Protein aggregation and hardening of nutritional bars as f(moisture)

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Typical Protein Bars

Nutrition Facts
• 150 - 300 calories
• 25 - 40g protein
• 10 - 30g CHO
• 0 - 5g fat
• Bodybuilders
• Special protein blend
Protein bar market

- Est. $930 million in sales in 2006 (Mintel)
- 35% sales growth from 2001-2006
  - Initial success followed by maturation

U.S. sales of nutrition and energy bars, 2001-06
Example protein nutritional bar

- **Moisture** ~ 15%(WB) 18%(DB)
  - $a_w \approx 0.55$
- **Protein**
  - Up to 40% of total weight
- **Protein sources**
  - Whey protein (WPI/WPC/WPH)
  - Caseinate/casein
  - Milk protein (MPI/MPC)
  - Soy protein (SPI/SPC)
  - Gelatin or hydrolysates
- **Sugar/sugar alcohols**
  - Glycerol (glycerine)
  - Maltitol and maltitol syrup
  - Oligofructose
  - Maltodextrin
  - Xylitol
  - Sorbitol

<table>
<thead>
<tr>
<th>Nutrition Facts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serving Size: 1 Bar (2.12 oz)</td>
</tr>
<tr>
<td>Servings Per Container: 12</td>
</tr>
<tr>
<td>Amount per Serving</td>
</tr>
<tr>
<td>Calories Total</td>
</tr>
<tr>
<td>from Fat</td>
</tr>
<tr>
<td>% Daily Value*</td>
</tr>
<tr>
<td>Total Fat</td>
</tr>
<tr>
<td>Saturated Fat</td>
</tr>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>Sodium</td>
</tr>
<tr>
<td>Potassium</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
</tr>
<tr>
<td>Dietary Fiber</td>
</tr>
<tr>
<td>Sugars</td>
</tr>
<tr>
<td>Protein</td>
</tr>
</tbody>
</table>

**Ingredients:**

Protein Blend (Whey Protein Isolate, Casein, Milk Protein Concentrate, Soy Protein Isolate, Calcium Caseinate), Glycerine, Maltitol, Hydrolyzed Gelatin, Modified Palm & Palm Kernel Oils, Water, Maltitol Syrup, Enriched Wheat Flour, Cocoa Powder (Processed with Alkali), Natural Flavor & Artificial Flavors, Soybean Oil, Oligofructose, Sunflower Seed Oil, Calcium Carbonate, Maltodextrin, Canola Oil, Xylitol, Vitamin & Mineral Blend (Ascorbic Acid, Copper Gluconate, Ferric Orthophosphate, dl-Alpha Tocopherol Acetate, Biotin, Niacinamide, Zinc Oxide, Beta Carotene, Calcium Pantothenate, Vitamin A Palmitate, Pyridoxine Hydrochloride, Manganese Sulphate, Riboflavin, Thiamin Mononitrate, Potassium Iodide, Cyanocobalamin, Chromium Chloride, Folic Acid, Sodium Molybdate, Sodium Selenite), Mono and DiGlycerides, Sorbitol, Cocoa Extract, Salt, Soy Lecithin, Color (Titanium Dioxide), Chocolate Liqueur, Sucralose (Splenda Brand), Sodium Bicarbonate, Mixed Tocopherols, May Contain Traces of: Peanuts, Various Nuts, and Seeds.
Problems with protein bars

- Taste
- Maillard Reaction
- Texture – bar hardening
- Loss of nutritional value
Whey protein stability in nutritional bar

- Problem - hardening of nutritional bar during storage
  - Bar model test formula
    - 35% WPI
    - 25% Corn syrup
    - 25% HFCS
    - 10% Peanut butter
    - 5% Glycerol
  - Moisture: ~15%
  - $a_w$: ~0.55
  - $Q_{10}$ ~ 3.6
  - Note initial jump presumed due to redistribution of water & humectants into protein particles as $a_w$ equilibration occurs but continues at slower rate in long storage
Possible mechanisms for hardening

- 1. Moisture & humectant redistribution into protein particles.
- 2. Protein-protein interactions → aggregation
- 3. Humectant effects on mobility, local viscosity/glass transition, and protein interaction
- 4. Maillard reaction effects on color & texture
Why does hardening occur?

1. Initial redistribution of water/humectants

Stage A: Redistribution of water/humectants in matrix and particles after mixing

EVIDENCE: slight reduction in water activity over 1st 5 days
2. Protein-protein interaction

- Driving forces
  - Covalent bonding
    - Disulfide bonds
  - Non-covalent interactions
    - H-bonding, hydrophobic interactions, electrostatic interactions

<table>
<thead>
<tr>
<th></th>
<th>% of whey proteins</th>
<th>SH</th>
<th>S-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactoglobulin</td>
<td>~50</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>~20</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>BSA</td>
<td>~10</td>
<td>1</td>
<td>17</td>
</tr>
</tbody>
</table>

β-Lactoglobulin  α-Lactalbumin  BSA
Example BSA % solubility loss @ 37°

Figure 12: Bell-shaped relationship between residual water and solid-state aggregation (evidenced by a reduction in solubility) of bovine serum albumin. Solubility was measured following a 24-hour incubation at 37°C. From Liu et al.84
Example rbST stability @ 47°C


Figure 11. Increase in the initial zero-order rates of degradation for recombinant bovine somatropin (rbST) in a lyophilized formulation stored at 47°C, measured by reverse-phase HPLC. From Hageman et al.13
Insulin aggregation as a function of water sorption of the protein

- Figure (A). The sorption isotherm at 50°C ($m_0$ is 4.6 g water/100 g protein)

- Figure (B). Aggregation of insulin after 24 hr at 50°C as (%RH)

Costantino and others 1994, Pharm Res 11(1): 21
Simplified bar model system

- Bar simplified model system (WB): WPI and buffer system
  - BioPRO® whey protein isolate from Davisco
    - Protein, 97.4% on dry basis
    - Lactose, < 1% of dry basis (minimal Maillard reaction)
    - Fat, 0.3% of dry basis (lipid oxidation minimal)
  - Phosphate buffer (10 mM, pH 7)
  - WPI/buffer: 3/2 (WPI, 60% of the total weight)
    - 0.67:1 water to protein ratio
  - Sodium Azide (0.05% of the total weight)
Extent of whey protein aggregation as $f(t,T)$

$Q_{10}$ aggregation $\sim 3.3$ from 23 to 45 °C or 11 fold factor

So 1 months at 45 °C = 11 months at 23 °C

thus $\sim 2.5$ weeks at 45 °C = $\sim 6$ months at room temperature
Aggregate particle formation

Changes in micro-structure (scanning electron microscopy)

Fresh control

Storage at 45 °C for 3 months
(Particle diameter 50~100 nm)
Changes in the conformation of whey protein molecules by DSC

![Graph showing changes in conformation](image)

- **Control**: 45°C, 100 days
- **α-Lactalbumin and BSA**: 45°C, 100 days
- **β-Lactoglobulin**: 45°C, 100 days

Temperature (Degree C)
Confirmation by FTIR for WPI
Mechanisms of protein aggregation

- Solubility of aggregates in various solutions

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Aggregate solubility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (10 mM, pH 7)</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>Buffer with 0.1% SDS</td>
<td>7.2 ± 0.5</td>
</tr>
<tr>
<td>Buffer with 6 M guanidine HCl</td>
<td>10.9 ± 0.7</td>
</tr>
<tr>
<td>Buffer with 8 M urea</td>
<td>11.6 ± 1.7</td>
</tr>
<tr>
<td>Buffer with 10 mM dithiotreitol</td>
<td>92.2 ± 0.9</td>
</tr>
<tr>
<td>Buffer with SDS and dithiotreitol</td>
<td>97.1 ± 1.7</td>
</tr>
</tbody>
</table>

- Non-covalent
- Covalent (Disulfide bond)
Changes in the texture of whey protein bar model system

- Texture measurement
  - TA-XT2 texture analyzer
  - Plunger: 3mm diameter
  - Compression speed: 1mm/s
  - Deformation strain: 50%
  - Hardness is recorded as the maximum force during the compression
Aggregation and Hardness of whey system vs time

- Formation of aggregates
- Hardening in texture

Graph showing storage time vs hardness and aggregation over days.
Suggested primary mechanism for texture change in whey bars due to disulfide bond interactions

- Fresh bar base
- Formation of separate aggregates between tiny particles
- Formation of aggregate network
3. Potential Influence of sugars/polyols (humectants) on hardening in whey bar systems

- Bar systems need an $a_w$ of < 0.7 to prevent microbial growth otherwise antimicrobial agents required
- Accomplished by replacing water with sugars or sugar alcohols (polyols) as plasticizers in whey system
  - Lowers $a_w$ and will influence:
    - Local viscosity of liquid phase which controls mobility & thus reaction rates (find maxima in $a_w$ 0.6 to 0.8 range)
    - $T_g$ of system which affects molecular mobility and texture
    - Protein conformation
    - Maillard reaction (browning) if humectant has reducing groups (HFCS)
    - Crystallization (graining) if use sucrose to control Maillard
Modified from Roos and Karel Food Tech 45(12): 66 1991

State Diagram

- **Crystal melt line**
- **Boiling line**
- **Freezing line**
- **Solution**
- **Glassy state**
- **Rubbery state**
- **Vapor**

**Key Points**

- **T = 23°C**
- **Tm**
- **Tg**
- **A = soft 15%wb**
- **B = soft @ 8%wb**
- **Added humectant**
- **Reaction rate & mobility as f(T-Tg)**

**Legend**

- Water activity
  - 0.8
  - 0.7
  - 0.6
  - 0.5
  - 0.3
  - 0

**Graphical Elements**

- A, B: Points on the diagram representing different conditions.
- Graph showing reaction rate and mobility as a function of water activity.

**Additional Information**

Effects of sugar/polyols on lowering of $a_w$
Effects on texture after 1 week @ 45°C
Effects of sugar/polyols on whey protein aggregation after 1 week @ 45°C

PG causes significant amounts of protein aggregation
Other sugar/polyols delay the protein aggregation
4. Maillard reaction in the whey bar

- Driving forces
  - Whey proteins are rich in lysine (>10 g/100 g protein)
  - The presence of reducing sugars such as fructose/glucose
  - The $a_w$ of most nutritional bars is between 0.5 ~ 0.7, in the reactive range for Maillard reaction
Maillard browning problem

Storage at 45 °C for 7 Days

Polyol/sugar fraction | A 0% | B 33% | C 50% | CD 60% | D 67% | E 78% | F 83% | G 100%
Non enzymatic browning in protein bars

Fig 31. Color development in the nutritional bar model system containing different amounts of sugars or polyols.
Previous study – lysine loss

Model system:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-sorbate</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>10</td>
</tr>
<tr>
<td>Glycerol</td>
<td>20</td>
</tr>
<tr>
<td>Whey protein</td>
<td>30</td>
</tr>
<tr>
<td>Apiezon B oil</td>
<td>20</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>20</td>
</tr>
<tr>
<td>Water</td>
<td>18.53g/100 g solid</td>
</tr>
</tbody>
</table>

- Initial water activity: 0.78
- 25 days to 50% lysine loss (FDNB method) at 35°C

Minolta Chromameter Model CR-200

- L values: 0 (black) to 100 (white)
- Previous study: 100% WPI bar 83 → 50 in 30 days at 35ºC
Small scale study – color changes

- HFCS/CS bars stored at 35 C

Weeks of storage time

0 1 2 3 4 5
Storage study – HFCS/CS bars at 35 °C

Remaining reactive lysine in protein bars stored at 35 C vs time

\[ y = 78.1 e^{-0.3531x} \]

\[ R^2 = 0.8785 \]

\[ t_{1/2} = 10.5 \text{ days} \]
Remaining reactive lysine of protein bars stored at 45 °C vs time

\[ y = 91.581e^{-0.2545x} \]

\[ R^2 = 0.9543 \]

\[ t_{1/2} = 2.25 \text{ days} \]

\[ Q_{10} = 4.7 \]

1 day AT 45 °C = 1 mo. @ 23 °C
NEB effects on protein quality @ $a_w = 0.65$
NEB effects on protein quality

Reactive lysine of protein bars after storage at 45C

Days storage

Mg lysine/ml solution

Maltitol-sv

HFCS-CS
HOW to make and keep a soft bar and of high nutritional quality?

- Potential solutions:
  - 1. Add reducing reagents and thiol-blocking reagents if allowed
  - 2. Add whey protein hydrolysates (Lowering the Tg of bar system)
  - 3. Control the types and ratio of humectants
1. Reducing or thiol blocking reagents

- Try to slow down protein aggregation and hardening
  - Reducing reagents: Cysteine, glutathione
  - Thiol blocking reagents: N-ethylmaleimide

<table>
<thead>
<tr>
<th>Model ID</th>
<th>Protein (6g)</th>
<th>Buffers (4g)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>WPI</td>
<td>10 mM phosphate buffer</td>
<td>Control</td>
</tr>
<tr>
<td>C-L</td>
<td>WPI</td>
<td>0.06 M L-cysteine</td>
<td>Reducing</td>
</tr>
<tr>
<td>C-H</td>
<td>WPI</td>
<td>0.45 M L-cysteine</td>
<td>Reducing</td>
</tr>
<tr>
<td>G-L</td>
<td>WPI</td>
<td>0.06 M L-glutathione</td>
<td>Reducing</td>
</tr>
<tr>
<td>G-H</td>
<td>WPI</td>
<td>0.45 M L-glutathione</td>
<td>Reducing</td>
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<tr>
<td>E-L</td>
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<td>0.06 M N-ethylmaleimide</td>
<td>Thiol blocking</td>
</tr>
<tr>
<td>E-H</td>
<td>WPI</td>
<td>0.45 M N-ethylmaleimide</td>
<td>Thiol blocking</td>
</tr>
</tbody>
</table>
Storage of whey model WB at 45 °C

30 day equivalent to 1 year at 23 °C
2. Addition of protein hydrolysates

H1 = 5.2% hydrolyzed
H2 = 8.8%
H3 = 14.9%

Hydrolysate effectiveness is not related to greater water holding capacity
Whey protein hydrolysates (Davisco)

- Bar model formula (25% replacement of WPI with whey protein hydrolysates)
  - 40% total protein
  - 30% corn syrup
  - 20% HFCS
  - 10% glycerol
  - ~ 15% water
  - $a_w = 0.65$

Degree of hydrolysis

- W: 5.2%
- WH1: 8.8%
- WH2: 14.9%
- WH3: 14.9%
Hardness development at 45 °C  Day 7 vs Day 0

WCFG model system with 40% proteins  ~7 day at 45°C equivalent to 3 months at 23 °C
Hydrolysate substitution lowers Tg so softer based on T-Tg

\[ WPI: y = -47.051 \ln(x) + 205.86 \quad R^2 = 0.9643 \]

\[ H1: y = -54.61 \ln(x) + 199.22 \quad R^2 = 0.9948 \]

\[ H2: y = -65.562 \ln(x) + 212.89 \quad R^2 = 0.9975 \]

\[ H3: y = -64.534 \ln(x) + 200.85 \quad R^2 = 0.996 \]
Solutions reviewed

- Add either reducing agents or thiol blockers
  - The reducing agents do NOT work for the model system
  - Cannot find a food grade thiol blocking agent yet

- Partly replace WPI with 25% whey protein hydrolysate
  - Makes a softer bar, partially slowing down the hardening

- Control type and ratio of humectants
  - No propylene glycol
  - Eliminate HFCS, use sucrose instead for sweetness or artificial
  - Use glycerol in combo with sorbitol, maltitol, and xylitol
Questions and/or comments?