Effect of Milk Proteins on Hardening of High Protein Nutrition Bars

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Center for Dairy Research “Solution Based Research Backed by Experience, Passion and Tradition”
Research Overview

- High Protein Food Systems
  - High Protein Nutrition Bars (HPNB)

- Part 1: Survey Study of Milk and Whey Protein Ingredients
  - Ingredient compositional, functional, & chemical properties
  - HPNB texture & sensory properties

- Part 2: Probing the Mechanism of Hardening
  - Texture, $A_w$, color, Native/SDS-PAGE, bond type analysis,
Research Questions

• What effect do various milk protein ingredients have on a HPNB model system?

• Can we use the ways in which ingredient variability influences the system to begin to understand the mechanism of hardening?
Production of HPNB

<table>
<thead>
<tr>
<th>WPC/H</th>
<th>MPC</th>
<th>Protein Level</th>
<th>Time Point</th>
<th>Duplicate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td></td>
<td>0M</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>X</td>
<td>6M</td>
<td>1</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Similar proximate compositions (% dry basis) with exceptions of WPH 2,4 w/ ~85% protein
### Model System: HPNB Formulation

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Fructose</td>
<td>32.03</td>
</tr>
<tr>
<td>Protein Blend</td>
<td>30.00</td>
</tr>
<tr>
<td>Peanut Flour</td>
<td>9.46</td>
</tr>
<tr>
<td>Peanut Butter</td>
<td>7.56</td>
</tr>
<tr>
<td>Sugar</td>
<td>7.56</td>
</tr>
<tr>
<td>Honey</td>
<td>6.56</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>5.56</td>
</tr>
<tr>
<td>Vanilla Extract</td>
<td>1.27</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Carb: Protein: Fat

40: 30: 30
44: 30: 26

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPH</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>SPI</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>MPC</td>
<td>15</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>
Functional Analyses of Protein Ingredients

- Foaming ability and stability
- Heat Stability Index (HSI)$^{11}$
- Water Holding Capacity (WHC)$^{12}$
- Turbidity$^{13}$
- % Insolubility$^{14}$

Sensory Evaluation of HPNB

**Property**
- Firmness
- Crumbliness
- Stickiness
- Hardness
- Denseness
- Fracturability
- Cohesiveness
- Tooth Packing
- Sweet
- Acid
- Salty
- Bitter
- Umami

**In-hand texture**
- Whey
- Rancid
- Cooked soy
- Peanutty
- Cardboard
- Oxidized
- Cereal/grainy
- Metallic
- Astringent
- Burn
- Residual
- Flavor

**In-mouth texture**
- Flavors

**Basic Tastes**
Analysis of Ingredients: Degree of Hydrolysis

- Generally, DH of 5.4%-16% have been associated with increased interfacial functionality\textsuperscript{15}

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Degree of Hydrolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPH 1</td>
<td>20.7\textsuperscript{a}</td>
</tr>
<tr>
<td>WPH 2</td>
<td>7.8\textsuperscript{c}</td>
</tr>
<tr>
<td>WPH 3</td>
<td>3.9\textsuperscript{d}</td>
</tr>
<tr>
<td>WPH 4</td>
<td>16.8\textsuperscript{b}</td>
</tr>
<tr>
<td>WPH 5</td>
<td>16.3\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{15}Luck et al., 2002.
Results Summary

- Low WPH HPNB
  - Most firm, hard, crumbly, fracturable
- High WPH HPNB
  - Least firm and hard; most dense, chewy, sticky
- WPH 3
  - Most firm, hard, fracturable HPNB
  - Higher WHC, lower solubility, higher viscosity (hydrated & in bev.), lower DH
Hypothesis

• Degree of hydrolysis influences the chemical and surface properties of the protein ingredient thereby influencing their interactions in the HPNB system
## Model System

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Fructose (75%)</td>
<td>52</td>
</tr>
<tr>
<td>Protein Powder (80%)</td>
<td>37.5</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>10.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Meets common composition of 30% protein with ~50% carb and 20% fat.

### Storage Conditions
- Storage at 33.5 °C/ambient humidity for 31 days
Types of Evaluation

• Ingredient Compositional and Functional Analyses
  – Proximate Analysis, Degree of hydrolysis, peptide Mw
  – WHC, foaming, solubility, turbidity, hydrophobicity
• Ingredient solubility in the presence of fructose
• Color, $A_w$ & Texture Analysis of HPNB (Colorimeter; Aqua Lab 4TE; TA.XT Plus)
• Formation of insoluble aggregates during bar storage
• Bond types in insoluble aggregates (Exposure to buffers)
• Bond types in in sol. & insol. aggregate (SDS and Native PAGE)
Texture Profile Analysis

![Graph showing the hardness as force (N) against hours of storage at 33.5°C. Different symbols represent different DH levels: No DH (diamond), Low DH (square), Mid DH (triangle), High DH (circle).]
Pre-Storage

- Color similar for all HPNB at initial manufacture
- Texture varied immediately

No DH
Hardness: 230 N

Mid DH
Hardness: 50 N

Low DH
Hardness: 308 N

High DH
Hardness: 20 N
Day 31 of Storage

Color changed

Drastically for Mid & High DH
Moderately for No DH HPNB
Very little for Low DH HPNB
Summary of Functional Data and Results (1)

- Mid and High DH WPH produced no foams
- Water Holding Capacity did not directly correlate to hardness
- Ingredient solubility did not change in presence of fructose
- Softer bars were less soluble = aggregation not cause of hardening
- Bar solubility did not change over time, but the trend was towards an increase NOT a decrease

\[16\text{Zhou et al., 2013.}\]
Summary of Functional Data and Results (2)

→ $A_w$ stable through storage for No (0.616) and Low DH (0.630) HPNB

→ $A_w$ increased for the Mid and High DH HPNB (~0.585 to 0.615)

→ Covalent bonds not cause of hardening – softest HPNB had highest prevalence

→ Aggregate formation appears mostly due to non covalent bonds (likely hydrophobic interactions) in all bars

→ Browning rxns not cause of hardening as previously reported\textsuperscript{16}

\textsuperscript{16}Zhou et al., 2013.
Conclusions

- Hydrolysis can reduce HPNB hardening
- Protein/peptide aggregation in higher DH WPH (covalent or non-covalent)\textsuperscript{17}
  - PAGE, HPNB solubility, ingredients lower solubility/viscosity/foaming\textsuperscript{18,19,20}
- Protein-sugar interactions increased in higher DH WPH
  - Maillard browning

\textsuperscript{17}Akkermans, 2008. \textsuperscript{18}Otte et al., 1996. \textsuperscript{19}Ryan et al., 2012. \textsuperscript{20}Verheul et al., 1998.
Bar Production
Acknowledgements

• Dr. Scott Rankin
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• Sensory panelists
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