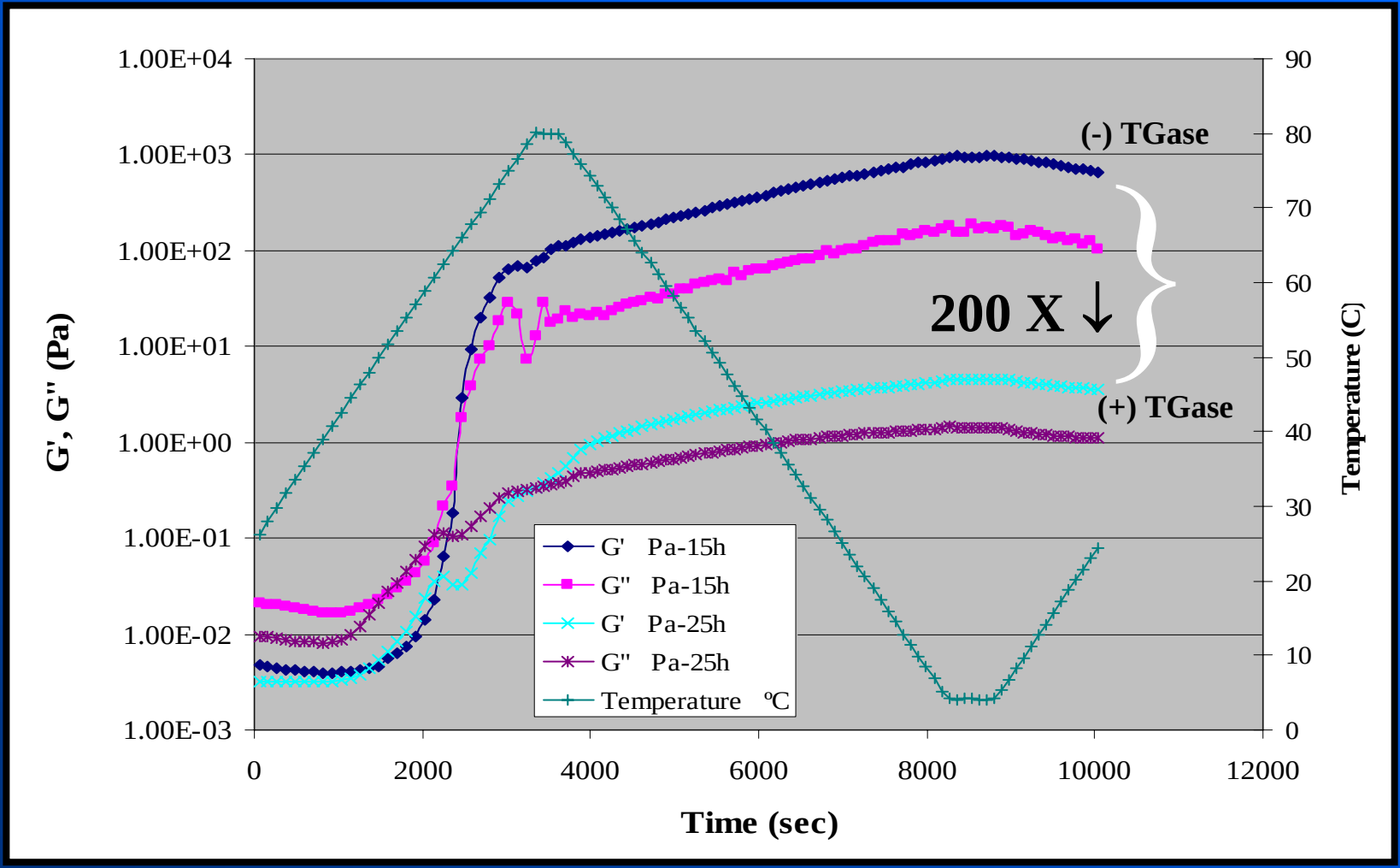
Gelation temperature ↑ ~14°C after catalysis for 10 hours

Effect of TGase Crosslinking on GEL STRENGTH 15h vs. 25h



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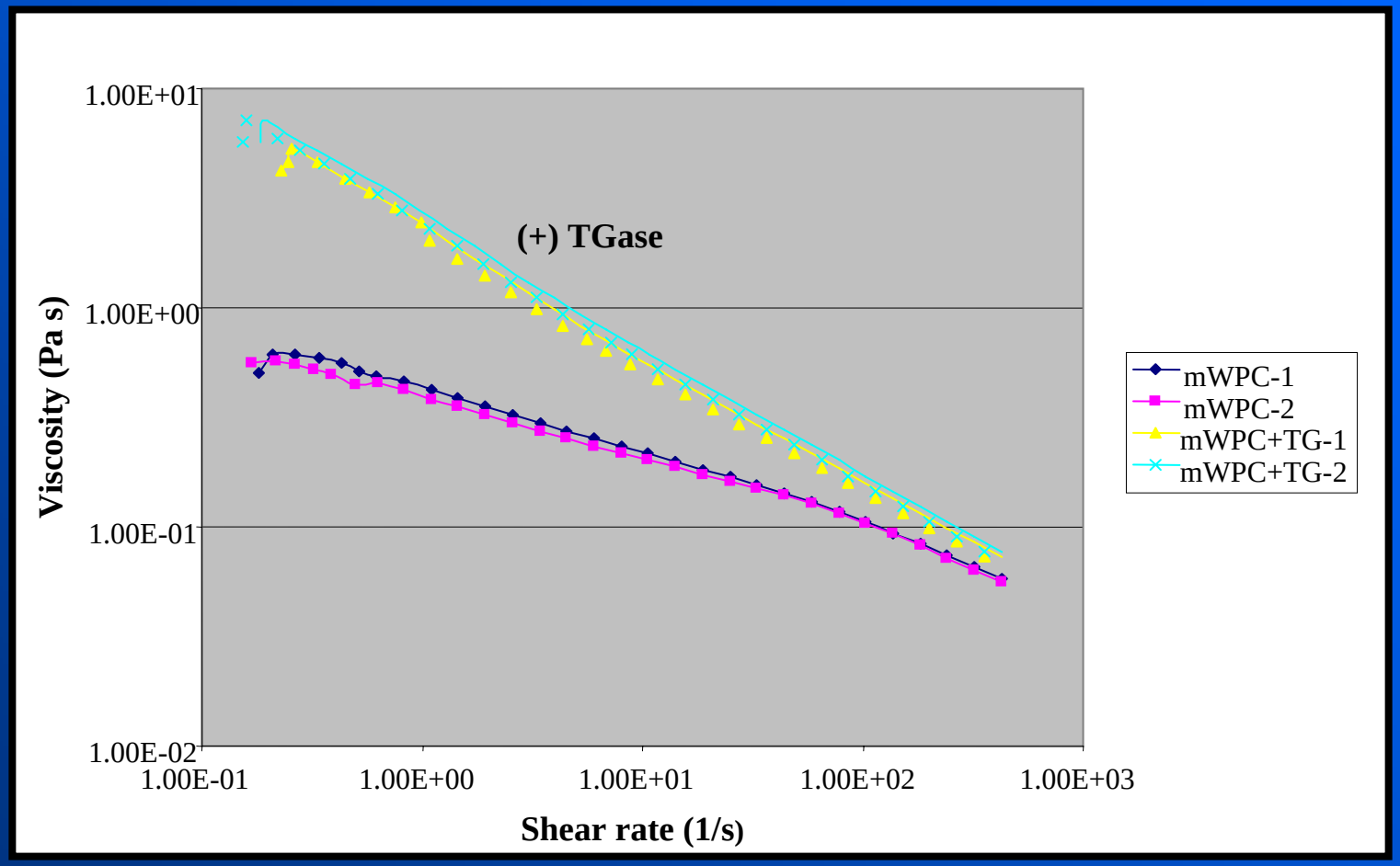
GEL STRENGTH was ↓ ~200 fold after catalysis for 10 hours

Limited HYDROPHOBIC interactions

Effect of TGase Crosslinking on EMULSION VISCOSITY 25°C & 4°C



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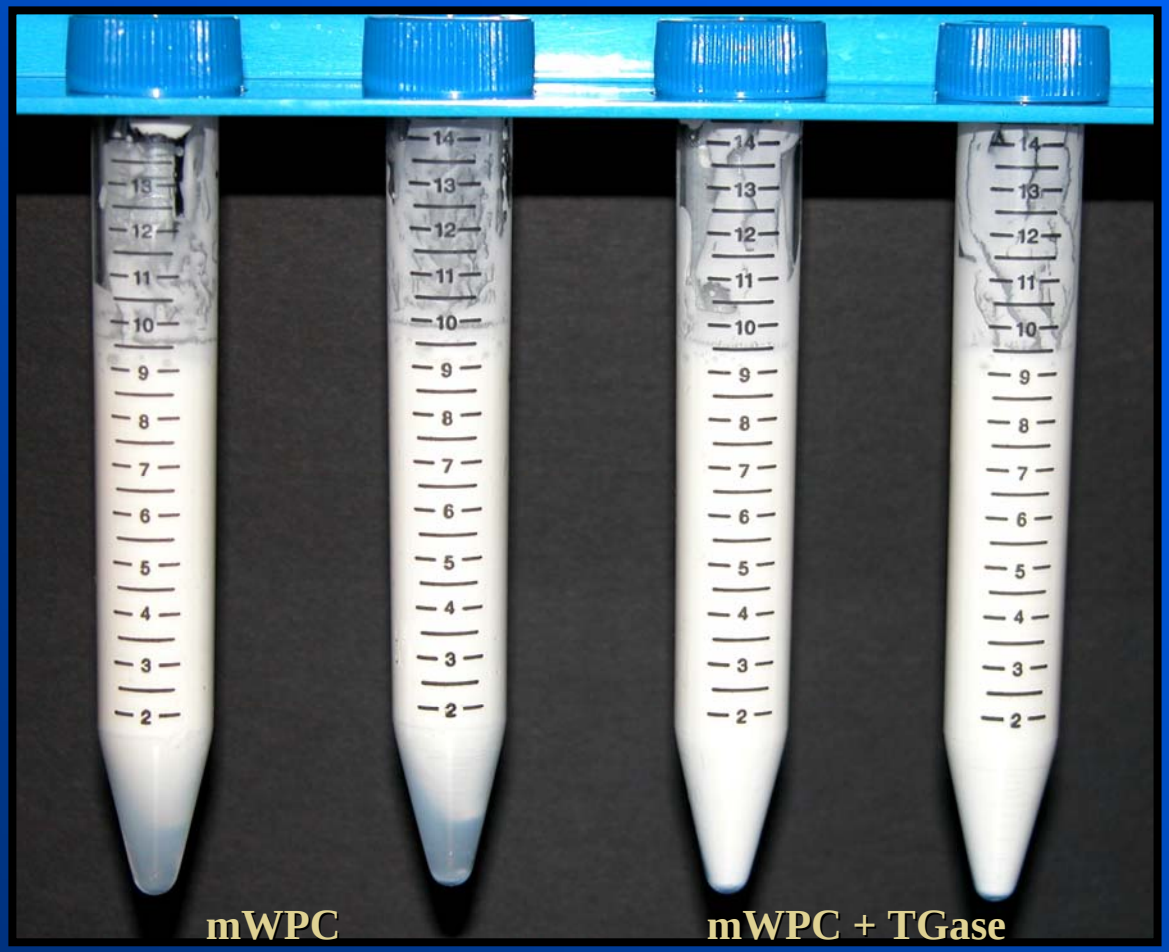
EMULSION VISCOSITY was ↑ (~ 10X) after polymerization with TGase

EMULSION STABILITY of TGase-treated mWPC dispersions



Emulsion stability of TGase-treated mWPC dispersions was MUCH IMPROVED over non-treated samples

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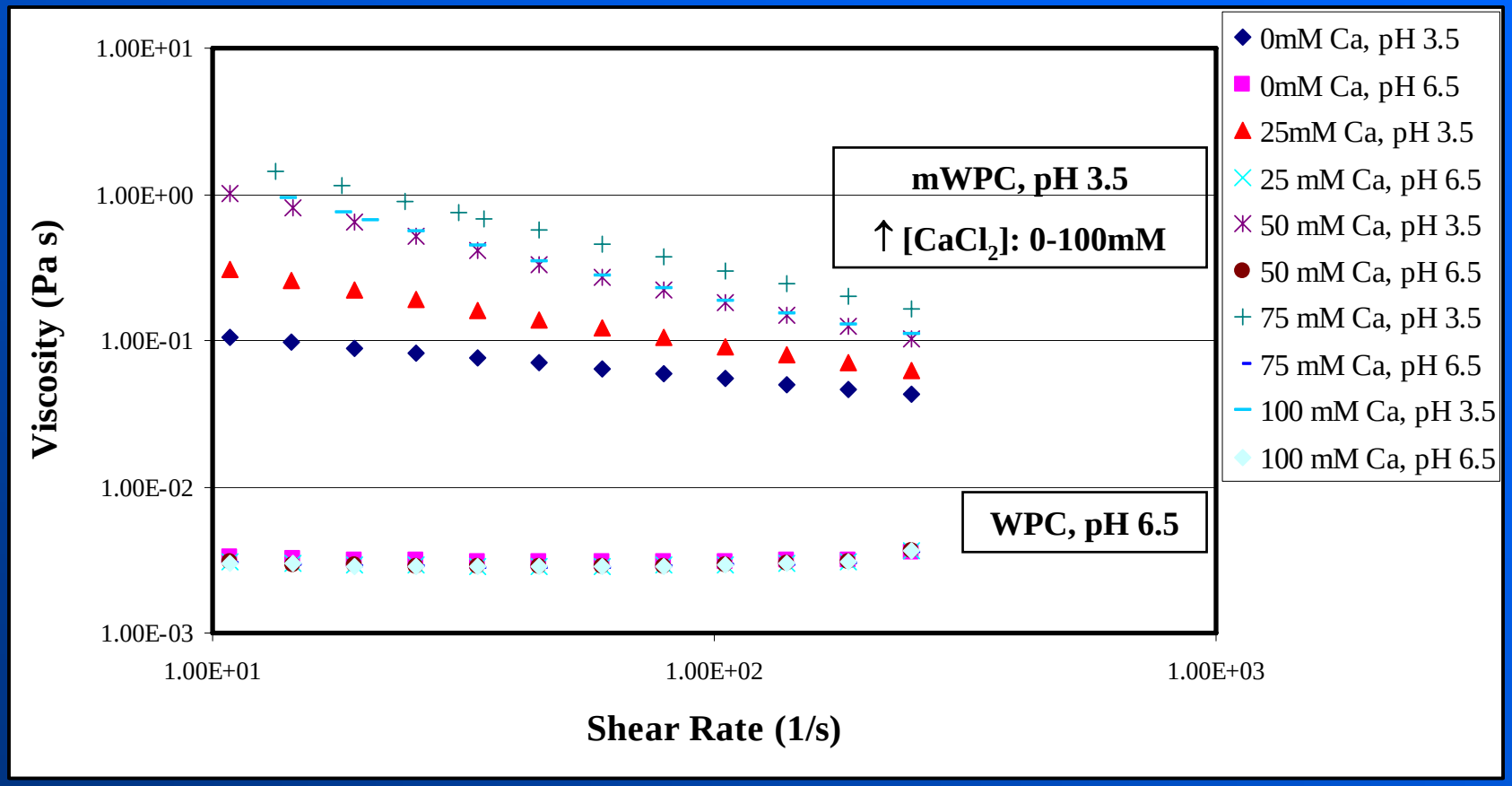
Viscosity measurement upon varying supplemental [$CaCl_2$] to 5.6% mWPC protein dispersions, pH 3.5 and pH 6.5



↑ [$CaCl_2$] ↑ Viscosity

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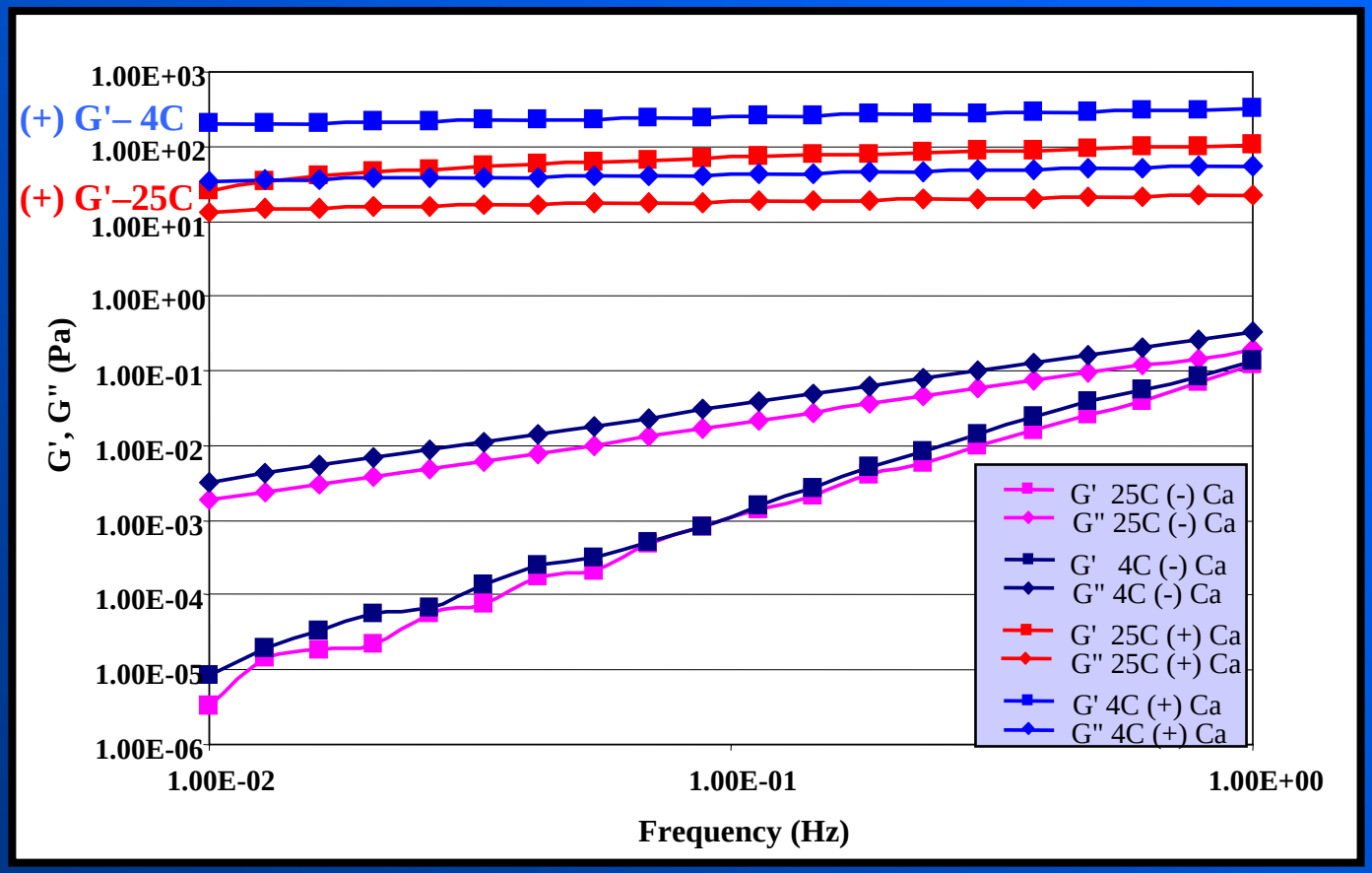
Changes in the storage modulus (G') and loss modulus (G'') of mWPC dispersions due to $CaCl_2$ and temperature effects



} Ca^{++}

- Gel strength was DRAMATICALLY ↑ upon addition of $CaCl_2$ at 4°C and 25°C
- Lowered amount of protein for gelation

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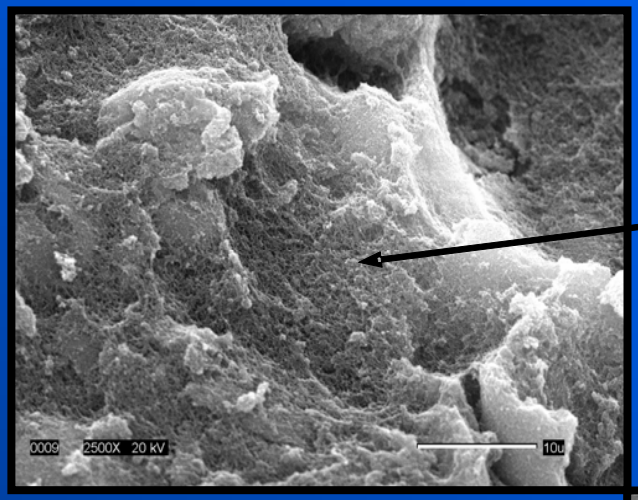
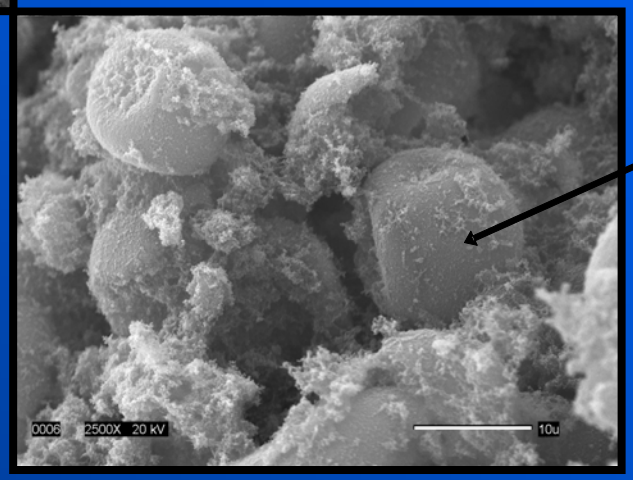
Scanning Electron Micrographs of mWPC and mWPC samples containing $CaCl_2$

mWPC, pH 3.5



mWPC fibrils

mWPC (+) 100 mM $CaCl_2$, pH 3.5



Spherical Structure

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CONCLUSIONS

- WPC, itself, exists naturally as a GLYCOCONJUGATE, highly lactosylated due to manufacturing conditions
- Preparation of the mWPC ingredient ↓ the degree of lactosylation
- The viscosity of mWPC-lactose dispersions ↑ due to Maillard reactions
- Gel strength and viscosity ↑ upon heating SPI , however, further heat modifications (i.e., Maillard reaction between SPI:carbohydrates) were deleterious
- Transglutaminase polymerization of mWPC dispersions resulted in:
  - ↑ Gelling temperatures
  - ↓ Gel strength
  - ↑ Emulsion stability
- Calcium addition to mWPC dispersions:
  - ↑ Viscosity, Gel strength, Water holding capacity
  - Requires less protein for gelation

Purpose : Expand Gelation Capabilities

- Develop modified WPC/SPI ingredients for:
  - ready reconstitution
  - gelation at ambient temperatures
  - enhanced heat stability

- Utility in
  - heat sensitive foods (pasteurized, low heat treatment)
  - Shelf stable pudding / yogurt powder

Acknowledgements

- NC State University Food Rheology Lab
- United States Department of Agriculture (Funding)
- Grande Cheese
  - Drs. Tom Helm and David Taylor
- SPI – NCSU Plant Sciences

John Lillard – mWPC

Grace Cramp - SPI

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