



Research and Development March 2012

# Development of New Whey Ingredients

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Director of Research



a world of nutritional ingredients and cheese

# Development of Dairy Ingredients

## Functional Applications

- Emulsifiers (0.25-2%), Salad dressings, sauces,
- Texture modifiers (0.25-2%) increase viscosity of sauces, soups etc
- Anti-oxidants (0.3%), decrease lipid oxidation
- Clean label trends

## Functional and Nutritional Applications

- Heat stable (Functional) whey protein concentrates and isolates for beverage applications that deliver 5-40 grams of protein (Nutritional).
- Nutritional bar texture (short, chewy, nougat etc) and deliver protein (5-25 grams)
- RTMs
- Protein systems for Greek Yogurts

## Future of Whey and Dairy Applications

- Bone health
- Muscular health
- Bioactive peptides (vaso-dilation)
- Cosmetic applications
- Specific peptide or protein fractions
- Lactoperoxidase
- Lactoferrin
  - a. Wound Healing

Basic Milk and Whey Protein Chemistry

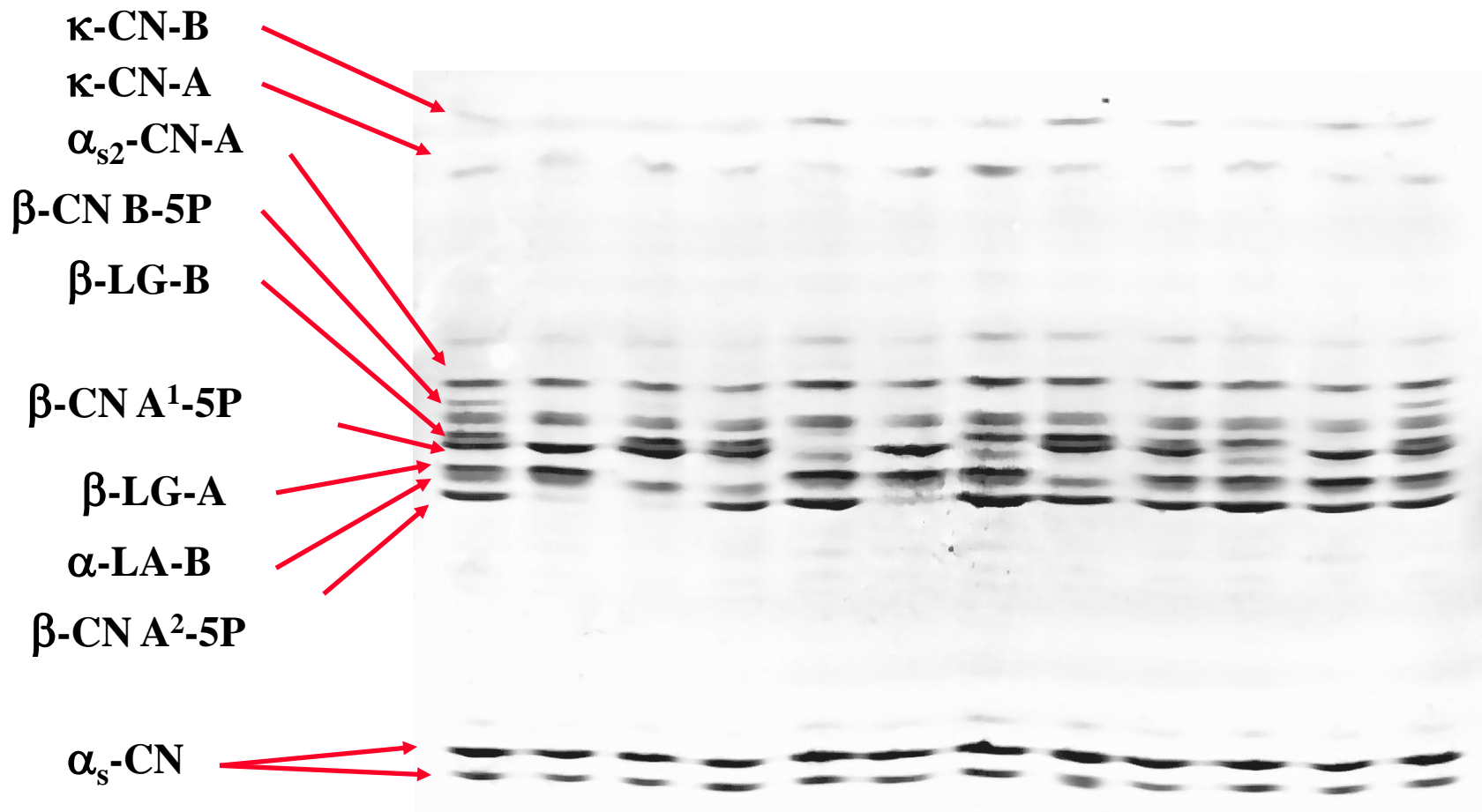
# Milk Components

Component	Average Percentage
Water	86.6
Fat	4.1
Protein	3.6
Lactose	5.0
Ash	0.7

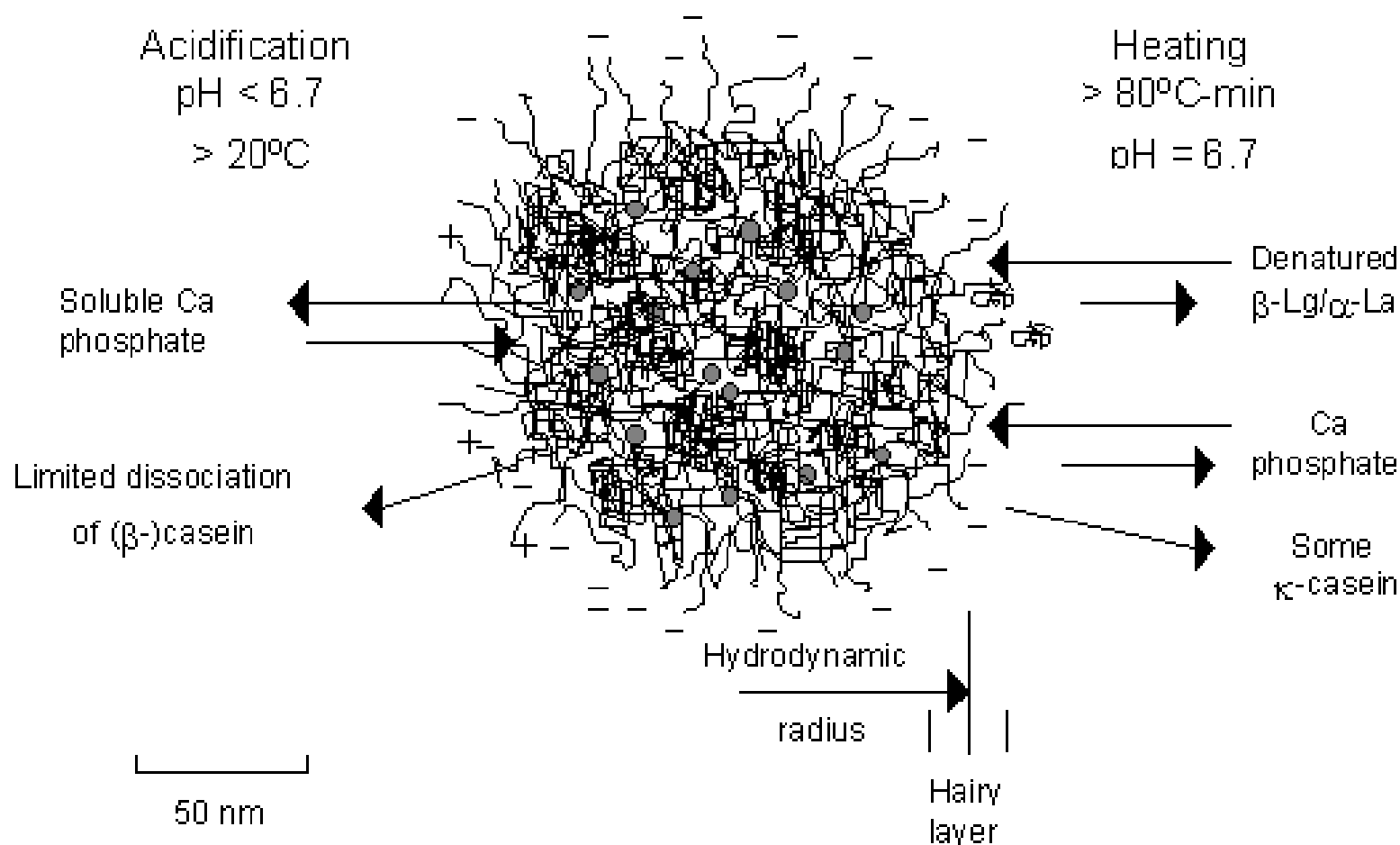
# Milk Proteins

Protein	Concentration g/L	Approximate %
Caseins	24-28	80
Alpha-casein	15-19	
Beta-casein	9-11	
Kappa-casein	3-4	
Gamma-casein	1-2	
Whey Proteins	5-7	20
Beta-lactoglobulin	2-4	
Alpha-lactalbumin	1-1.5	
Serum albumin	0.1-0.4	
Immunoglobulins	0.6-1.0	

# Isoelectric Focusing of Whole Milk



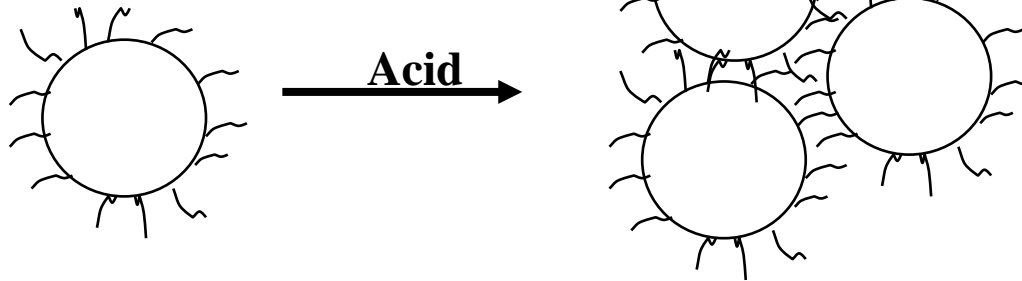
# Casein Micelle



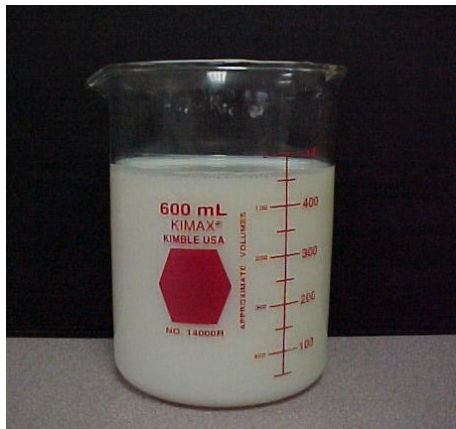
# Precipitation by Acidification

Neutral Charge pH 4.6

Negative Charge pH 6.7



Milk



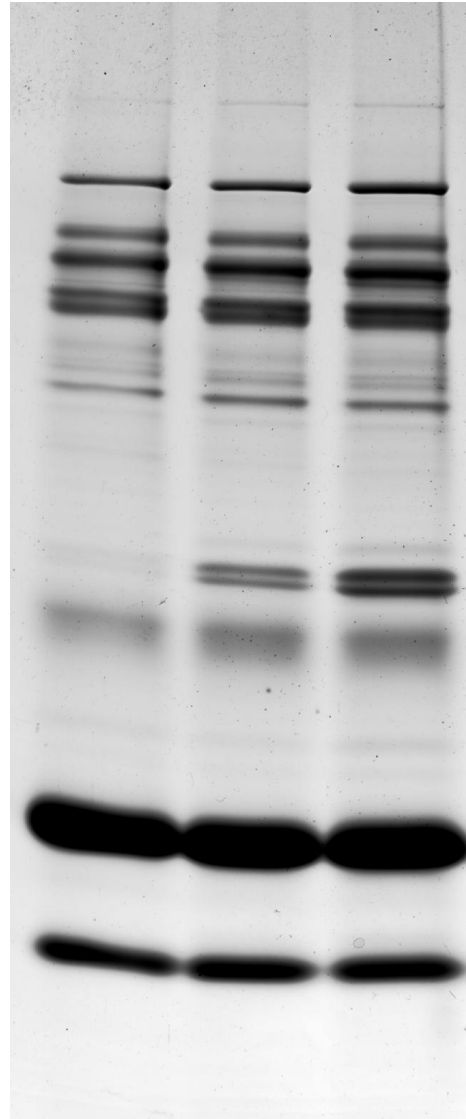
Slow Acidification



Fast Acidification



# Gel Electrophoresis of Whey Samples



**Ig's**

**LF/LP**

**BSA**

**$\alpha, \beta$ -casein**

**$\beta$ -lactoglobulin**

**$\alpha$ -lactalbumin**

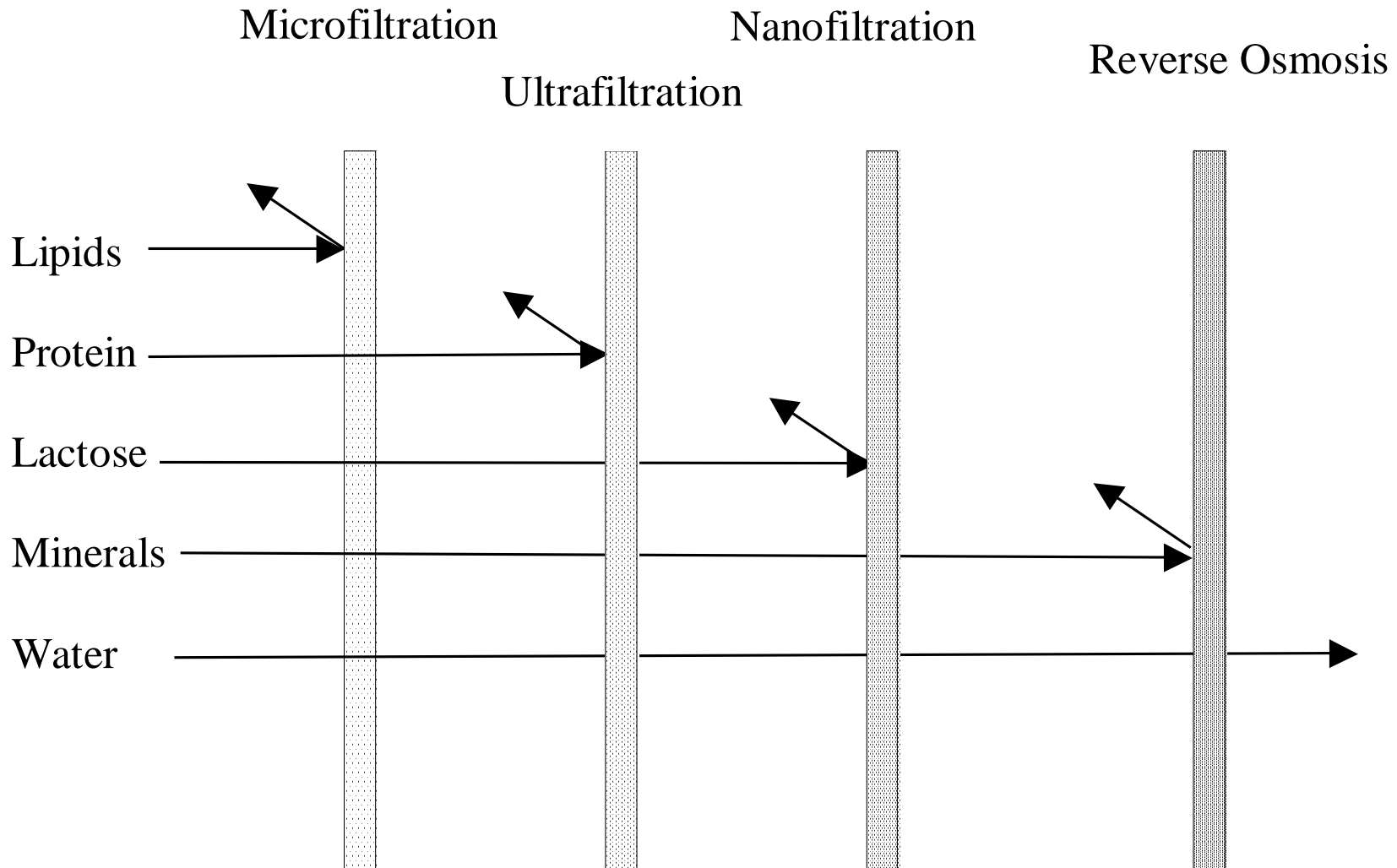


# Whey Composition

Component	Percentage
Water	93.5%
Lactose	5%
Protein	0.8%
Minerals	0.7%
Fat	0.07%

# Component Size Comparison

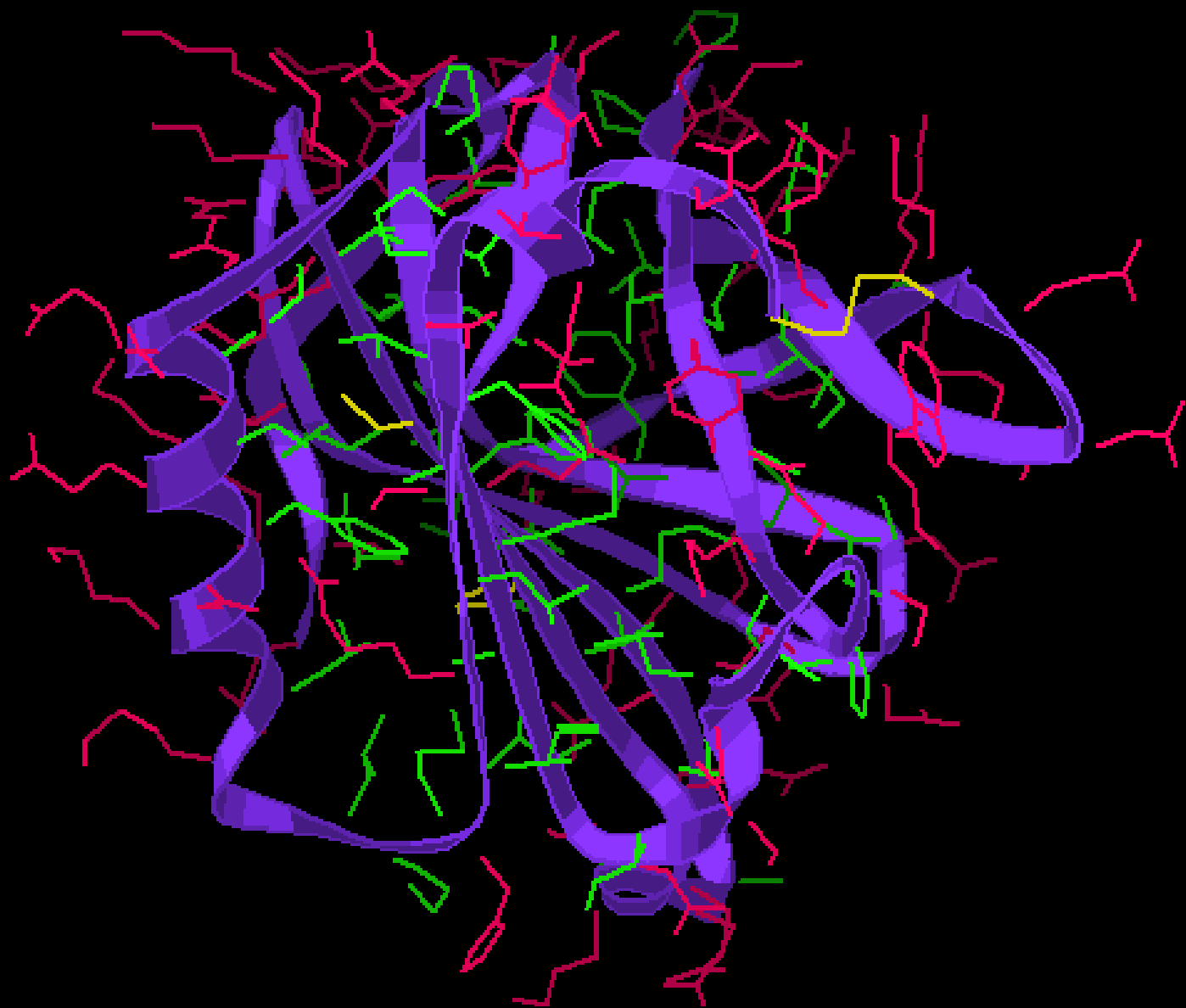
Component	Micron
Water	.0003
Cl <sup>-</sup> , Ca <sup>2+</sup>	.0004
Lactose	.0008
Whey Proteins	.003-.005
Casein Micelles	.025-.3
Fat Globules	.1-10
Bacteria	.2-8



<b><u>Ingredient</u></b>	<b><u>% Water</u></b>	<b><u>% Fat</u></b>	<b><u>% Protein</u></b>	<b><u>% Casein</u></b>	<b><u>% Whey</u></b>	<b><u>% Lactose</u></b>	<b><u>% Ash</u></b>	<b><u>% Ca</u></b>
<b>Milk</b>	<b>87.4</b>	<b>3.5</b>	<b>3.2</b>	<b>2.5</b>	<b>0.7</b>	<b>4.9</b>	<b>0.7</b>	<b>0.12</b>
<b>Skim Milk Powder</b>	<b>4</b>	<b>1</b>	<b>35</b>	<b>28</b>	<b>7</b>	<b>52</b>	<b>8</b>	<b>1.2</b>
<b>MPC 42</b>	<b>4</b>	<b>1</b>	<b>42</b>	<b>35</b>	<b>7</b>	<b>45</b>	<b>8</b>	<b>1.2</b>
<b>Rennet Casein</b>	<b>10</b>	<b>.5</b>	<b>80</b>	<b>80</b>	<b>-</b>	<b>1</b>	<b>8</b>	<b>2.7</b>
<b>Sweet Whey Powder</b>	<b>4</b>	<b>1</b>	<b>12</b>	<b>-</b>	<b>12</b>	<b>73</b>	<b>8</b>	<b>0.7</b>
<b>Delac Whey Powder</b>	<b>4</b>	<b>2</b>	<b>23</b>	<b>-</b>	<b>23</b>	<b>56</b>	<b>16</b>	<b>0.85</b>
<b>Demin Whey Powder</b>	<b>4</b>	<b>2</b>	<b>13</b>	<b>-</b>	<b>13</b>	<b>80</b>	<b>1</b>	<b>0.08</b>
<b>WPC 34</b>	<b>3.5</b>	<b>3</b>	<b>35</b>	<b>-</b>	<b>35</b>	<b>52</b>	<b>8</b>	<b>0.54</b>
<b>WPC 50</b>	<b>3.5</b>	<b>4</b>	<b>50</b>	<b>-</b>	<b>50</b>	<b>35</b>	<b>5</b>	<b>0.5</b>
<b>WPC 80</b>	<b>3</b>	<b>6</b>	<b>80</b>	<b>-</b>	<b>80</b>	<b>6</b>	<b>2</b>	<b>0.64</b>
<b>WPI</b>	<b>3.5</b>	<b>0.5</b>	<b>91</b>	<b>-</b>	<b>91</b>	<b>1</b>	<b>3</b>	<b>0.7</b>
<b>Lactose</b>	<b>0.15</b>	<b>0.1</b>	<b>0.2</b>	<b>-</b>	<b>0.2</b>	<b>99</b>	<b>0.1</b>	<b>0.1</b>
<b>Milk Calcium</b>	<b>6.0</b>	<b>1.0</b>	<b>7.0</b>	<b>-</b>	<b>7.0</b>	<b>7</b>	<b>73</b>	<b>23</b>
<b>Thermax</b>	<b>3.5</b>	<b>3</b>	<b>35</b>	<b>-</b>	<b>35</b>	<b>52</b>	<b>8</b>	<b>0.54</b>

# Whey Proteins

Protein/Fraction	Level g protein/Liter whey	Molecular weight
$\alpha$ -lactalbumin	1.0	14,176
$\beta$ -lactoglobulin	3.0	18,363
BSA	0.3	66,267
immunoglobulins	0.6	
Glycomacropeptide	0.8	6,754-7,280
Lactoferrin	0.0012-0.1	77,000
Lactoperoxidase	22,000 umole/min/L whey	77,500
Lysozyme	0.00013	18,000
Total whey protein	5.7	







# Development of Dairy Ingredients

## Functional Applications

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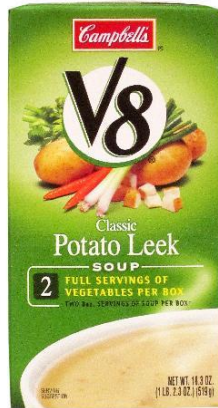
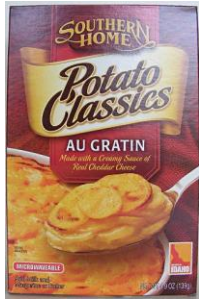
# Whey Protein Concentrate



Cultured Pasteurized Lowfat Milk; Sugar; Whey Protein Concentrate; Nonfat Milk Solids; Whey; Vanilla Extract



# Potatoes and Whey

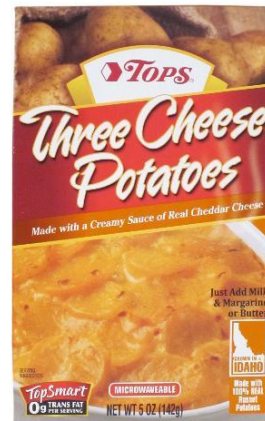


Potatoes (1); Whey (2); Whey Protein Concentrate (10);

Water; Potatoes(2); Dehydrated Potatoes(3); Dehydrated Onion; Butter; Cream; Milk(6); Whey Protein Concentrate (10);

Potatoes (1);Whey (6);

Whey Protein Isolate (5); Potato Starch (11);



Potatoes (1); Reduced Lactose Whey (3); Whey (4);

Potatoes (1); Whey (5);

Idaho Potatoes (1); Modified Corn Starch (2); Whey From Milk (3);

Red Potatoes (1); Russett Potatoes (2); Milk Blend (3); Milk (4);

# Functional Whey Protein Concentrate

## TYPICAL ANALYSIS

Protein, dry basis	>78%
Moisture	<5.0%
Fat	<10%
Minerals	<5.0%
Lactose	<5.0%
pH	6.0 - 6.5

## SUGGESTED LABELING

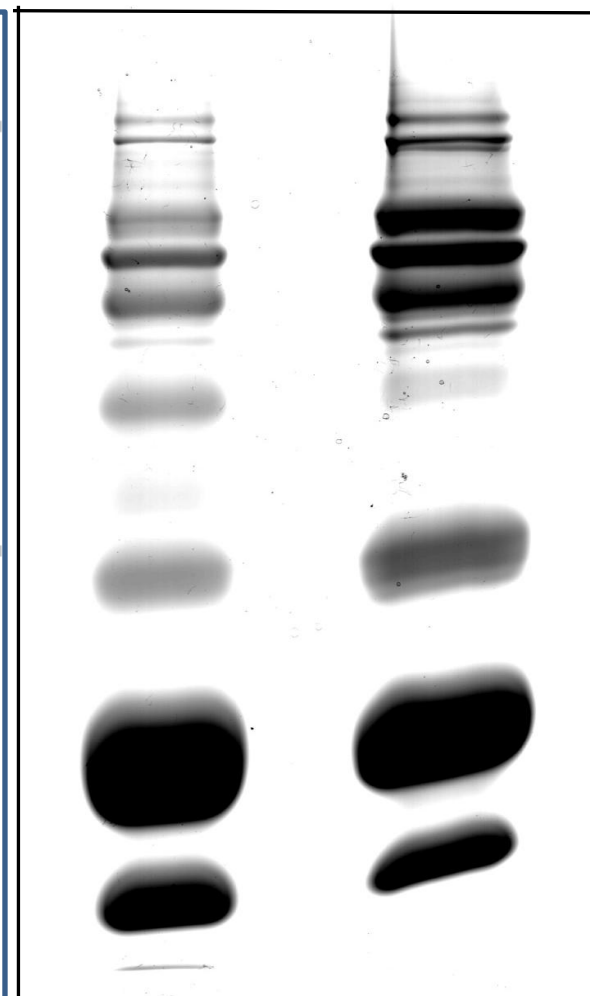
- Whey protein concentrate
- Allergen information: contains milk ingredients
- Kosher and Halal approved

## PRODUCT CHARACTERISTICS

- Excellent emulsion stability
- UHT/retort stable
- Contributes to opacity
- Improves water binding/moisture retention
- Enhanced mouthfeel
- Adds fat-like character

## SUGGESTED USES

- Sauces
- Salad dressings
- Yogurt
- Dairy-based dips
- Creamy soups
- Bakery products
- Beverages

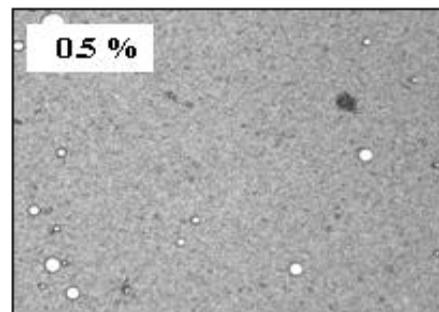
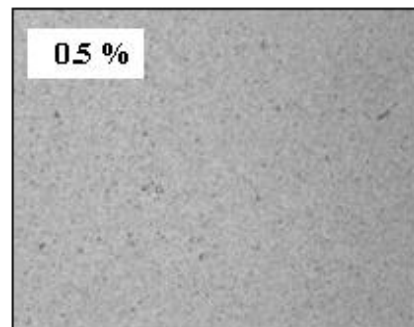
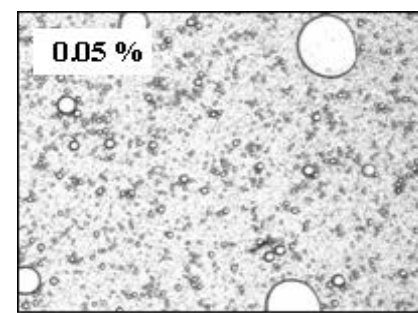
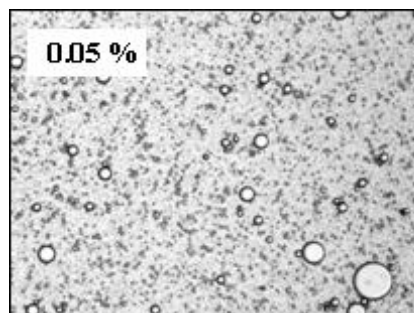


# Emulsion Comparison

- Protein concentration from 0.05-2 wt%
- 10 % corn oil
- 5 passes at 5,000 psi
- Surface load at saturation
  - CWPC = 5 mg/m<sup>2</sup>
  - FWPC = 4 mg/m<sup>2</sup>

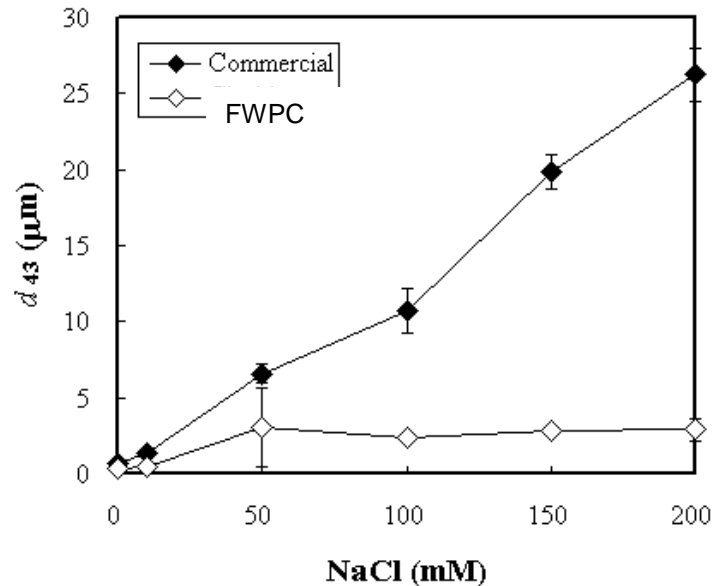
FWPC

CWPC



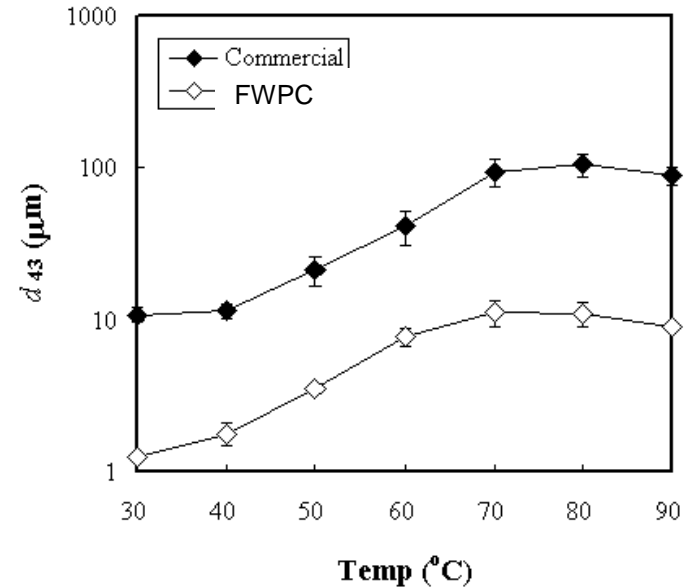
# Emulsion Stability

## Susceptibility to ionic stress



- Droplet concentration of 5%
- 0-200 mM NaCl
- Stored room temperature for 24 hours

## Susceptibility to temperature



- Droplet concentration of 5%
- Held for 30 minutes at each temperature
- Evaluated after 24 hours of storage at room temperature

# Reduced Fat Ranch Dressing

**FWPC 0.5%**

**Egg Yolk 1.5%**

- Testing parameters
  - 10 cycle freeze thaw
  - 2.5 weeks with cycles staggered for minimum 24 hours
  - Freezer Temperature = 28 F
  - Thawed in 140 F water





# Development of New Whey Ingredients

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## Bioactive Ingredients Neutraceutical Ingredients

- Bone health
- Muscular health
- Bioactive peptides (vaso-dilation)
- Cosmetic applications
- Specific peptide or protein fractions
- Lactoperoxidase
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  - a. Wound Healing



# Whey Protein Isolate and Concentrate in Beverage Applications

Truncated ingredient list



9 g protein

Whey Protein Concentrate



6 g protein

Sucrose; Almonds; Oat Flour; Invert Syrup; Whey Protein Isolate;



15 g protein

Purified Water; Whey Protein Isolate; Cane Sugar;



8 g protein

Water; Whey Protein Isolate; Phosphoric Acid;



24.6 g protein

Undenatured Whey Protein Concentrate; Flavoring;



25 g protein

Filtered Water; Diafiltered Skim Milk; Proprietary Protein Blend; Milk Protein Concentrate; Whey Protein Concentrate;



10 g protein

Water; Dairy Protein Blend; Milk Protein Concentrate; Calcium Caseinate; Whey Protein Concentrate;



6 g protein

Organic Pear; Water; Organic Prune; Organic Whey Protein Concentrate;



80 g protein

Protein Blend; Calcium Caseinate; Whey Protein Concentrate; Whey Protein Hydrolysate; Whey Protein Isolate

# Functional Beverage Categories



	Chocolate Meal-Replacement	ProNos Vanilla Protein Shake
Serving Size	8 FL OZ	17 FL OZ
Protein Content	10 g	35 g
Protein Type	Milk Protein Concentrate	Whey Protein Isolate & Concentrate
% Protein	~4%	~7%

**Neutral – pH 6.0-7.0**

Sports Nutrition  
Meal replacements  
Dairy Beverages

Key Characteristics for Ingredients:

- Heat stable at Neutral pH
- Good flavor profile



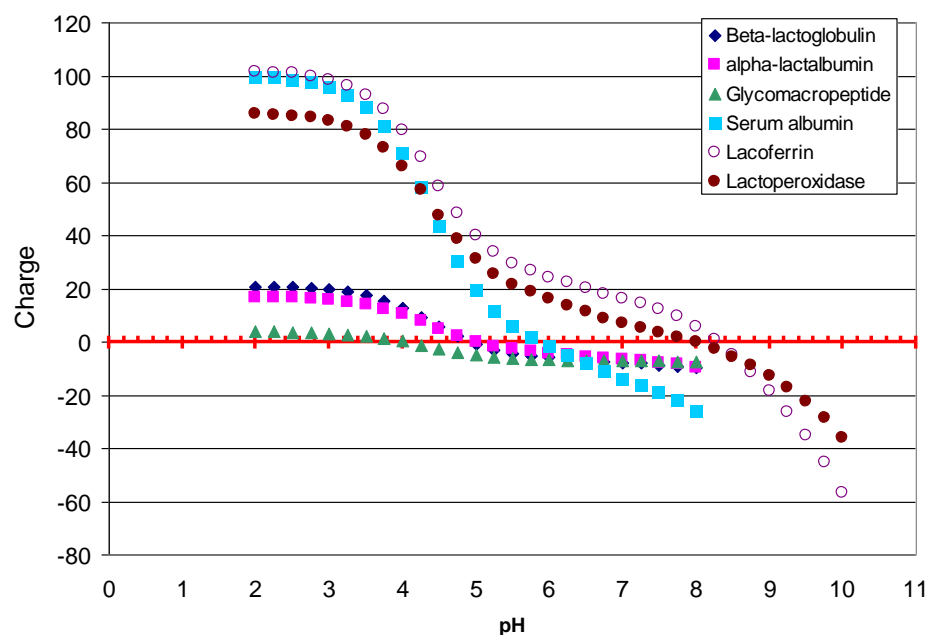
# Functional Beverage Categories



Intermediate – pH 3.5–4.2

Smoothies  
Yogurt-based beverages  
Rehydration

	Tropical Lime Protein Smoothie	Mix One Protein & Antioxidant Drink
Serving Size	8 FL OZ	11 FL OZ
Protein Content	12 g	15 g
Protein Type	Whey Protein Isolate	Whey Protein Isolate
% Protein	~5%	~4%



# Functional Beverage Categories



**Acid – pH <3.5**

Sports drinks  
Isotonics

Juice-based beverages  
Protein Waters

	Plumberry Protein Tea	Calcium-Fortified Protein Punch	IsoPure Protein Tea
<b>Serving Size</b>	<b>20 FL OZ</b>	<b>12 FL OZ</b>	<b>20 FL OZ</b>
<b>Protein Content</b>	<b>10 g</b>	<b>15 g</b>	<b>40 g</b>
<b>Protein Type</b>	<b>Acidified Whey Protein Isolate</b>	<b>Acidified Whey Protein Isolate</b>	<b>Acidified Whey Protein Isolate</b>
<b>% Protein</b>	<b>~2%</b>	<b>~4%</b>	<b>~6%</b>



# Whey Protein Isolate and Concentrate

## Truncated ingredient list



Chickory Root Extract; Sugar; Palm Kernel Oil; Palm Oil; Skim Milk Powder; Alkali Processed Cocoa Powder; Whole Milk Powder; Whey Protein Concentrate; g protein



Invert Syrup; Milk Protein; Cocoa Mass; Sugar; Protein Hydrolysate; Whey Protein Isolate;



6 g protein

Whey Protein Concentrate; Peanuts; Soluble Corn Fiber;



7.6 g protein

Brown Rice Malt; Dried Bananas; Whey Protein Isolate; Rice Flour



10 g protein

Whey Protein Crisps; Whey Protein Isolate; Whey Protein Concentrate; Tapioca Starch



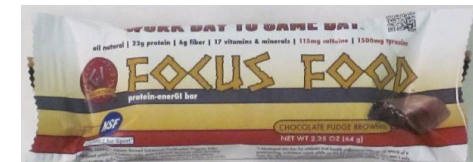
30 g protein

Chocolate Coating; Whey Protein Concentrate; Palm Kernel Oil;



30 g protein

Protein Blend; Whey Protein Isolate; Calcium Caseinate; Milk Protein Isolate; Milk Protein Concentrate; Micellar Casein; Egg Albumen; Hydrolyzed Collagen; Soy Protein Isolate;



22 g protein

Chocolate Flavored Coating; Sugar; Whey Protein Concentrate; Fractionated Palm Kernel Oil;

# Nutritional Bars



## Proteins

Whey Protein Isolate

Soy Protein Isolate

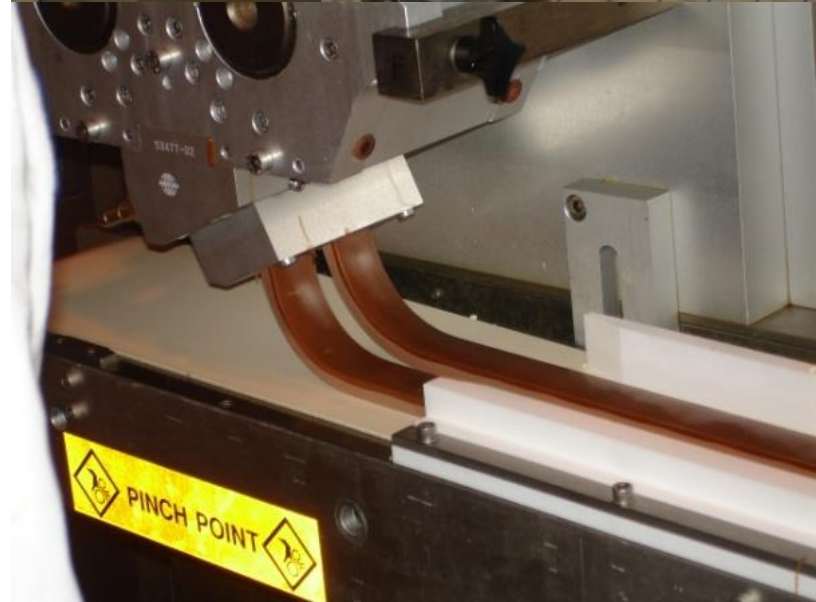
Milk Protein Isolate

Calcium Caseinate

Rice Protein (80%)

Collagen

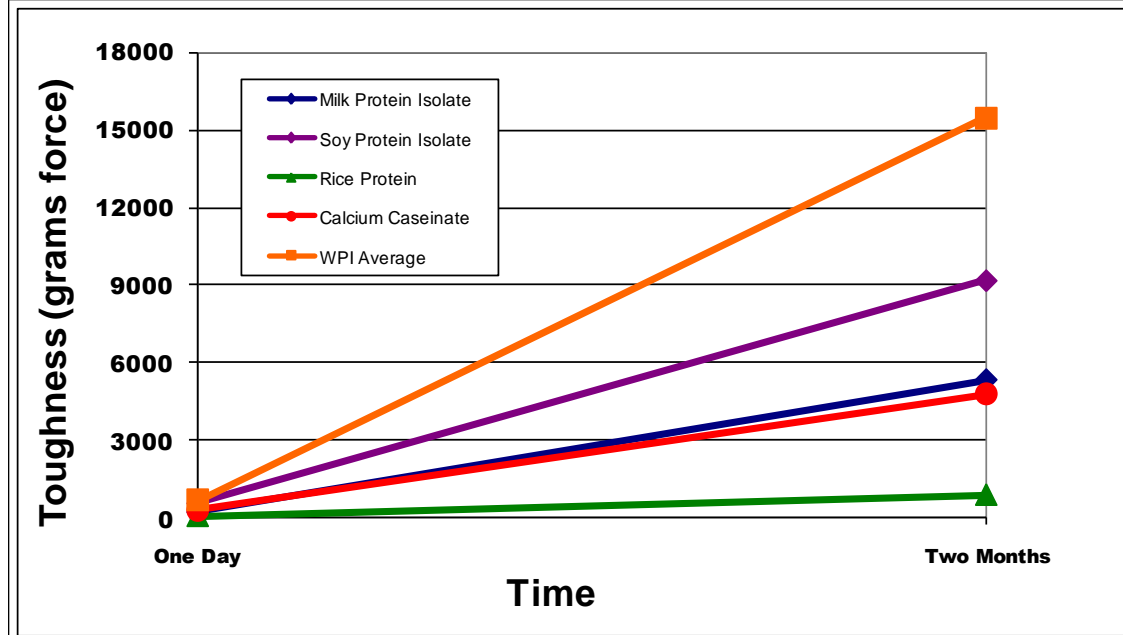
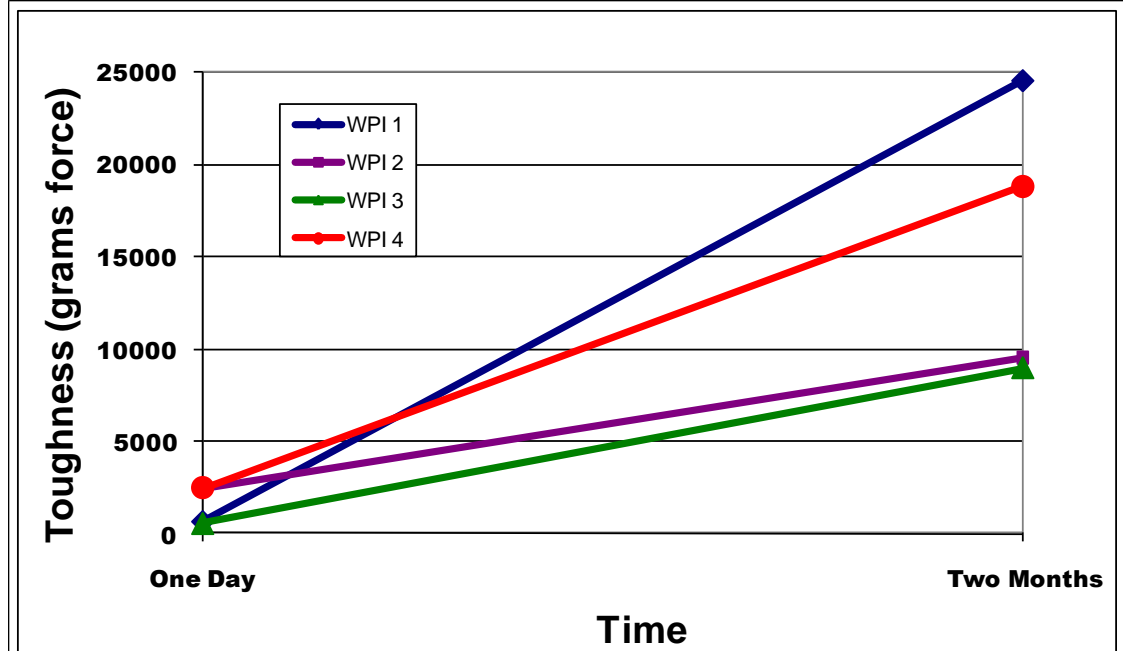
Hydrolyzed Proteins



# Whey Protein Isolate

- **Challenges**

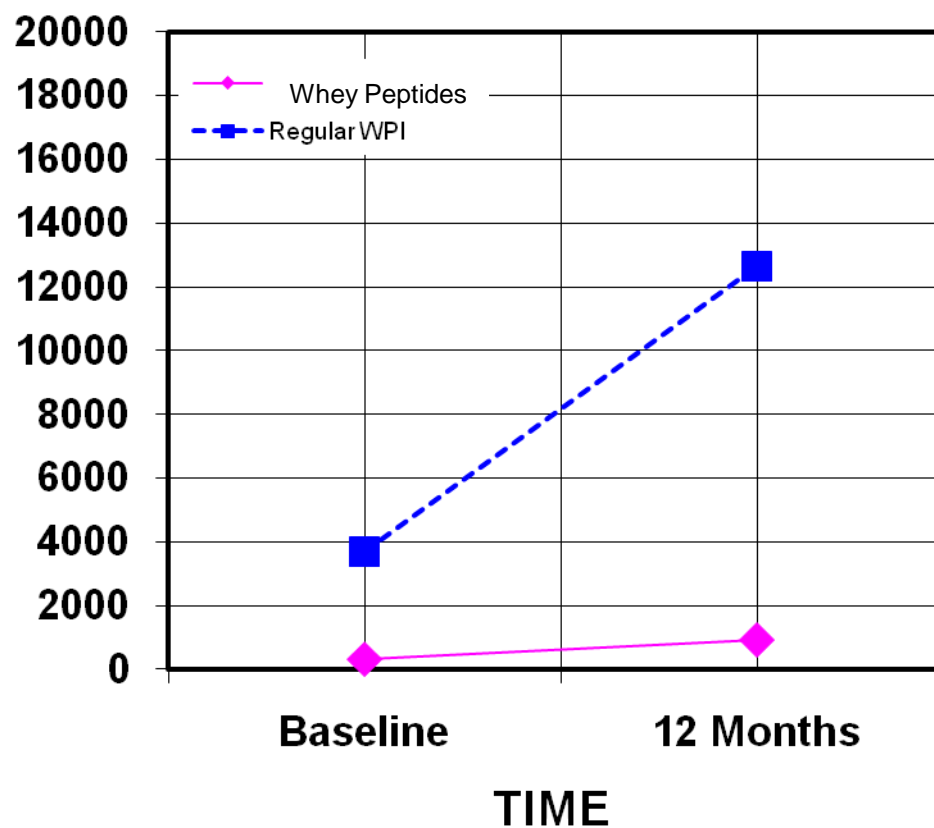
- Hardening over time
- Stickiness
- Longer texture
- Tooth pack



## Influence of whey peptides on Protein Bars



HARDNESS (g force)





# Development of New Whey Ingredients

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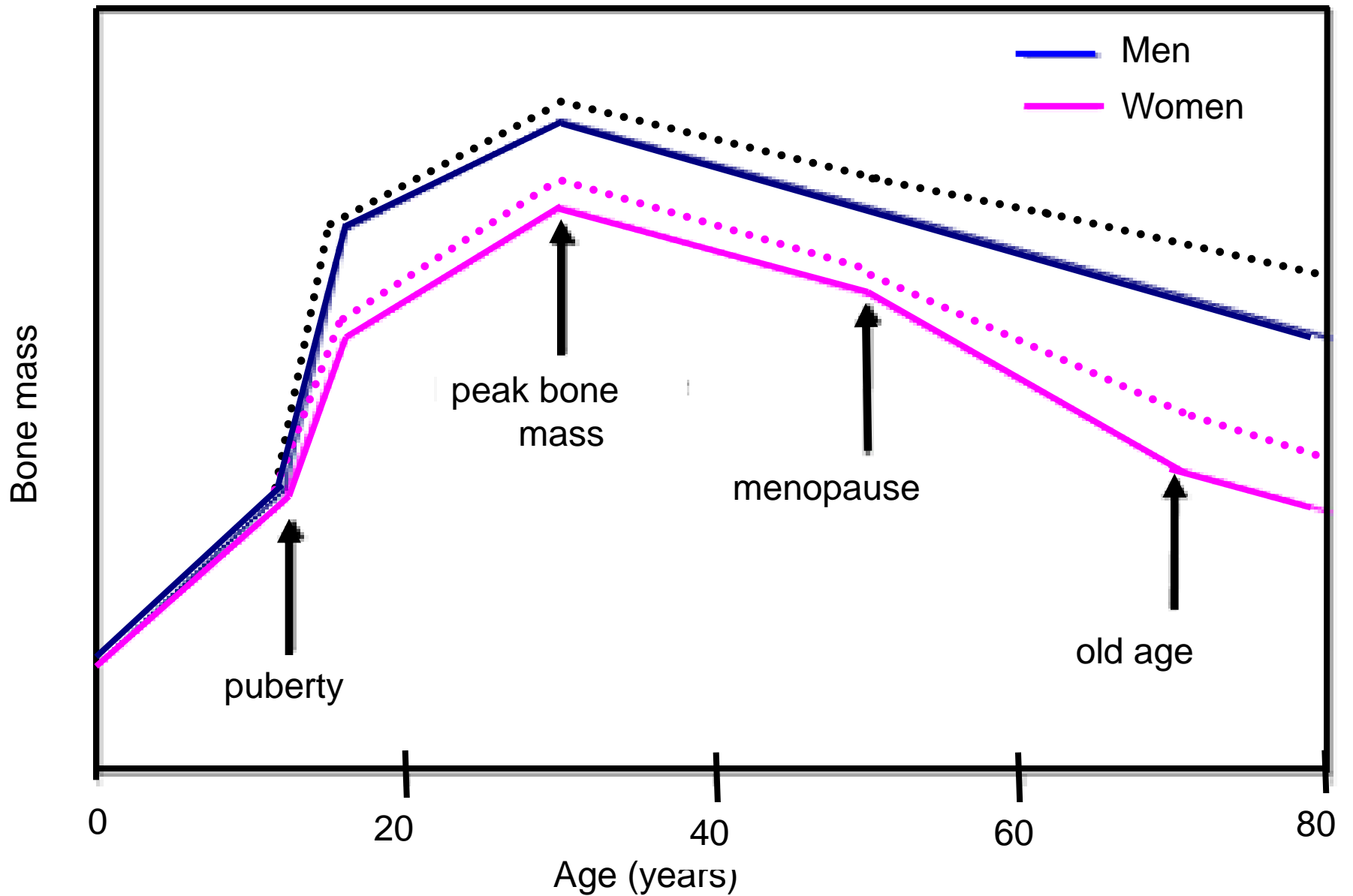
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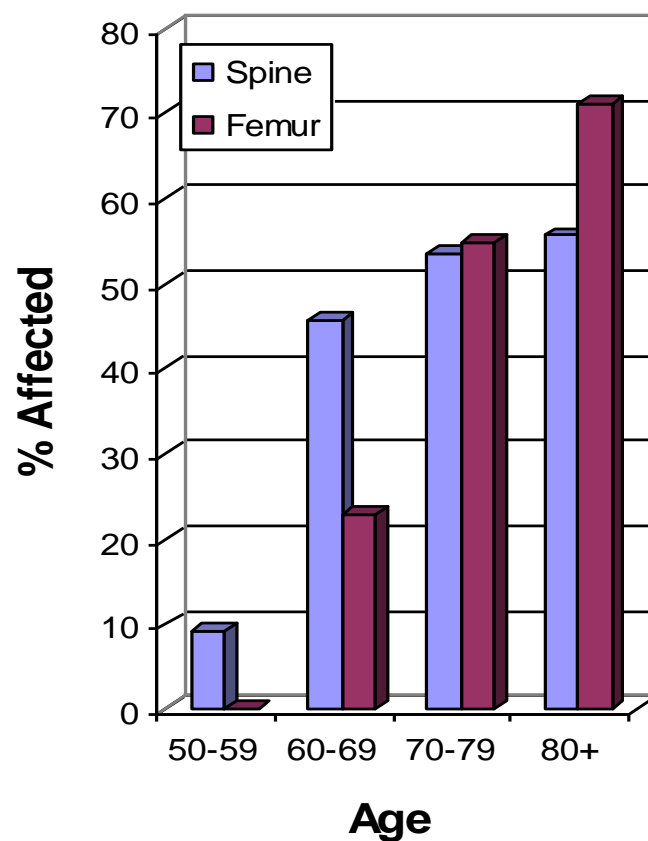
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# Bone Mass During Aging



- In 2005 in the USA, there were predicted over 2 million fractures costing \$17 billion. Men accounted for 29% of fractures and 25% of the total cost burden. By 2025, annual fractures and costs are projected to increase by 50% and \$25 billion, respectively (6).
- Osteoporosis affects an estimated 75 million people in Europe, USA and Japan (1).
- 1 in 3 women over 50 will experience osteoporotic fractures, as will 1 in 5 men (2,3,4).





Drive Consumer Sales With The Real Calcium Symbol On Your Package.



## Balanced Approach

Mineral	Bone	TruCal
Ca	25%	24%
P	12%	12.5%
Mg	0.37%	1.5%
K	0.7%	0.8%
Zn	0.009%	0.008%
Cu	0.0005%	0.0004%

## PERFECT BALANCE.

### Milk Mineral Complex... Calcium and Essential Minerals.

New from Glanbia, TruCal™ Milk Mineral Complex: Designed to optimize bone health through a balanced combination of calcium and essential minerals (P, Mg, zinc, and K).

New clinical studies have shown that mineral balance is critical to developing and maintaining optimum bone density and strength.

Bone is a dynamic system consisting of living cells, embedded in an intercellular matrix of calcium, phosphate, magnesium, citrate and zinc. Supplementation with only pure calcium can

create a mineral imbalance and stimulate leaching of magnesium, potassium and the other minerals from bone, causing hypermineralisation of calcium and bone brittleness.

Harvested from nature through advanced technology, TruCal.™

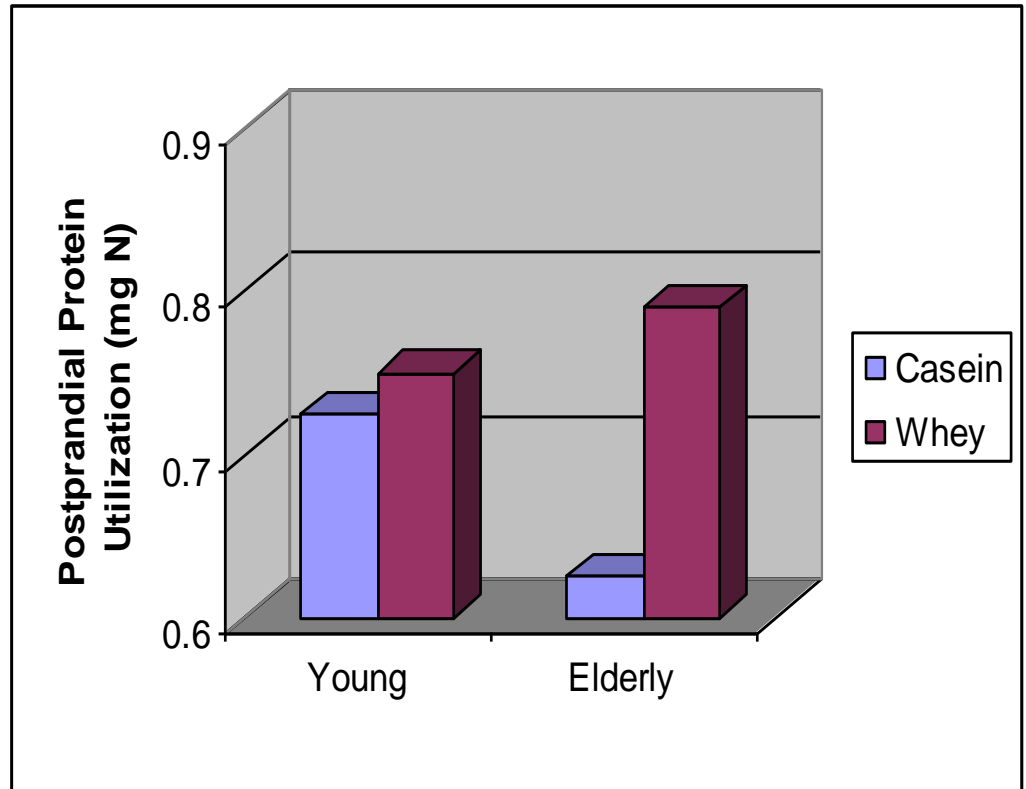
Available in three forms:  
TruCal™ Original Milk Mineral Complex  
TruCal-FX™: Lactose Free  
TruCal-FP™: With Bioactive Peptides



Call 1-800-336-2183 or visit our website [www.glanbiausa.com](http://www.glanbiausa.com)

# Protein Synthesis Gains Whey vs. Casein

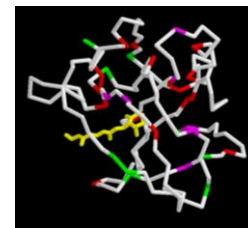
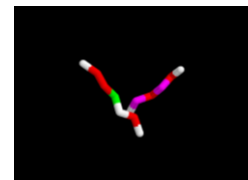
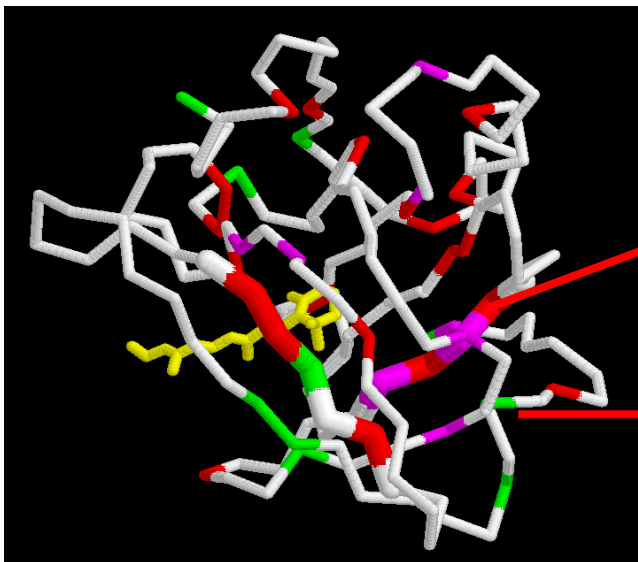
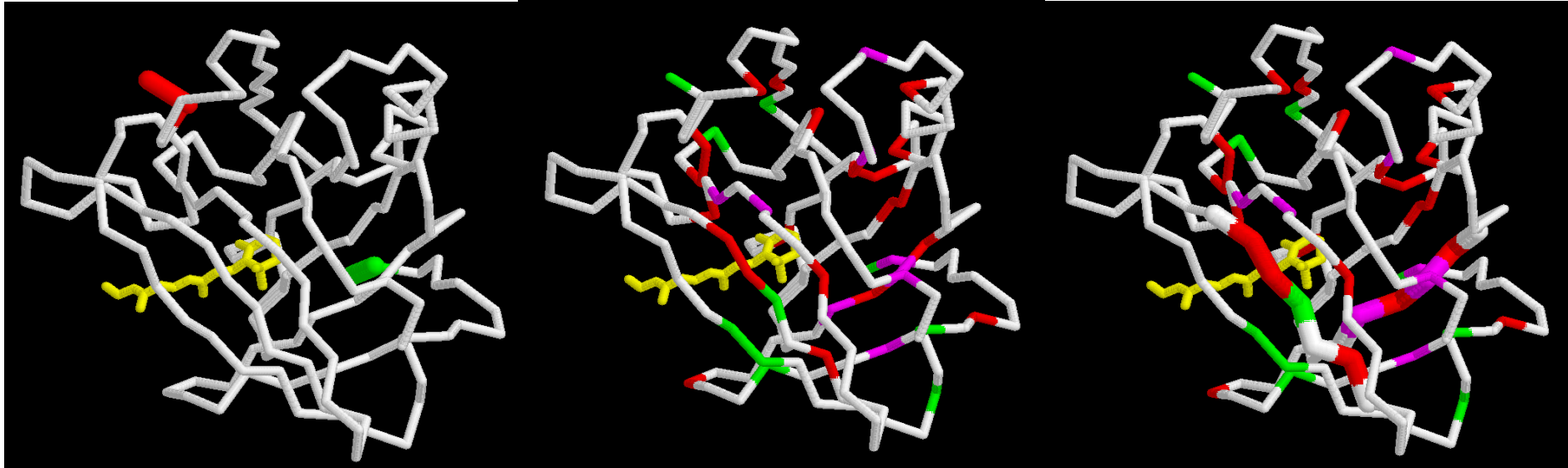
- Young (~24 yrs old) versus elderly (~72 years old) response to approximately 30g of whey protein or casein
- *Greater protein synthesis following whey in the elderly*



# Bioactive Peptides Derived from Whey Proteins

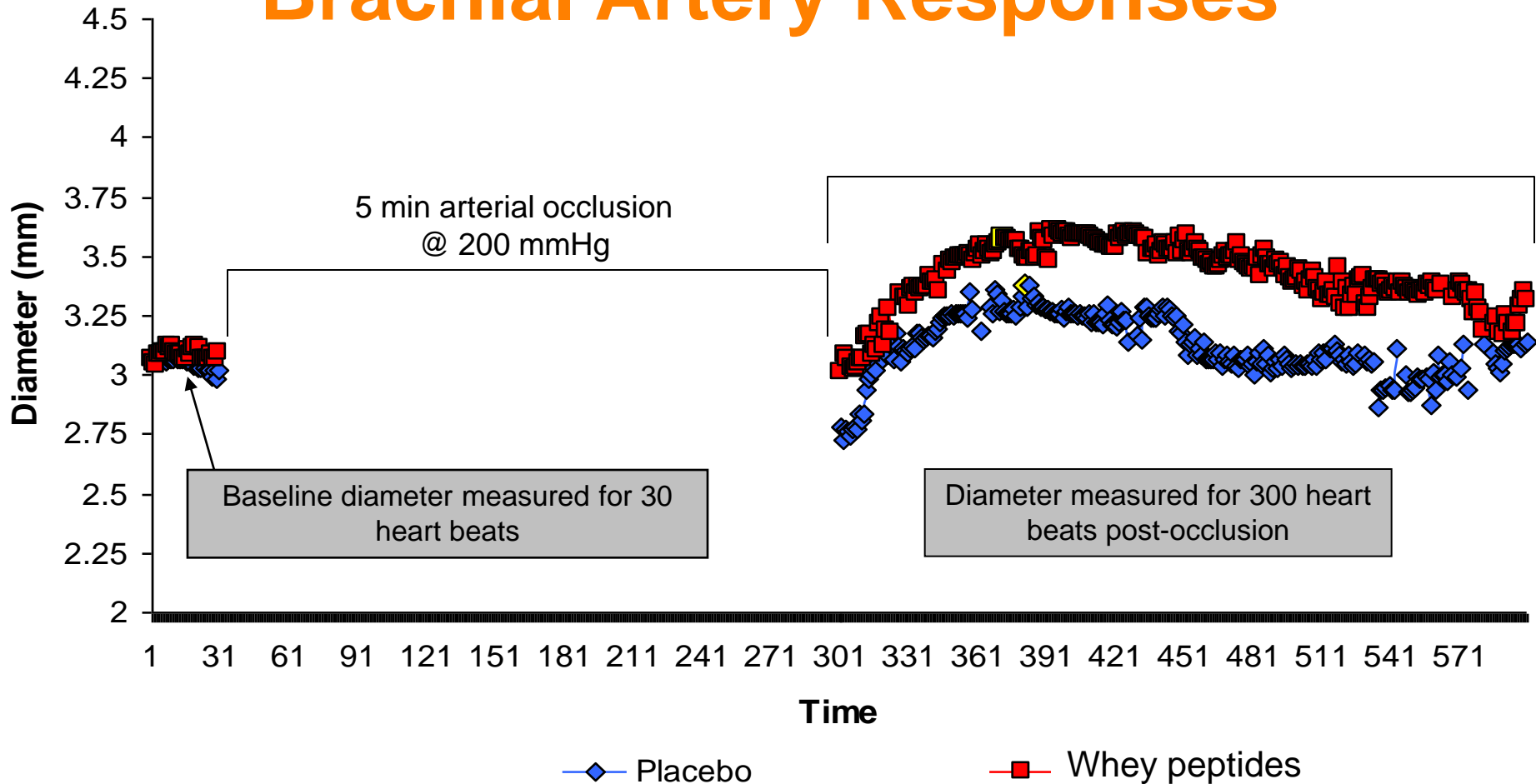


# Novel peptides from whey protein



— Leucine  
— Isoleucine  
— Valine

# Brachial Artery Responses



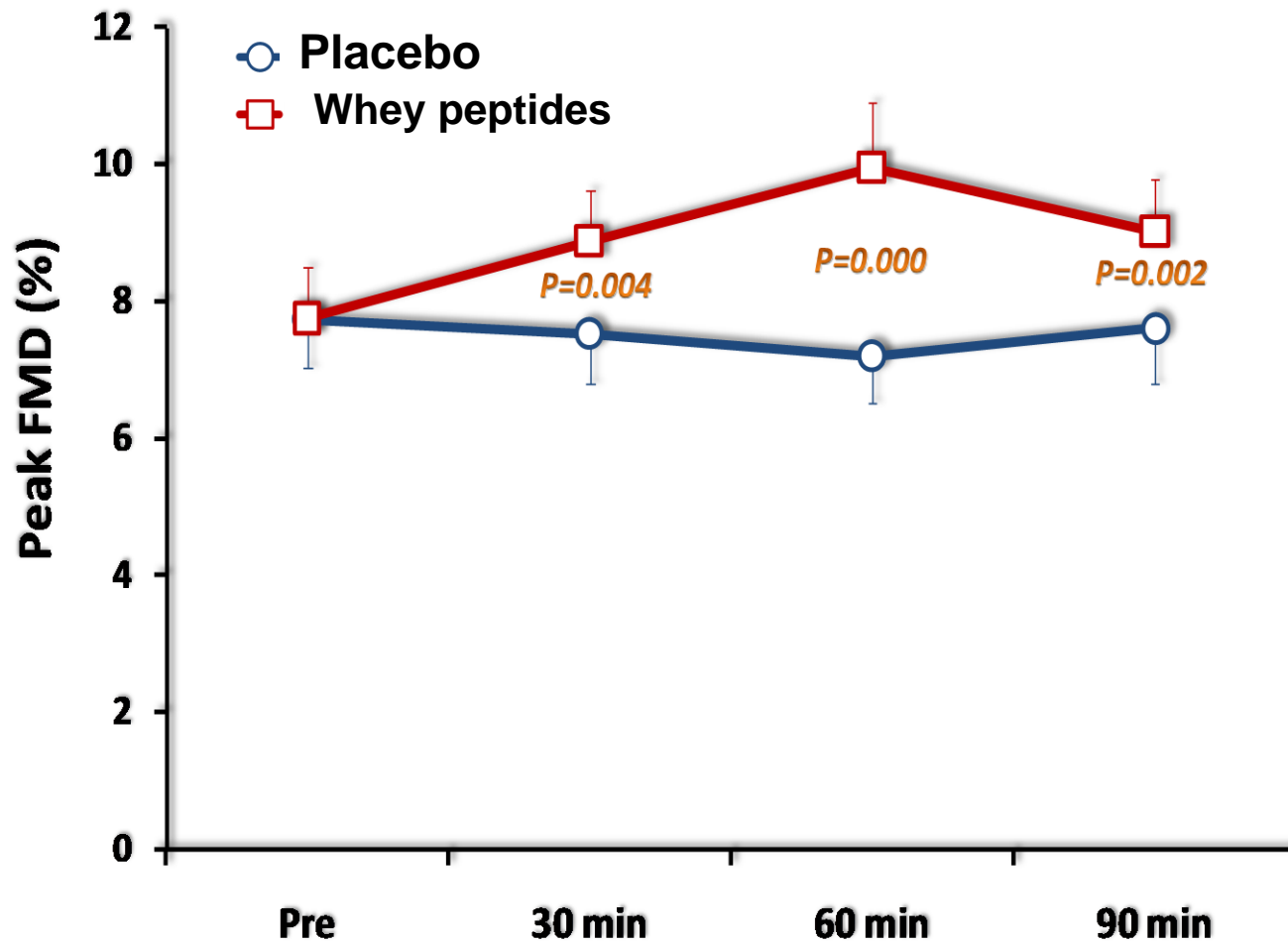
\*Peak diameter determined from average of 5 frames before and 5 frames after visually determined maximal diameter

Flow Mediated Dilation =  $\left[ \frac{(\text{Peak } d - \text{Baseline } d)}{\text{Baseline } d} \right] * 100$

**60 min Placebo FMD =  $\left[ \frac{(3.31 - 3.05)}{3.05} \right] * 100 = 8.5\%$**

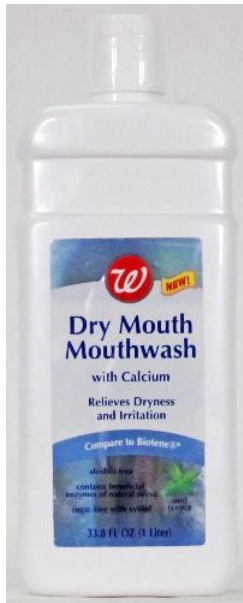
**60 min Whey FMD =  $\left[ \frac{(3.55 - 3.08)}{3.08} \right] * 100 = 15.3\%$**

# Brachial Artery Flow Mediated Dilation



†P<0.000 for time x trial interaction effect

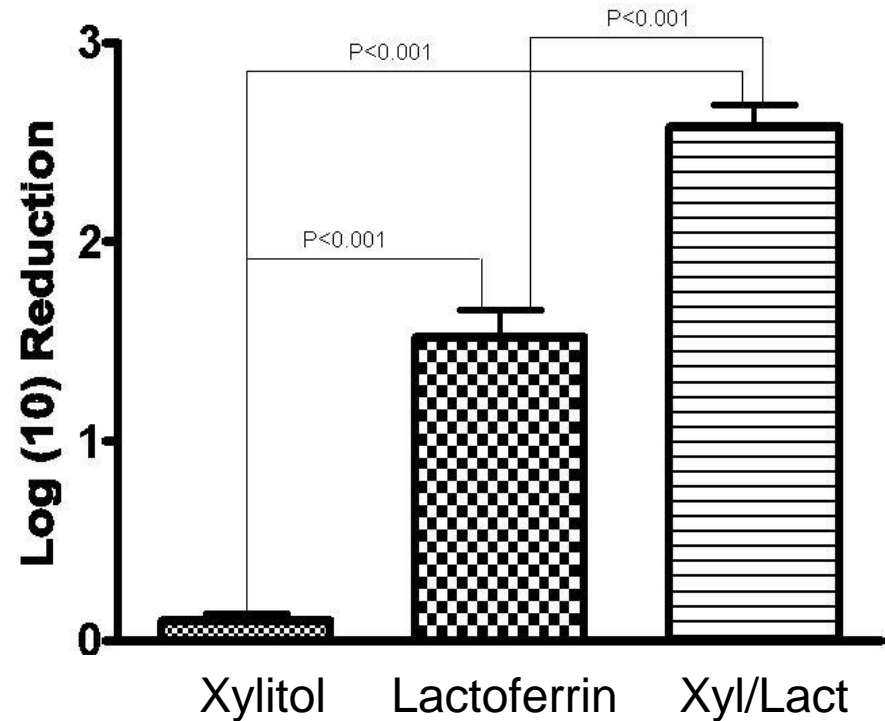
# Lactoferrin



# Lactoferrin as anti-biofilm for wound gels

## Materials and Methods

- CDC reactors (Designation number E 26 4708)
- 24 hour attachment/biofilm development
- 2.7 ml/min flow after 24 hour
- Coupons collected vortex, sonicate, vortex to release bacteria.



# A study of biofilm-based wound management in subjects with critical limb ischaemia

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# Study Overview

- 4500 subjects admitted Aug 2002-Jan 2006.
- 190 subjects included based upon high potential for limb amputation ( $\text{TCpO}_2 < 20$  mmHg) and more than five visits.
- Total Patients 190
  - CLI without diabetes or osteomyelitis (33)
  - CLI and osteomyelitis (101)
  - CLI and diabetes (135)
  - CLI with osteomyelitis and diabetes (79)
- Lactoferrin and xylitol as the primary anti-biofilm agents
  - Ultrasonic debridement/Sharp debridement
  - Opening tunnels and remove undermining
  - Removal of devitalized tissue
  - Debridement of bone  $\rightarrow$  osteomyelitis
  - Complementary agents  $20\text{mg}/\text{cm}^3$  lactoferrin and  $50\text{ mg}/\text{cm}^3$  xylitol

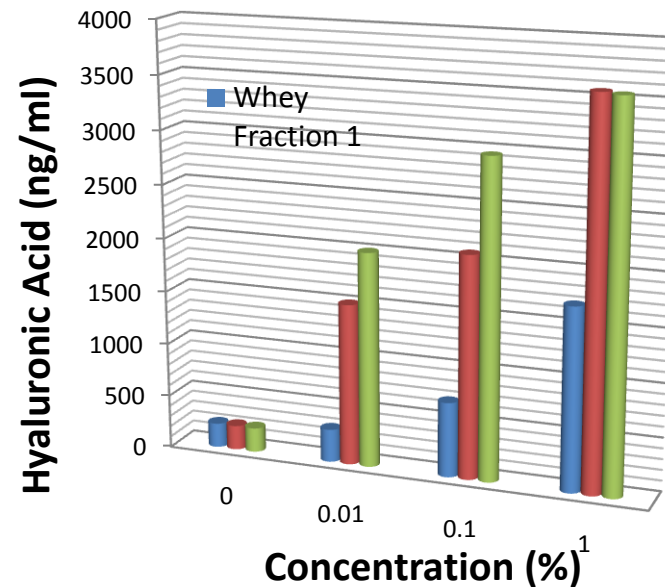
## Results of biofilm-based wound-care strategies in patients with critical ischaemic wounded limbs

	Healed	Not Healed	Total	Percent Healed
All CLI patients	146	44	190	77%
CLI without diabetes or osteomyelitis	30	3	33	91%
CLI and osteomyelitis	68	33	101	67%
CLI and diabetes	101	34	135	75%
CLI with osteomyelitis and diabetes	53	26	79	67%

Under the current standard of care, most of these wounds would have been deemed ‘unhealable” and would have resulted in a major limb amputation

# Cosmetics

- **Hyaluronic acid** naturally occurs in human skin and is capable of holding 1000 times its own weight of water. Although the amount of HA in skin decreases with age, supplementing the body's natural supply can help offset visible signs of aging. HA acts like an "instant facelift," retaining essential moisture in the skin layers to reduces wrinkles caused by dehydration and aging.
- Seeded into 12 well plate with FGM
- Cell viability
- Pro-collagen, elastin, hyaluronic acid
- Hyaluronic acid (anti-skin ageing and "moisture magnet"



# Development of New Whey Ingredients

## Functional Ingredients

- Emulsifiers (0.25-2%), Salad dressings, sauces,
- Texture modifiers (0.25-2%) increase viscosity of sauces, soups etc
- Anti-oxidants (0.3%), decrease lipid oxidation

## Combination of Functional and Nutritional

- Heat stable (Functional) whey protein concentrates and isolates for beverage applications that deliver 5-40 grams of protein (Nutritional).
- Nutritional bar texture (short, chewy, nougat etc) and deliver protein (5-25 grams)
- Protein systems for Greek Yogurts

## Bioactive Ingredients Neutraceutical Ingredients

- Bioactive peptides (vaso-dilation)
- Cosmetic applications
- Specific peptide or protein fractions
- Lactoperoxidase
- Lactoferrin
  - a. Wound Healing
  - b. Iron homeostasis

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