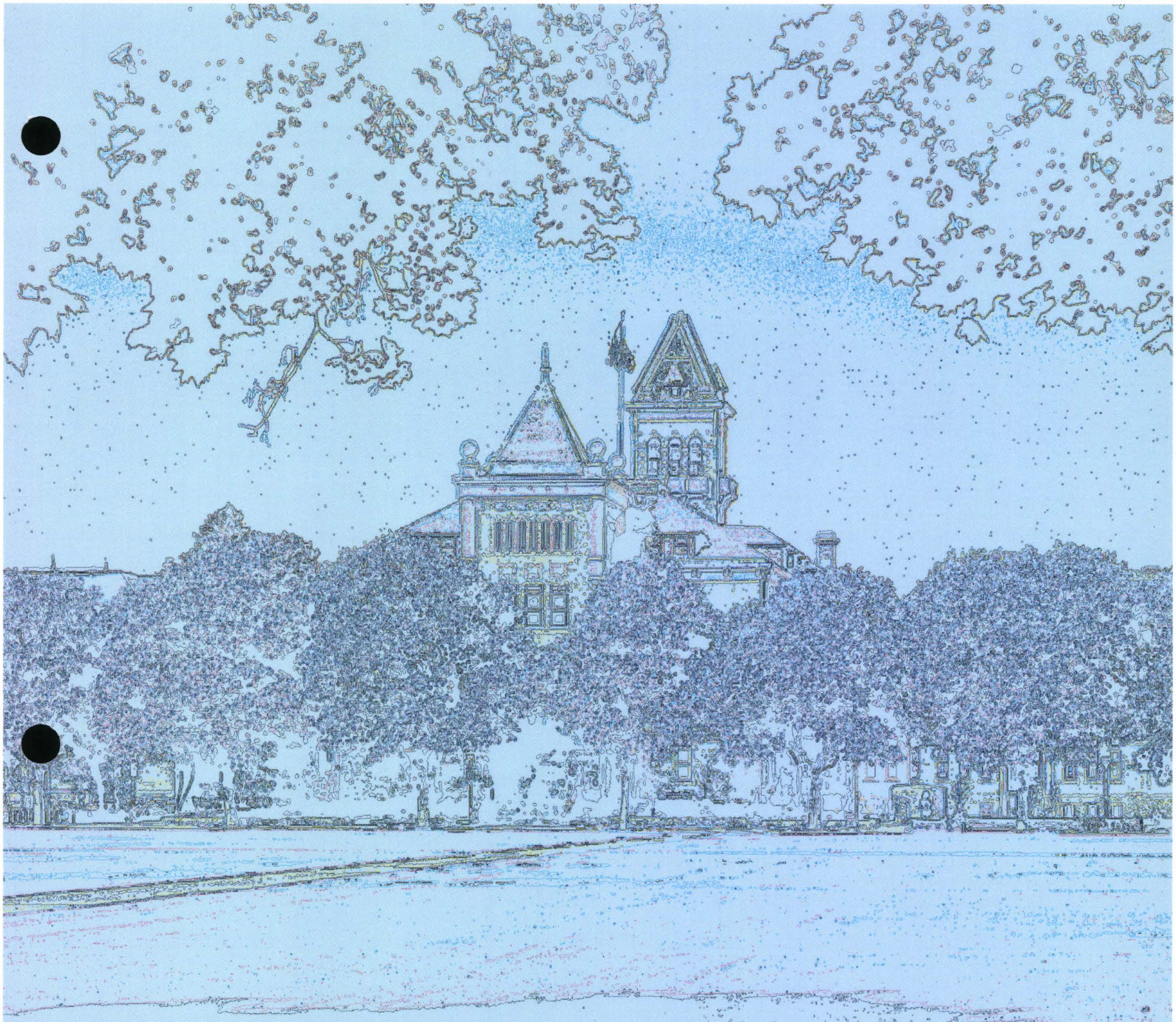
2004

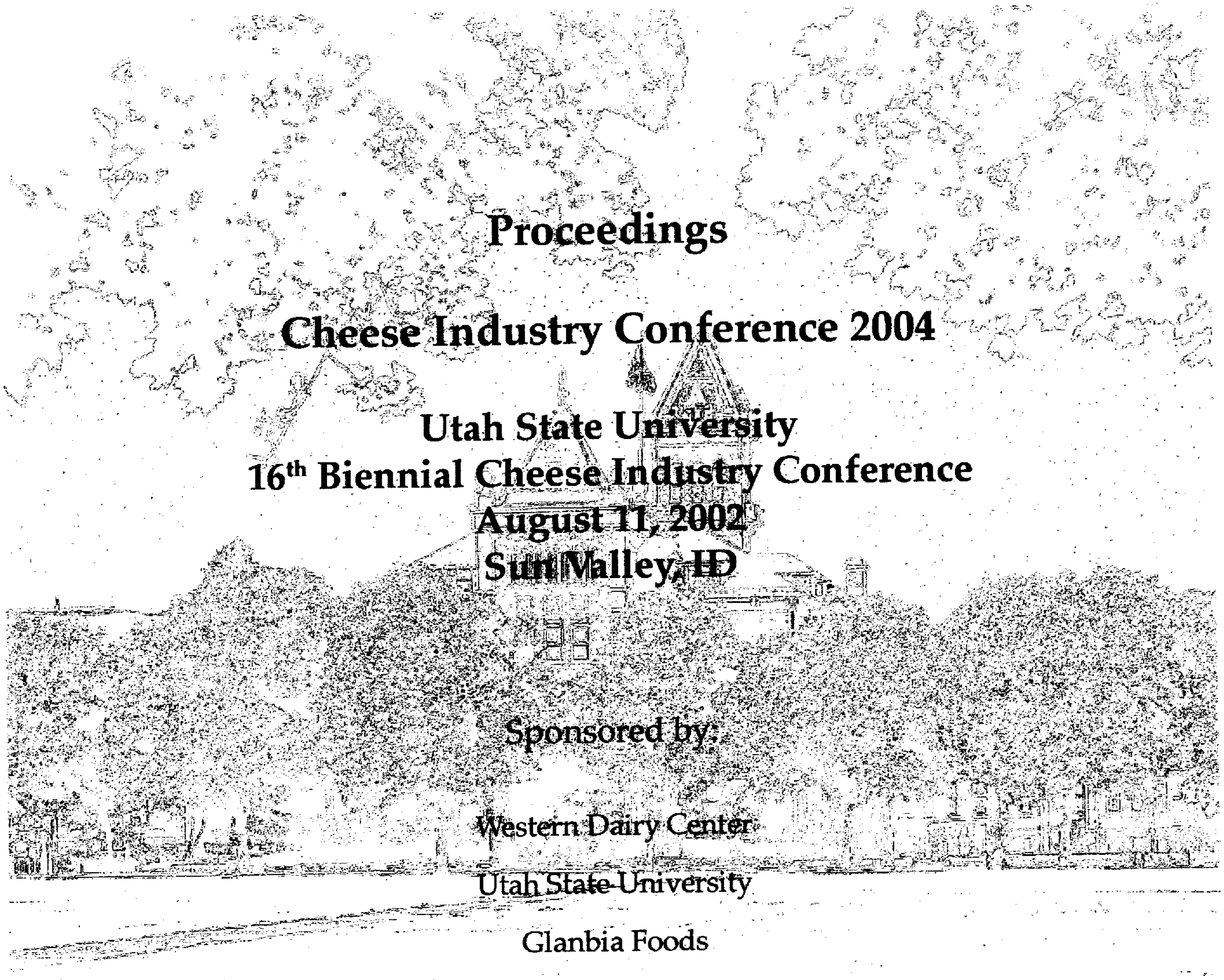
16th Biennial Cheese Industry Conference

Various Authors



Utah State University
16th Biennial
Cheese Industry Conference

August 11, 2004 Sun Valley, ID



Proceedings

Cheese Industry Conference 2004

Utah State University
16th Biennial Cheese Industry Conference
August 11, 2002
Sun Valley, ID

Sponsored by:

Western Dairy Center

Utah State University

Glanbia Foods

Chr. Hansen, Inc.

Scherping Systems

APV

Cheese Reporter

Idaho Milk Processors Association

Utah State University
Logan, Utah 84322-4815

Sixteenth Biennial Cheese Conference - 2004

August 11, 2004
Sun Valley, Idaho

Wednesday, August 11
Salon C, Sun Valley Inn

7:30 a.m. Registration & Continental Breakfast

8:10 a.m. Welcome - Carl Brothersen, Associate Director, Western Dairy Center

Session One, Chair, Jeff Broadbent, Utah State University

8:20 a.m. Changes in the Standard of Identity and the use of milk protein concentrate in dairy products
Bob Fassbender, T.C. Jacoby & Company Inc.

9:10 a.m. Technology for concentrating milk,
Lars Nielsen, APV, Denmark

10:00 a.m. Milk break - sponsored by **Chr. Hansen, Inc.**

10:30 a.m. How protein fortification affects milk coagulation
Don McMahon, Western Dairy Center, Utah State University

11:20 a.m. Comparison of different methods of milk protein fortification on Cheddar cheesemaking efficiency
Tim Guinee, Teagasc Dairy Products Research Centre, Ireland
Sponsored by **Glanbia Foods.**

12:30 p.m. Lunch - sponsored by **Scherping Systems**

Session Two, Chair, Don McMahon, Utah State University

1:30 p.m. Milk pricing in an unregulated environment
Bill Schiek, Economist, Dairy Institute of California

2:20 p.m. Cheese cultures for accelerated ripening of Cheddar cheese
Dave McCoy, Chr. Hansen, Inc.

3:10 p.m. Milk Break - Sponsored by **Chr. Hansen, Inc.**

3:30 p.m. Flavor development in accelerated ripened Cheddar cheese
Carl Brothersen, Western Dairy Center, Utah State University

4:20 p.m. Application of microbial genomics to cheese technology
Jeff Broadbent, Western Dairy Center, Utah State University

5:10 p.m. Adjourn

CONTENTS

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Western Dairy Center

Utah State University

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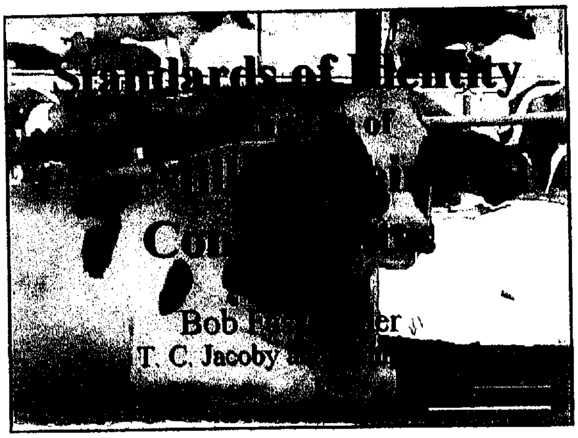
Changes in the Standard of Identity and the Use of Milk Protein Concentrate in Dairy Products

Bob Fassbender
T.C. Jacoby & Company Inc.



- Membrane Primer
- Standards of Identity
- Current Situation

Definitions:

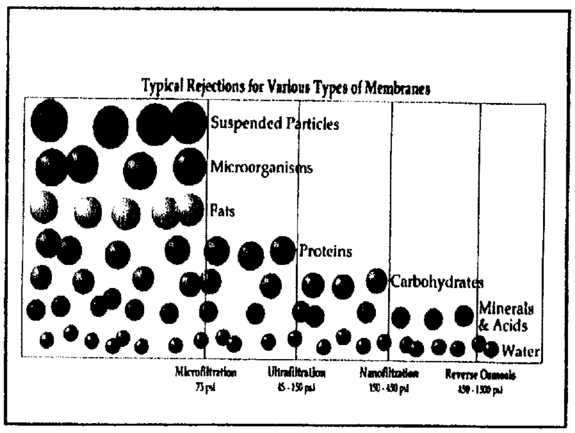
- Concentrated Output of a Membrane System

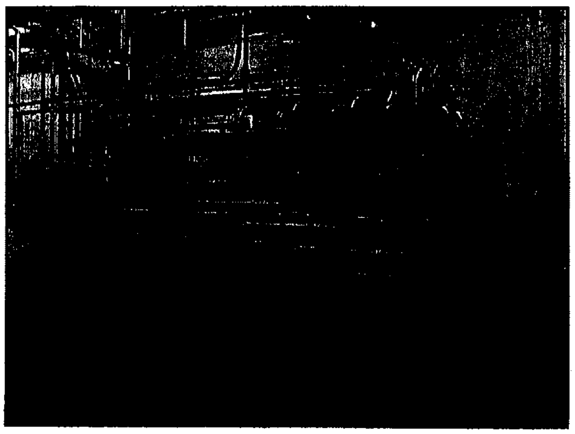
Definitions:

- Dilute Byproduct of a Membrane System
- The Product that Passes Through the Membrane

Definitions:

- Concentration Process
- Water Removal Only





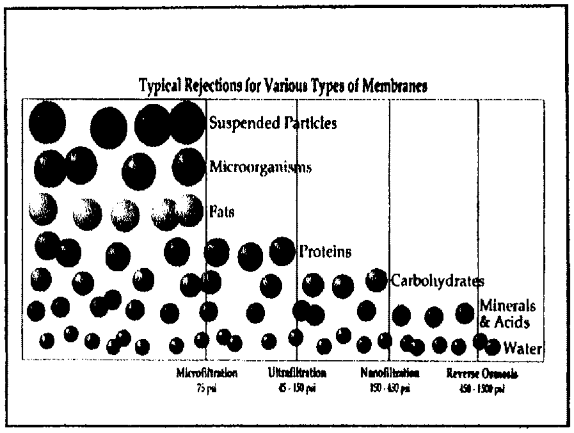
RO COMPOSITION

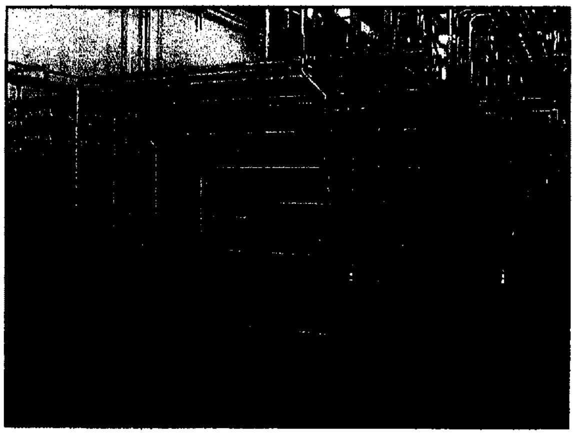
- Concentration	2.5X
- % Feed TS	12.2
- % Production TS	30.5
- % Fat	9.0
- % Protein	7.5
- % Lactose / Ash	14.0

Definitions:

- Fractionation Process

-Water, Lactose and Minerals Removed





UF COMPOSITION

-Concentration	3.5X
- % Feed TS	12.2
- % Production TS	28.0
- % Fat	11.25
- % Protein	10.25
- % Other Solids	6.50

Definitions:

- Dry Form of UF Skim Milk
- Water, Lactose and Minerals Removed

Typical COMPOSITION

-% Feed TS	12.2
- % Production TS	96.0
- % Fat	2.5
- % Protein	59.0
- % Lactose	27.5
-% Ash	7.0

Typical Protein Levels

- 42%
- 56 %
- 70 %

Definitions:

-Additional Lactose Removal by the Introduction of Water into the Retentate and Refiltering

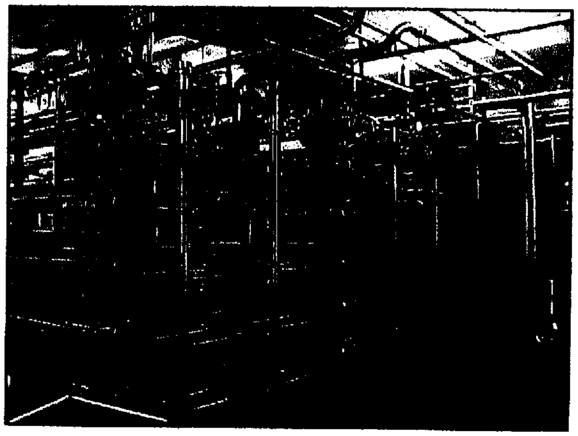
Definitions:

- Legal definition of various foods
-Found in Code of Federal Regulations (CFR)

- Details manufacturing parameters & composition standards, including ingredients and additives

- Established to "Promote honesty and fair dealing in the interest of consumers"

- About 250 Different Standards
- 97 Standards Pertain to Dairy
- 72 % of the Dairy Standards Relate to Cheese & Cheese Products
- Found in CFR Title 21, Part 133



CONCERNS:

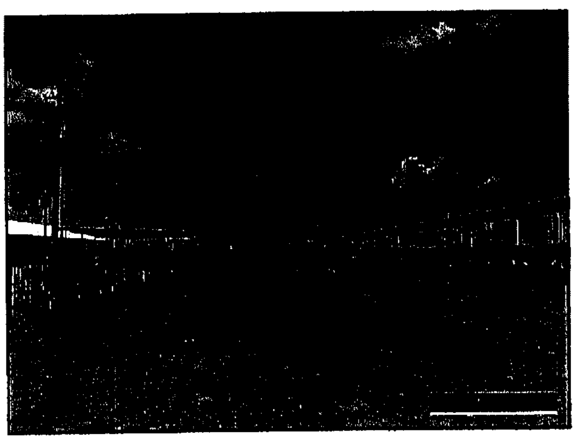
- IMPORTED Product
- May be Blends of Whey and Casein

Permitted Uses of MPC or UF Milk

- Non-Standardized Products
- Yogurt
- Cottage Cheese Dressing
- Low Fat Sour Cream Varieties
- In Plant Applications

Non-Permitted Uses of MPC or UF Milk

- Standardized Dairy Products
- Cheese
- Cottage Cheese Curd
- Fluid Milk Products



APPLICATIONS

- Alternate Make Provision

"by any other procedure which produces a finished cheese having the same physical and chemical properties"

APPLICATIONS

-"Regulatory Discretion"

"Until an Enforcement Strategy can be developed, or the Standards of Identity are amended, FDA is NOT taking any enforcement action."

APPLICATIONS

- NCI Citizen Petition - 2000

- "...FDA intends to publish a proposed rule this fiscal year to amend section 133.3 to provide for the use of fluid UF milk in standardized cheese ..."

APPLICATIONS

- Temporary Marketing Permit
- FDA has authority under Section 130.17 of the CFR to allow "investigations of potential advances in food technology..."

APPLICATIONS

- TMP to be issued in 2004 for Cottage Cheese

APPLICATIONS

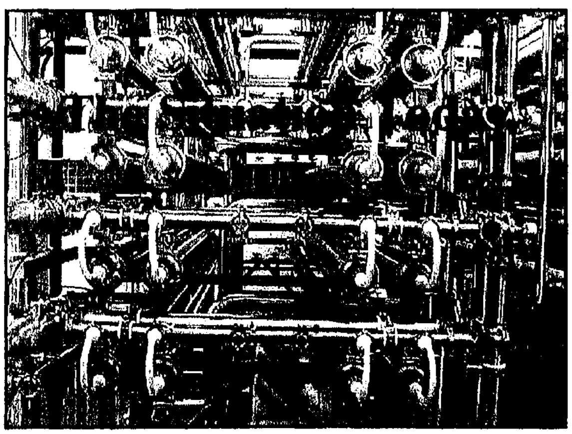
- Non-Standard Products, Must be "Labeled"

The Situation Today

- 2003 IMS Conference sets minimum membrane processing parameters for UF Systems
- Effective 2004 -2005

The Situation Today

- Proposal 169 Study Committee to Evaluate Membrane Filtration and Develop Uniform Guidance Principles for FDA



- At Least 9 Commercial Operations Producing UF Milk

- At Least 1 Commercial "Domestic" MPC Facility

2

Western Dairy Center

Utah State UNIVERSITY

Utah State University
16th Biennial
Cheese Industry Conference

Technology for Concentrating Milk

Lars Nielsen
APV
Denmark

16th Biennial
Cheese Industry
Conference

APV

Technology for concentrating milk

-Membrane Filtration

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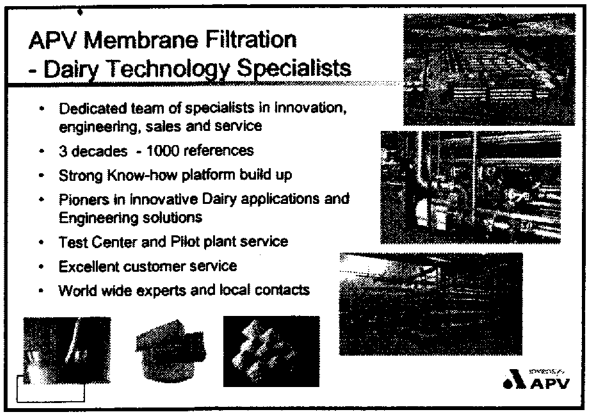
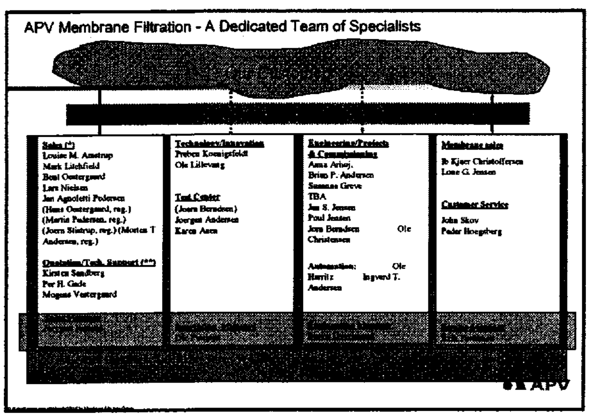
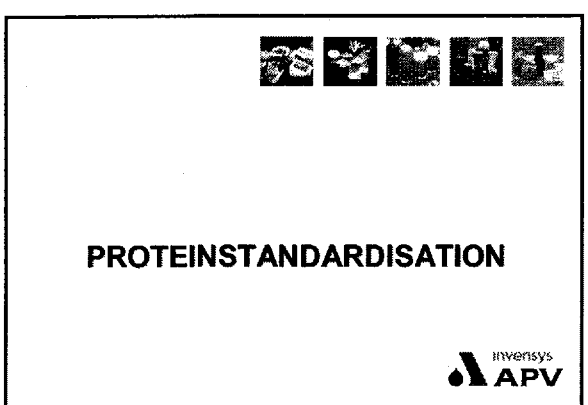
AGENDA

- Short presentation of APV Membrane Group
- Protein Standardisation by UF
  - Batch Proces
  - In Line Proces
  - Controlling Proces
  - Examples
- Protein Standardisation by "MF" Ceramic Membranes
  - In Line Proces
  - Comparison to UF

APV


APV Membrane Filtration
- Dairy Technology Specialists
• Dedicated team of specialists in innovation, engineering, sales and service
• 3 decades - 1000 references
• Strong Know-how platform build up
• Pioners in innovative Dairy applications and Engineering solutions
• Test Center and Pilot plant service
• Excellent customer service
• World wide experts and local contacts
APV


APV Membrane Filtration - A Dedicated Team of Specialists
Sales (*)
Louise M. Amstrup
Mark Litchfield
Bent Oestergaard
Lars Nielsen
Jan Agnoletti Pedersen
(Hans Oestergaard, reg.)
(Martin Pedersen, reg.)
(Joern Stistrup, reg.) (Morten T Andersen, reg.)
Quotation/Tech. Support (**)
Kirsten Sandberg
Per H. Gade
Mogens Vestergaard
Technology/Innovation
Preben Koenigsfeldt
Ole Lillevang
Test Center
(Joern Berndsen)
Joergen Andersen
Karen Aaen
Engineering/Projects & Commissioning
Anna Arhoj.
Brian P. Andersen
Susanne Greve
TBA
Jan S. Jensen
Poul Jensen
Jens Berndsen Ole Christensen
Automation: Ole Harritz Ingvard T. Andersen
Membrane sales
Ib Kjaer Christoffersen
Lone G. Jensen
Customer Service
John Skov
Peder Hoegsberg
APV


PROTEINSTANDARDISATION
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Protein Standardisation

- 2 Methods:

- Protein Standardisation by UF -
- Protein Fractionation- Pro-Frac™ /Standardisation by MF-

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PROTEIN STANDARDISATION

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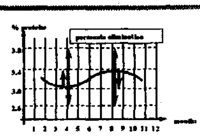
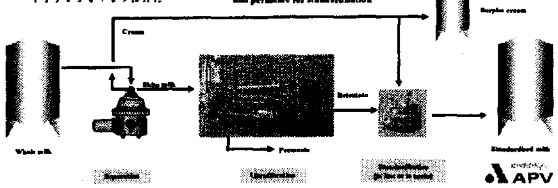
Milk Protein Standardisation by UF



- Batch standardisation in milk silo
  - raw milk at < 10° C
- In line standardisation
  - up stream at 5 - 10°C or after separation at 50° C
- Concentration of pasteurised milk to...
  - produce retentate (e.g. 6% TS)
    and permeate for standardisation



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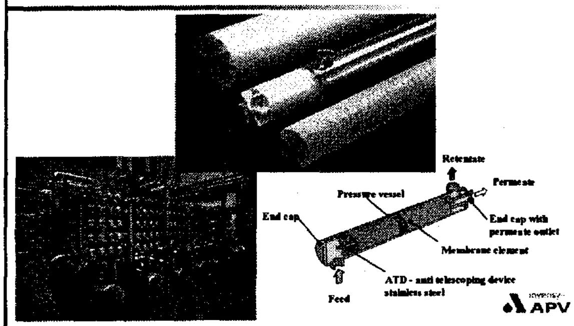
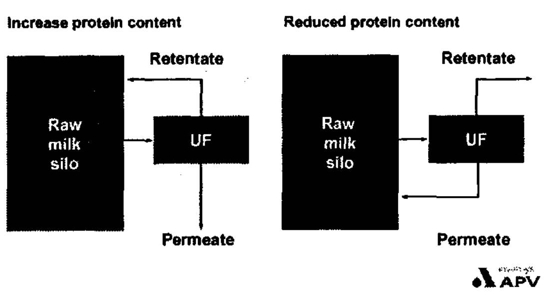
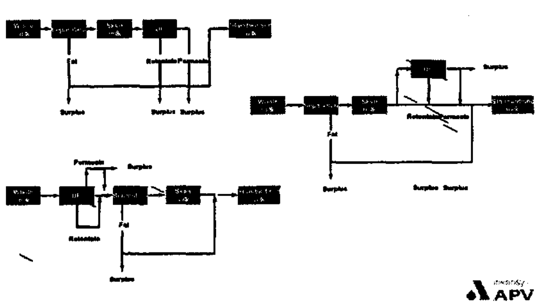
UF Spiral Wound System



Protein standardisation - Batch operation



Protein and fat standardisation continuous operation



Mass Balance - Example

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BOV 40.000 l/h

Customer	BOV Meyer[illegible]
Quotation no.	
Feed type	
Reference	[illegible]

Appendix II

UF Mass Balance

Feed Temperature	[illegible]	
Feed Rate	52000 kg/h	Total Feed/day [illegible] kg
Production Time	[illegible]	

Composition & Quantities of Products

Component		Feed	Retentate	Avg.Perm.	Powder
1) True protein	[%]	3.14	4.0[illegible]	[illegible]	[illegible]
1) NPN	[%]	[illegible]	[illegible]	[illegible]	[illegible]
Lactose	[%]	4.60	4.60	[illegible]	28.[illegible]
Acid	[%]	[illegible]	[illegible]	[illegible]	[illegible]
Total Ash	[%]	0.75	0.84	[illegible]	[illegible]
Fat	[%]	[illegible]	[illegible]	[illegible]	[illegible]
TS, sum	[%]	13.05	15.26	[illegible]	[illegible]
Cap. Avg.	kg/h	52000	40342	11658	[illegible]

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Controlling Proces

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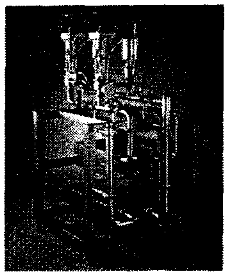
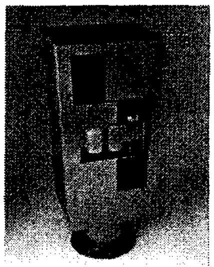
CompoMaster with ProcesScan FT





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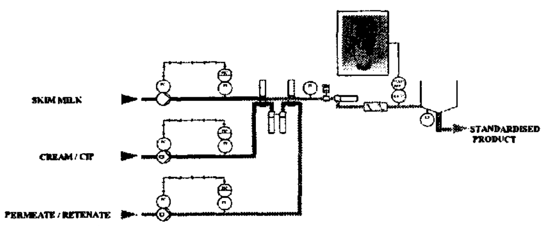
CompoMaster, Type KCC, In-Line System



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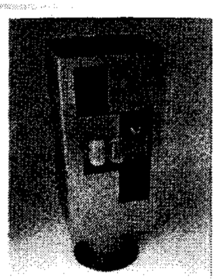
To be applied on Milk and Cream for determination of standard components:

- **Fat**
- **Protein**
- **Lactose**
- **SNF**
- **Total solids**



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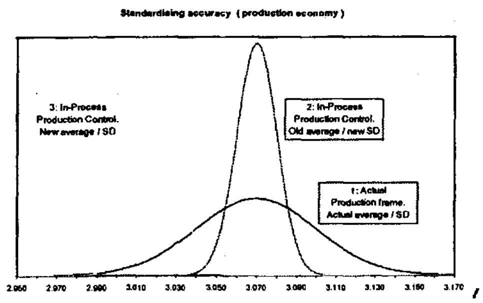
CompoMaster, Type KCC



Fully automatic standardising system

References

APV Unit Systems - Membrane Filtration

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PROTEINSTANDARDISATION - SOPROLE, CHILE
- UF plant, CompoMaster and ProcesScan

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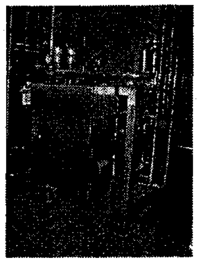
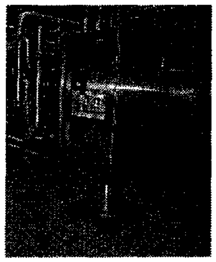
PROTEINSTANDARDISATION - SOPROLE, CHILE

CompoMaster for automatic
Fat and Protein Standardisation



ProcessScan for automatic
inline protein analysing



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CONTROL SYSTEM

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CONTROL SYSTEMS OVERVIEW
FOR APV MEMBRANE PLANTS

TYPE 0: Hand operated pilot plant (fully manual)
TYPE 1: Standard remote controlled plant
Type 2: Standard semiautomatic plant
Type 3: Customer specified fully automatic plant

LOW LEVEL — HIGH LEVEL

AUTOMATION LEVEL

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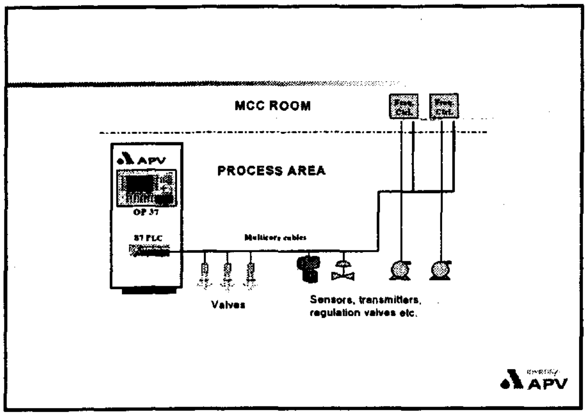
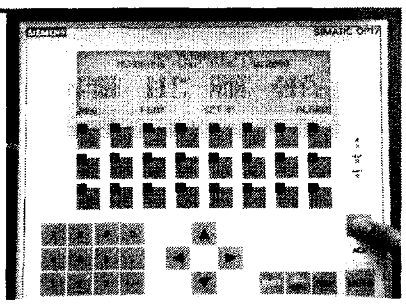
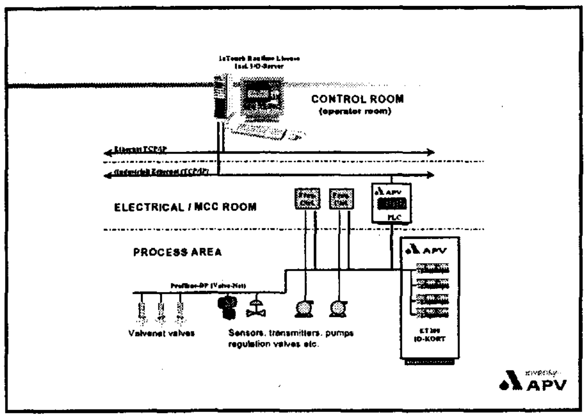
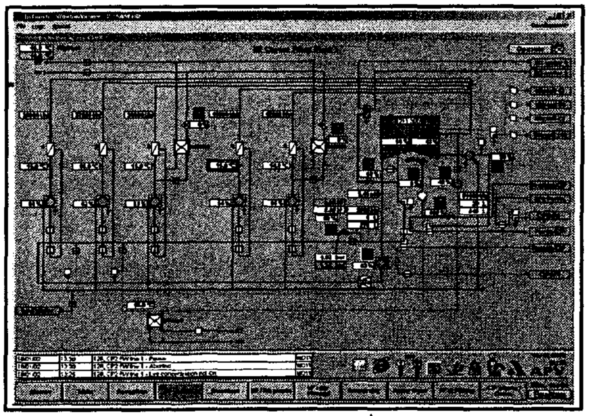


OP17 type operator screen







Economy

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Protein standardisation - Gouda/Edam Cheese - 1 mio kg. of cheese milk/day

- Protein % in milk: Min. 3.25 - Max. 3.55 - average 3.40 ⇨ stand. to 3.7% (approx. 8.5%)
- UF plant: 50 t/h x20 h, 6° C raw milk silo

– Investment (delivered/installed)	kEUR	260
– Operational cost/year	kEUR	90
– Capital cost/year	kEUR 1)	57
– Total costs/year	kEUR	147
Gains		
– Saving of rennet/year - 8.5%	kEUR 2)	140
– Increased yield/year - 0.25% (conservative)	kEUR 3)	198
– Total gains/year	kEUR	338

- Return of investment - (result/year) kEUR 191 = ~ 16 months
- Additional advantage, not capitalised
  - 8.5% higher cheese vat capacity
  - 85 t/day of high quality milk permeate for powder milk stand., or milk drinks and other products
  - And several other advantages...

1) Depreciating 10 years, Interest 6% p.a.

2) 20 ml. Rennet/100 kg milk - saving 8.5% x 23 EUR/kg

3) Cheese price 3.5 EUR/kg - 1.3 EUR/kg fines

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Advantages

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UF Protein Standardisation and Concentration..

- In cheese making:
  - Higher protein - less rennet, more cheese
  - Constant protein - better control of process and constant quantity
  - Constant quality and improved economy
- In market/fresh milk products:
  - Higher protein - calcium enriched milk and protein boosted milk drinks with flavour - New innovative milk drinks
  - Lower protein - improved economy in milk production
  - Yoghurt and dessert - control of consistency and quality
- In milk powder products:
  - Constant protein content - constant quality
  - Lower protein content (34% SNF acc. to Codex Alimentarius standard (Codex Stan 207-1999) - improved economy
  - Higher protein - MPC 50/80 or tailored milk protein ingredients

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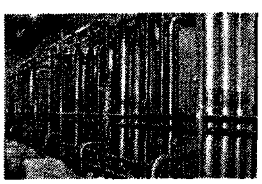
Fractionation of Milk Proteins

- The APV Pro-Frac™ Concept
- Possibilities and background



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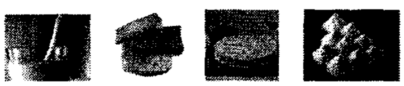
Protein Fractionation of Milk - What Is It?

- Skim milk is filtered by microfiltration over a membrane that allows passage of whey proteins, but not casein micelles. To achieve:
  - Casein enriched milk (MF retentate) and
  - "Ideal whey" (MF permeate)
- The fractionation effect (permeability of whey proteins) is the decisive parameter and is determined by for instance pre-treatment, membrane type, diafiltration as well as optimal flow and pressure conditions.

APV

New Possibilities with Pro-Frac™

- Pro-Frac™ opens up for innovative dairy products:
  - Pre-concentration and standardisation of casein in cheese milk
  - New Cheese types based on full concentration
  - Special milk drinks/fresh products
  - Native casein micelles as milk ingredient in food products and Nutraceuticals
  - High value MWPI (Milk Whey Protein Isolate) for food products and Nutraceuticals



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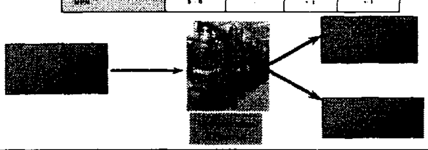
Fractionation of Milk Proteins
- Molecular Separation of Casein and Whey Proteins

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	76 - 78	4.6	1,000 - 100,000	120 (50 - 600)
[illegible]	21 – 22	-	-	-
[illegible]	3 - 4	4.2 - 4.5	14	3 - 6
[illegible]	10 - 12	5.2 - 5.4	36	3 - 5
[illegible]	2 - 3	5.5 - 8.3	IgG 150 IgA 385	6 - 15
[illegible]	0.3 - 1	4.7 - 5.1	66	3 - 5
[illegible]	5 - 6	-	< 1	< 1



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The Pro-Frac™ Concept and Membrane Systems

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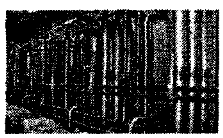
The APV Pro-Frac™ Concept

- Combines APV membrane systems and technology...
  - Microfiltration/Fractionation (MFF)
  - Ultrafiltration/Concentration (UF)
  - Diafiltration/Refinement (DF)

  ...for optimal processing and yield
- Five well proven references
- Customised design to reflect:
  - Desired ratio of casein/total protein and TS in retentate
  - Optimal integration with existing milk treatment system
  - MF bacteria and spore removal prior to protein fractionation



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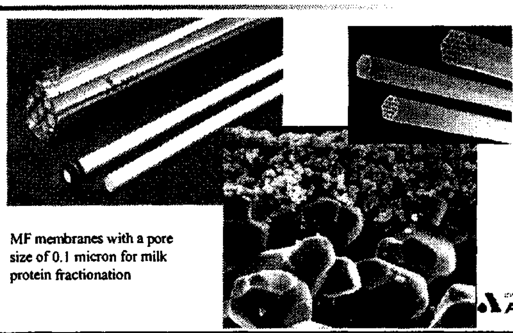
Ceramic Membranes



MF membranes with a pore size of 0.1 micron for milk protein fractionation

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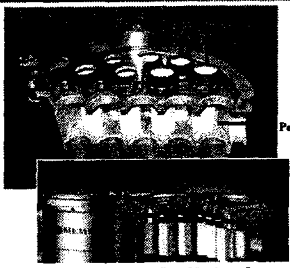
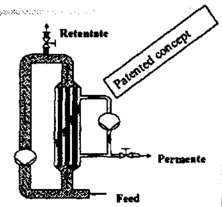
Microfiltration Module with UTP System

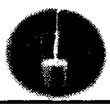




- Perfect regulation of the TransMembranePressure
- Stainless steel concept, 3A and FDA approved, easy to clean
- GP membranes is the future solution, eliminating permeate recirc.

Invensys APV

MF Ceramic Membrane System

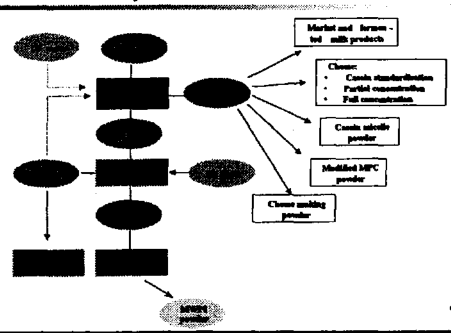


Invensys APV

The Pro-Frac™ Process and Dairy Products

Invensys APV

The Pro-Frac™ and MWPI Process
- for Innovative Dairy Products



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Pro-Frac™ in Cheese Making

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Pro-Frac™ - Innovation in Cheese Production

- Casein standardisation: MF-VCF 1.3 - 1.8
  - Use of traditional cheese equipment
  - All cheese types
- Partial concentration: MF - VCF 1.5 - 3.5
  - Partly replacement of the whey drainage through pre-concentration prior to the cheese making process
  - Requires cheese equipment that can handle heavy curd
  - Soft, semi-hard and hard cheeses
- Full concentration: MF + DF + UF - VCF 6 - 8
  - Requires special cheese equipment
  - New types of cheese
  - Yellow cast cheese, cheese base and pizza cheese

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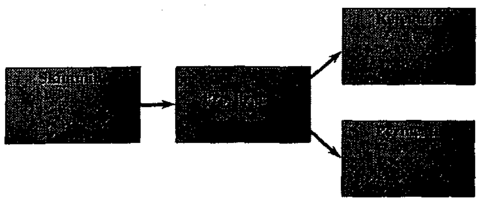
MF-Protein Fractionation
- for Casein Standardisation

Example of a Mass Balance VCF 3.3:



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Mass Balance Protein Fractionation
- VCF 3.2

Milk components		Skimmilk	Retentate	Permeate
Total protein	%	3.60	10.00	0.62
NPN	%	0.19	0.18	0.19
Casein	%	2.84	8.80	0.02
Whey protein	%	0.57	0.82	0.41
Fat	%	0.07	0.22	0.00
Lactose	%	4.64	4.55	4.77
Total ash	%	0.76	1.35	0.48
Acid	%	0.20	0.19	0.21
Total solids	%	9.27	16.31	6.09
Casein/total protein	%	78.9	88.0	
Volume	l/h	10,000	3,100	6,900

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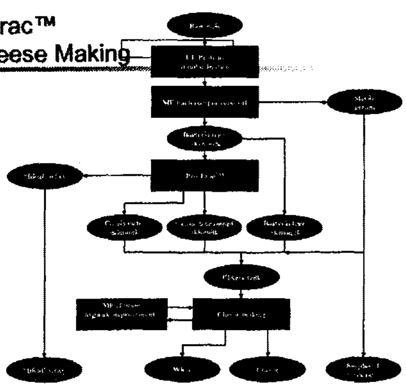
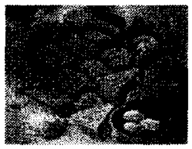
Pro-Frac™
in Cheese Making



APV

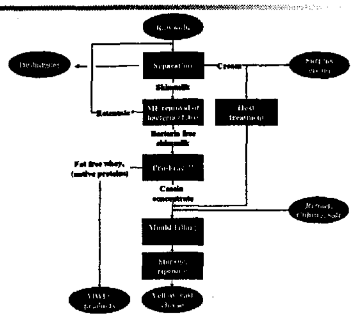
Casein Milk for Cheese Production

- Casein standardisation and concentration provides possibility for new cheese sorts and new MWPI products
  e.g. Mozzarella produced from milk with up to 5% casein
- Great possibility of avoiding the problems that may arise with curdling of high concentrated UF milk where whey proteins may result in:
  - Softer texture
  - More greyish colour
  - Slower maturation
  - Reduced melting qualities
- Pro-Frac™ for high quality



APV

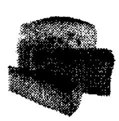
Pro-Frac™ Cheese by Full Concentration



APV

Advantage of Protein Fractionation in Cheese Making

- The advantages of protein fractionation in cheese making are:
  - lower cheese milk volume to handle
  - lower volume of classical cheese whey (from the cheese process)
  - reduced coagulation time
  - reduced amount of rennet
  - better firmness of the curd
  - increased trapping of casein fines and fat
  - slightly higher yield
  - innovative processes and cheese types
  - incorporation of microparticulated MWPI to achieve higher yield and low fat cheese with excellent taste



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Casein and MWPI Powder

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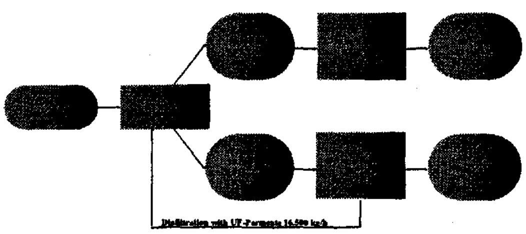
MF Fractionation and UF Concentration
- for Native Casein Micelle Powder and MWPI Powder

Example of a Mass Balance VCF 8.5:



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Cheese Whey and "Ideal Whey" - Comparison

Parameter	Cheese whey	Ideal whey
Fat%	0.05-0.07	<0.005
Total protein% True protein% NPN%	0.75 0.55 0.20	0.60 0.43 0.17
Denatured aggregated protein	Up to 15%	Under 7%
Cheese culture	Yes (heat resistant)	No
Nitrate	May occur	No
Rennin + GMP	Yes	No
Quality/history	Often mixture from different cheese factories	Homogeneous
Quantity	Approx. 90% of cheese milk	Approx. 60% of cheese milk

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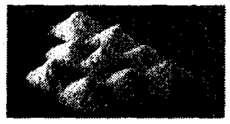
High Quality Whey Products Are Characterised by....



- Low fat content
- Low bacteria content
- Nitrate free
- High solubility
- High gel strength and water binding
- High whipping capability and foam stability
- Emulsifying qualities

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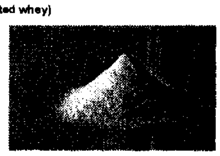
Adding Value to Whey
- MWPI is an excellent choice - because......

- Whey proteins are removed before the cheese production directly from the milk, which secures high quality whey for MWPI
- No need for whey treatment before UF
- High quality: Low spore and fat content, low denaturation
  - Allows range of high value products (WPI, Isolates, Hydrosylates, Microparticulated whey)
- High functionality:
  - high protein solubility
  - improved foam qualities
  - highest gel strength
- No remainders of:
  - rennet (and by-product GMP)
  - cheese culture and secondary flora
- Classical whey volume reduced



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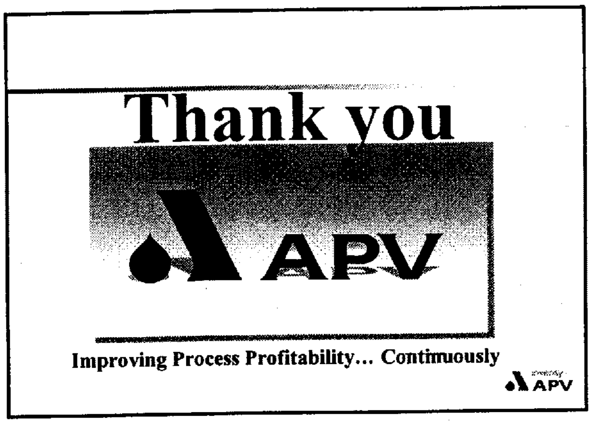
APV Pro-Frac™





Thank you
APV
Improving Process Profitability... Continuously
APV

3

Western Dairy Center

Utah State University

Utah State University
16th Biennial
Cheese Industry Conference

How Protein Fortification Affects Milk Coagulation

Donald McMahon
Professor
Utah State University

How Protein Fortification Affects Milk Coagulation

Donald J. McMahon
&
Bonney S. Oommen.
Utah State University

- Based on:
  - the Ph.D. Dissertation of Dr. Bonney Oommen, 2001-2004.
  - the electron microscopy techniques developed by William R. McManus

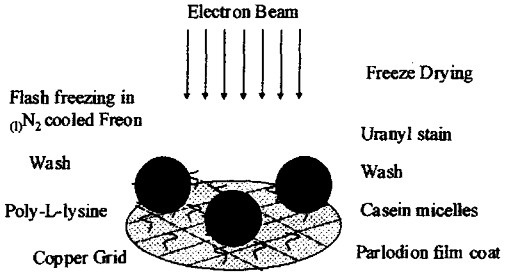
Outline

- Electron Microscopy
- Rehydration of milk protein powders.
- Rennet coagulation properties of protein fortified milk.
- Casein micelle structure.

Electron Microscopy

- A technique developed at Utah State University for viewing protein particles using transmission electron microscopy
  - Capture proteins on a plastic coated grid
  - Heavy metal stain the sample
  - Instantaneously freeze the sample
  - Sublimate water under vacuum
  - Image sample
- Protein particles remain as close to their native state as is possible, for viewing at very high magnifications.

Sample Preparation for Electron Microscopy



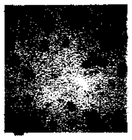
Rehydration of milk protein powders.

- Rehydration rate is influenced by
  - Size and shape of powder particles
  - Extent of shear applied during hydration
  - Time
  - Solubility of powder constituents
- Protein structures in rehydrated milk protein powders differs between
  - Skim milk powder
  - Sodium caseinate powder
  - Caclium caseinate powder

Rehydrating Skim Milk Powder

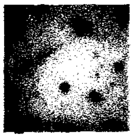
- When skim milk powder is hydrated,
  - Water penetrates into the powder particles at a rate that is dependent on the extent of mixing that is used.
  - Soluble components such as lactose are dissolved and move into the water phase.
  - The particles begin to disintegrate into their constituent insoluble (i.e., colloidal) particles–the casein micelles.
  - After 4 h of hydration at low shear, clumps of the casein micelles and other constituents of still remain and hydration is incomplete,

Rehydrating Skim Milk Powder



After 10 h of mixing at low shear

Individual rehydrated casein supramolecule



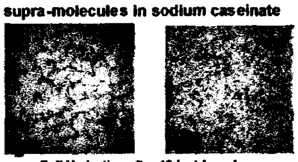
After 10 min of mixing at high shear and 1 h of hydration

- Longer Times and Higher Shear bring out complete rehydration, and dissociation of the powder particles into their individual constituents.
  - soluble lactose and minerals
  - soluble proteins
  - colloidal-sized casein supramolecules (casein micelles)

Rehydrating Sodium Caseinate

- Sodium caseinate is manufactured by
  - Acidifying milk so the caseins become insoluble and the milk coagulates.
  - Separating the acid casein from the milk serum and rinsing with water.
  - Neutralizing with sodium hydroxide to dissolve the casein coagulum
  - Drying to form a powder.
- **There are no casein supra-molecules in sodium caseinate**

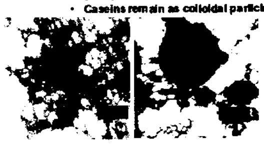
Partial Hydration 4 h at low shear



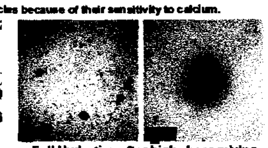
Full Hydration after 10 h at low shear Proteins present as small particles and chains

Rehydrating Calcium Caseinate

- **When acid casein is neutralized using calcium hydroxide**
  - **The caseins retain a spherical supramolecular structure similar in <u>size</u> to the casein micelles originally present in milk.**
  - **But its internal structure is different to native casein micelles.**
    - **While calcium is present, phosphate is absent so there is no colloidal calcium phosphate**
    - **Caseins remain as colloidal particles because of their sensitivity to calcium.**



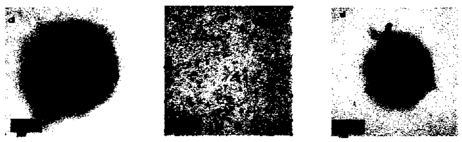
Partial Hydration after 4 h at low shear Caseins present as colloidal-size particles and strands of protein



Full Hydration after high shear mixing Caseins present as spherical colloidal particles of various sizes.

Calcium caseinate particles are more heavily stained with uranyl oxalate than casein micelles from milk.

Comparison of Supramolecule Structures



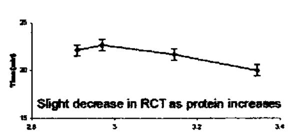
- Calcium caseinate forms colloidal particles that are "similar" to casein micelles in milk,
  - but have a "submicelle-type" internal structure.
- Sodium caseinate can be converted into colloidal particles by adding calcium.

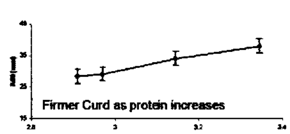
Rennet coagulation properties of protein fortified milk.

- Rennet coagulation time of milk and firmness of curd is influenced by;
  - Enzyme level
  - Temperature
  - Protein level
  - Calcium and phosphate concentration
  - pH
  - Heat treatment of milk
  - Milk quality
- Coagulation properties of protein-fortified milk depend upon
  - the protein level, and
  - the protein source.

Skim Milk fortified with Nonfat Dry Milk

- Fortified by adding 1%, 3% and 5% of NFDM slurry containing 12% protein.
- Coagulation time decreased slightly as the amount of added protein increased.
- Firmness of curd increased with added protein.

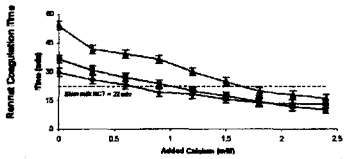




Skim Milk fortified with Sodium Caseinate

Compared to nonfortified skim milk, adding sodium caseinate delays coagulation:

22 min RCT Skim milk
30 min RCT 1% NaCN slurry added
54 min RCT 5% NaCN slurry added



Skim milk fortified by adding sodium caseinate slurry containing 12% protein.

- 1% --> 3.0% protein
- 3% --> 3.15% protein
- 5% --> 3.35% protein

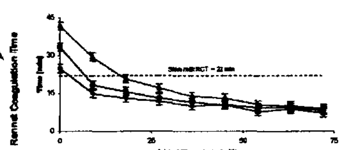
Adding calcium chloride restores coagulation time of sodium caseinate-fortified milk to original value of skim milk:

0.6 mM Ca required for 1% added NaCN slurry
1.6 mM Ca required for 5% added NaCN slurry

Skim Milk fortified with Calcium Caseinate

Compared to nonfortified skim milk, adding calcium caseinate delays coagulation:

22 min RCT Skim milk
25 min RCT 1% CaCN slurry added
42 min RCT 5% CaCN slurry added



Skim milk fortified by adding calcium caseinate slurry containing 12% protein.

- 1% --> 3.0% protein
- 3% --> 3.15% protein
- 5% --> 3.35% protein

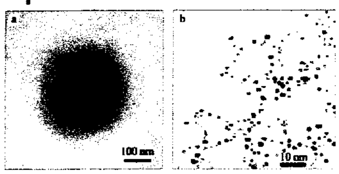
Adding potassium phosphate restores coagulation time of calcium caseinate-fortified milk to original value of skim milk:

2.6 mM phosphate required for 1% added CaCN slurry
16 mM phosphate required for 5% added CaCN slurry

Structure of Casein Micelles

- Casein proteins in milk are collected into colloidal particles
  - Size varies
    - 20 nm to 600 nm diameter
    - Average size about 150 nm diameter
  - Average casein micelle contains about 10,000 protein molecules
    - V α_{s1}-casein α_{s2}-casein β-casein κ-casein
  - Open structure that holds 4 to 8 g water per g protein
  - Spherical shape
  - Contains 2/3 of calcium phosphate in milk
    - Insoluble
    - Colloidal calcium phosphate
    - Present as nanoclusters
- Models for casein micelle structure
  - Submicelle models
  - Casein Polymerization models
  - Dual binding models

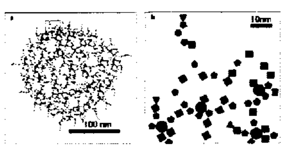
Electron Micrograph of Colloidal Casein Supramolecule from Milk



A single plane of electron-dense locations on the periphery of a casein supramolecule color coded according to their functionality (f), ie., number of particles to which they are closely associated.

red (f=1)	green (f=2)	blue (f=3)	black (f_4)

Schematic Model of Casein Supramolecule Structure



Irregular structure allows for all possible combinations of proteins.

Calcium phosphate

– formed into clusters because of low solubility.

–Prevented from nucleating into crystal form by being rapidly bound by the calcium-sensitive caseins.

–nanoclusters act as nodes that hold together chains of caseins.

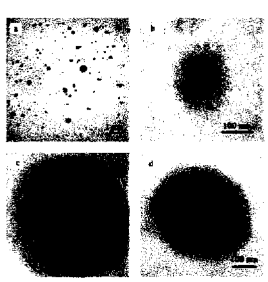
Chains of proteins grow until

- they encounter a chain terminating protein,
- bond with another chain, or
- become attached to another calcium phosphate nanocluster.

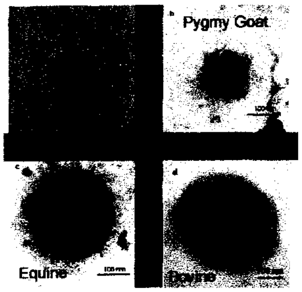
Limited to colloidal size by the chain-terminating influence of κ-casein.

Size Distribution of Casein Micelles

- Typical size variation observed for casein supramolecules in bovine milk.
- Inherent variation in protein arrangement occur within the casein supramolecule



Casein Supramolecules from Various Animal Species



- Differences in protein composition of milk by different species, produces different casein supramolecules.
- Principles of casein supramolecule assembly remain the same
  - Calcium phosphate nanoclusters act as nodes
  - Caseins bind to the nanoclusters and form chains and branched strands of protein
  - Chain termination limits size of supramolecule growth.

Conclusions

- Either high shear or long times are required to hydrate milk protein powders.
- Colloidal supramolecular structure of casein in milk requires calcium to be present.
  - Sodium caseinate does not form supramolecules.
- Adding caseinates to milk changes the calcium phosphate system in milk, and retard coagulation. To restore coagulation rates:
  - Add calcium if milk is fortified with sodium caseinate.
  - Add phosphate if milk is fortified with calcium caseinate.

Conclusions

- Supramolecular structure of casein micelles:
  - CaPhos nanoclusters functioning as nodes that hold together the strands of caseins forming filagreed loops and chains.
  - Casein molecules forming linear and branched chains.
  - Chain termination by κ-casein limits supramolecules to colloidal-sized spheres.
  - Interior and surface of casein micelle have same basic structure.
- This molecular model for the casein supramolecule satisfies the principles of
  - self aggregation,
  - interdependence, and
  - diversity

  that are often observed in nature

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Utah State University
16th Biennial
Cheese Industry Conference

Comparison of Different Methods of Milk Protein Fortification on Cheddar Cheesemaking Efficiency

Tim Guinee
Teagasc Dairy Products Research Centre
Ireland

Comparison of different methods of milk protein fortification on Cheddar cheesemaking efficiency

Timothy P. Guinee, B.T.O'Kennedy, P.M. Kelly
Dairy Products Research Centre,
Moorepark Teagasc,
Fermoy,
Co. Cork, Ireland.

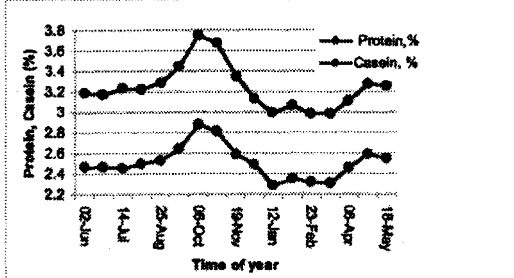
DPRC

NDP

Why fortify milk protein for cheese manufacture ?

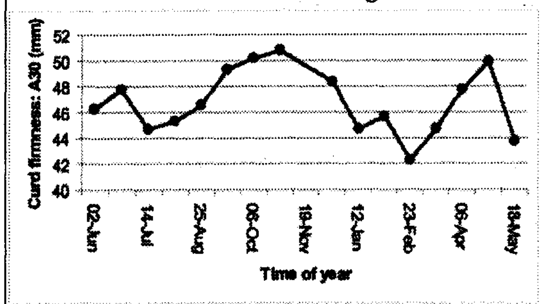
- Provides a means of standardizing protein content and protein/fat ratio
  - can reduce effect of seasonal variations in milk composition, which are conducive to inconsistencies in
    - rennet coagulability and curd firmness
    - yield
    - composition
    - quality

Seasonal variations in composition of Irish manufacturing milk



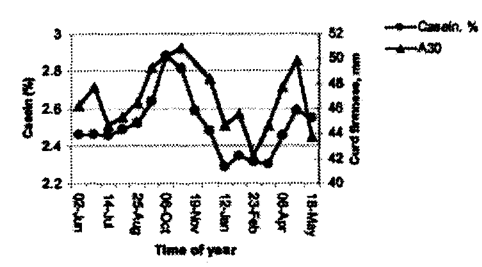
Data from O'Brien et al. (1999)

Seasonal variations in composition of Irish manufacturing milk

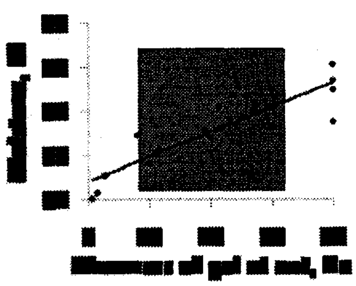


Data from O'Brien et al. (1999)

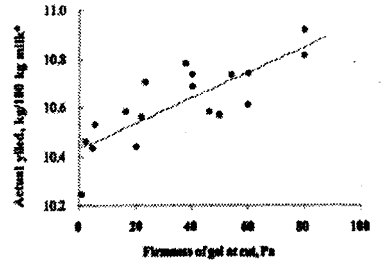
Relationship between casein level and curd firmness



Effect of gel firmness at cut on moisture content of Cheddar cheese

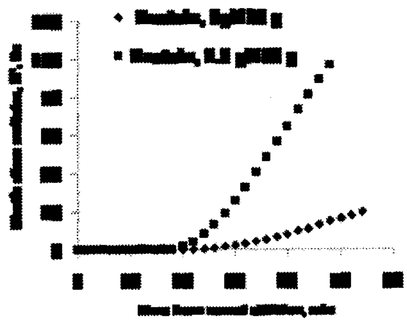


Effect of gel firmness at cut on yield of Cheddar cheese

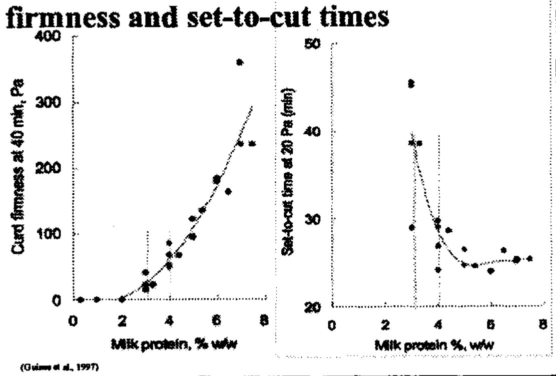


*Yield: per 100kg milk normalised to a fat + protein of 7.5% (w/w).

Effect of increasing milk protein on change in curd firmness with time



Effect of increasing milk protein on curd firmness and set-to-cut times



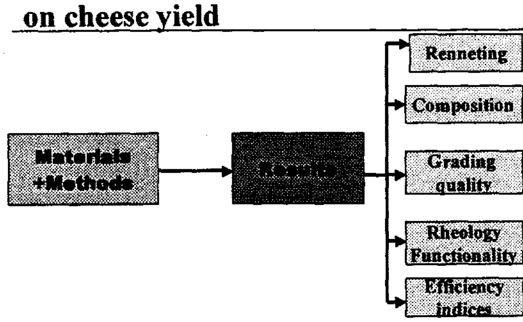
Why fortify milk protein for cheese manufacture ?

- Provides a means of standardizing protein content and protein/fat ratio
- Lessens effect of seasonal variation in milk protein level and associated inconsistencies in yield, composition and quality
- Allows cheese manufacturer to more effectively set SOPs to maximize cheese yield
- More consistent cheese composition and quality
- Higher cheese yields for a given volume milk?
- Greater, and more consistent, plant throughput

Work objectives of our study

- Effect of increasing milk protein from 3.3 % (Control, **C**) to 4.0% on cheese composition/yield of Cheddar cheese
- Protein increased by:
  - addition of **ultrafiltered milk retentate (UF)**
  - addition of spray dried **phosphocasein (PC)**
  - addition of spray dried **milk protein concentrate (MPC)**

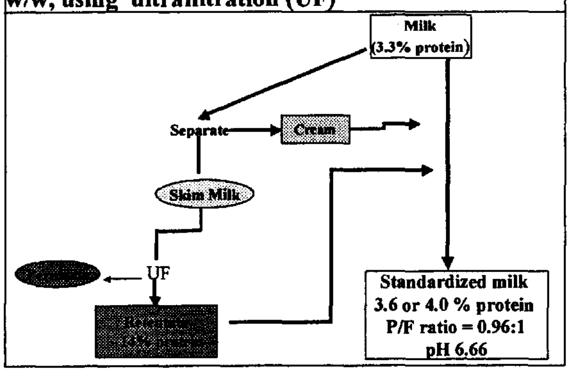
Influence of milk protein fortification on cheese yield



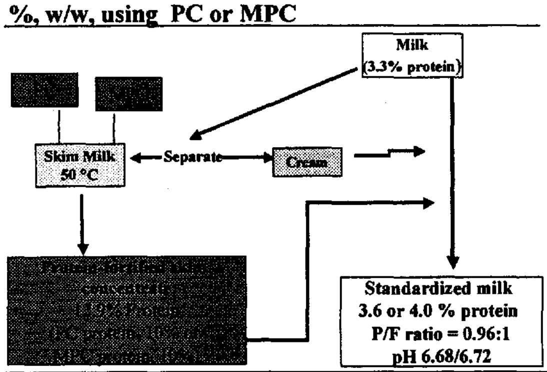
Definition of ingredients

- **Phosphocasein (PC)**
  - prepared from skimmed milk by microfiltration/diafiltration
  - spray dried
  - 84 % protein
  - protein = micellar casein
  - pH ~ 7.1
- **Milk protein concentrate (MPC)**
  - prepared from skimmed milk by ultrafiltration/diafiltration
  - spray dried
  - 87 % protein
  - protein = casein + native whey protein, as in milk
  - pH ~ 6.8

Increasing milk protein level from 3.3 (C) to 4.0 %, w/w, using ultrafiltration (UF)



Increasing milk protein level from 3.3 (C) to 4.0 %, w/w, using PC or MPC



Some details on cheesemaking practice

- Standardization of
  - protein-to-fat ratio: 0.97
  - pasteurization at 72 for 26 s
  - **rennet and starter added on protein basis**
  - **starter: bulk, added for 30 min before set**
  - pH at renneting/set: 6.6 - 6.55 *(lactic acid adjustment)*
  - **temperature at set 31 °C**
  - **cut at constant firmness: 54 Pa**
  - **cut programme and heal time: constant**
  - **stirring: increased from 10 to 25 rpm on cooking**
  - **cook to 39°C at a rate of 0.2°C/min**
  - **whey drainage: pH 6.15**
  - **curd milling: pH 5.25**
  - **mellow: 20 min**

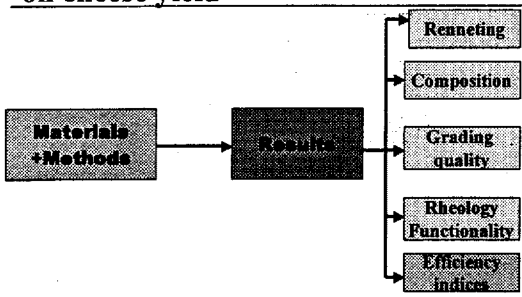
Experimental design/protocol

Treatment	Protein %	Fat, %
Control milk : C	3.3	3.4
PC fortified milk: C+PC	4.0	4.15
MPC fortified milk: C+MPC	4.0	4.15
UF fortified milk:C+UF	4.0	4.15
Replicate trials	**4**	

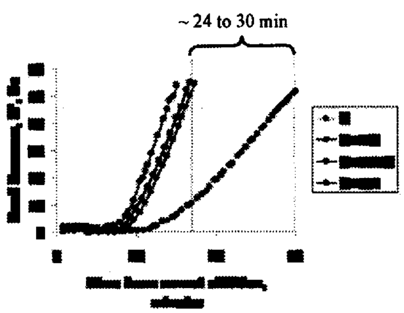
Experimental design/protocol

- Full mass balance for each treatment
- Measured compositions of ingredients, milk and whey streams, and cheese
- Cheese
  - stored at 4 °C x 30d, and 8 °C x 240 d
  - tested for proteolysis, rheology, flowability on storage
- Cheeses scored by cheese grader at 180 and 270d for body/texture + flavour/aroma

Influence of milk protein fortification on cheese yield



Effect of increasing milk protein from 3.3 (C) to 4.0 % by PC, MPC or UF on curd formation



Effect of increasing milk protein from 3.3 (C) to 4.0 % by PC, MPC or UF on cheese composition

	C	C+ PC	C+MPC	C+UF
Moisture, %	37.5[a]	36.3[b]	36.2[b]	35.8[b]
MNFS, %	54.1[a]	53.2[b]	53.3[b]	53.0[b]
Protein, %	26.4	26.5	26.1	26.1
FDM, %	49.9[b]	49.9[b]	50.3[a]	50.4[a]
Salt, %	1.7	1.8	1.8	1.8
Ca (mg/g protein)	28.9	29.5	29.5	29.5
pH	5.07[b]	5.13[b]	5.17[a]	

5.19[a]

- Composition typical for all cheeses
- Milk Protein increase - no major effects except for moisture/MNFS

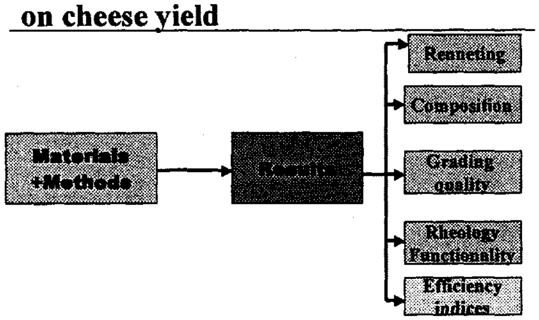
Effect of increasing milk protein from 3.3 (C) to 4.0 % by PC, MPC or UF on cheese quality

- Little effect on primary or secondary proteolysis
- Melt properties
  - C slightly higher flowability
  - little difference between C+PC, C+MPC and C+UF
- Rheological Properties
  - C had lower fracture stress, fracture strain and firmness; softer/shorter than other cheeses
  - little difference between C+PC, C+MPC and C+UF

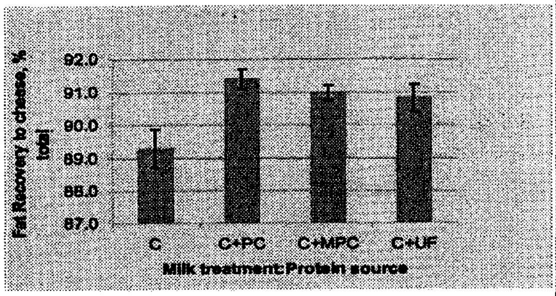
Effect of increasing milk protein from 3.3 (C) to 4.0 % by PC, MPC or UF on cheese quality

- All cheeses good quality: body/texture ≥ 33 and flavour/aroma ≥ 39.5 at 180 and 270 d
- Grades
  - C+PC and C+MPC higher body/texture scores than C or C+UF
  - C+PC and C+MPC similar flavour/aroma scores to C
  - C+UF lower flavour/aroma scores than C

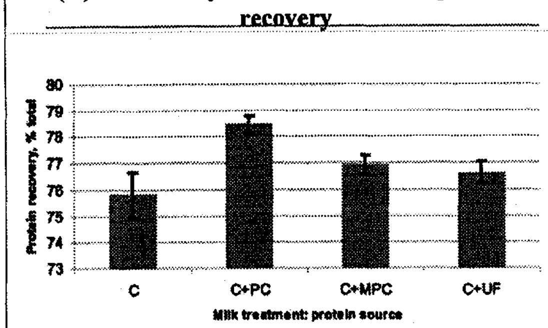
Influence of milk protein fortification on cheese yield



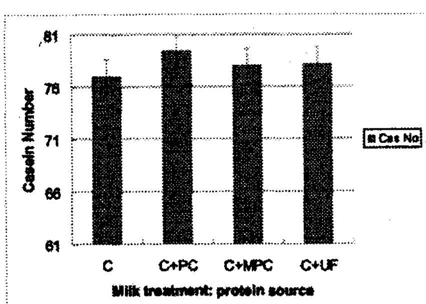
Effect of increasing milk protein from 3.3 (C) to 4.0 % by PC, MPC or UF on fat recovery to cheese



Effect of increasing milk protein from 3.3 (C) to 4.0 % by PC, MPC or UF on protein recovery



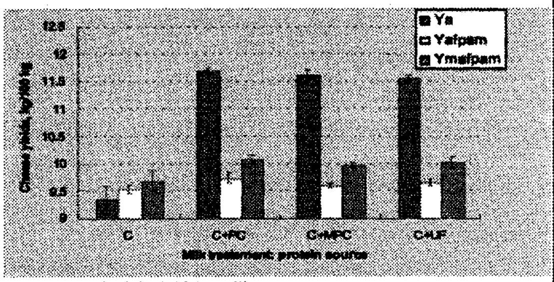
Effect of increasing milk protein from 3.3 (C) to 4.0 % by PC, MPC or UF on casein in milk



Cheese Yields

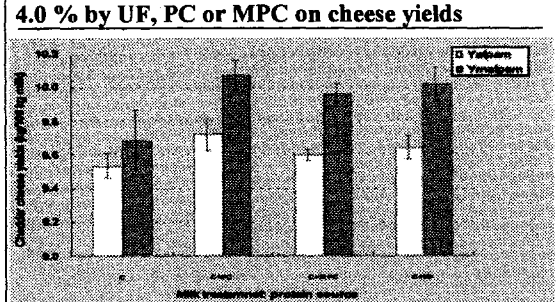
- Actual Yield: **Ya**
  - kg cheese /100 kg cheese milk
- Actual Yield normalized: **Yafpam**
  - kg cheese /100 kg cheese milk normalized to a common fat + protein of 6.7%
- Moisture-adjusted normalized: **Ymafpam**
  - kg cheese with moisture adjusted to 38.5%/100 kg milk normalized to a common fat + protein of 6.7%

Effect of increasing milk protein from 3.3 (C) to 4.0 % by UF, PC or MPC on cheese yields



- Ya, actual yield, kg/100 kg milk;
- Yafpam = Ya/100 kg milk normalized for fat + protein level (6.7%)
- Ymafpam = Yma/100 kg milk normalized for fat + protein level (6.7%

Effect of increasing milk protein from 3.3 (C) to 4.0 % by UF, PC or MPC on cheese yields



- Yafpam = Ya/100 kg milk normalized for fat + protein level (6.7%)
- Ymafpam = Yma/100 kg milk normalized for fat + protein level (6.7%)

Effect on cheese yields of increasing milk protein from 3.3 (C) to 4.0 % by PC, MPC or UF: Percentage Increase over control

Cheese Yields (kg/10, 000 kg milk)	C	C+PC	C+MPC	C+UF
Ya: Actual ,	-	24.8*	24.0*	23.4*
Yafpam, Normalized,	-	1.9*	0.7	1.1
Ymafpam: Normalized, Moisture-adjusted	-	4.0*	2.9*	3.4*

For Ya, Fat + protein for milk = 6.7 for C and 8.2 for C+PC, C+MPC, C+UF

** Statistically significant, $P < 0.05$*

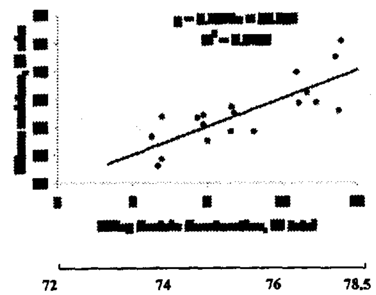
Cost-Benefit analysis: for use of PC to increase milk protein to 4 %

- **Benefit of increased Ya with PC**
  - ≈ € 693 /10, 000 kg milk for the extra cheese, 231 kg/10, 000 kg milk
- **Cost of adding PC**
  - ≈ € 370 for 74 kg PC added to10,000 kg milk
  - ≈ € 260 € per 10, 000 g milk for the 64.8 kg extra butter fat to balance extra protein
- **Net benefit**
  - **≈ € 39 /10, 000 kg milk**
  - ≈ € 1.2 M for 30, 000 tonne Cheddar plant
  - ≈ 0.4 c/L milk

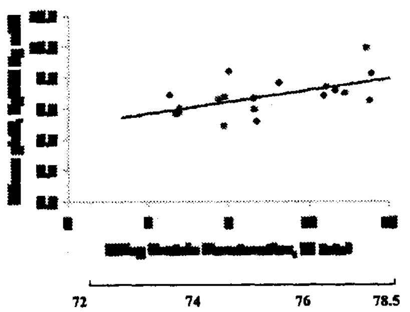
How can the full financial advantage of fortifying with ingredients be realised?

- Increasing the moisture in the cheeses from the protein-fortified milks to same level as the control
- How? Alteration of:
  - *Pasteurization temperature*
  - pH at set
  - *gel firmness at cut*
  - cut programme
  - cut size
  - scalding rate, and scalding temperature
  - others

Effect of pasteurization temperature on the moisture content of Cheddar cheese



Effect of pasteurization temperature on the yields of experimental Cheddar cheese



Conclusions

- Milk protein fortification from 3.3 to 3.6 or 4%
  - lower cheese moisture,
  - moisture can be easily increased by process intervention
- The use of PC
  - gave a cheese yield higher than that expected from the increased protein and fat solids in milk
  - extra yield benefit = €39 per tonne cheese on fortifying milk protein to 4% protein

Acknowledgements

- Glanbia Foods
- Project team members: E. Mulholland, C. Mullins, J. Kelly, D.O'Callaghan
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Comparison of different methods of milk protein fortification on Cheddar cheesemaking efficiency

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Western Dairy Center

Utah State University

Utah State University
16th Biennial
Cheese Industry Conference

Milk Pricing in an Unregulated Environment

Bill Schiek
Economist
Dairy Institute of California

Milk Pricing In the West

Bill Schiek
Dairy Institute of California

Western Milk Pricing Is Undergoing Adjustments

- Western milk markets have become increasingly dominated by manufacturing usages (cheese, whey, butter, nonfat milk powders).
- Federal orders west of the Rockies covered large areas and have limited fluid milk usage. Regulation has been more tenuous than elsewhere.
- California, which accounts for 21% of U.S. milk production, has its own unique regulated system, but is under pressure.

Western Milk Pricing Undergoing Adjustments..

- Termination of the Western Federal Milk Marketing Order has introduced an extra element of uncertainty to the pricing and marketing of milk west of the Rockies.
- In order to understand changes brought about by marketing milk in an unregulated market. We first need to review the characteristics of regulated milk pricing as we know it.

Regulated Pricing of Milk: General Principles

- Processors Pay for milk according to how it is used
  - Class I - packaged fluid milk products
  - Class II - cultured and frozen dairy products
  - Class III - cheese products
  - Class IV – butter and dry milk products.
- Class I is usually the highest price. Other classes are usually lower, but not always.

Regulated Pricing of Milk: General Principles

- Producers receive a "pooled price" for their milk, which is conceptually an average of the different prices in the market weighted by the volume of milk used in each class.

How Pooling Works--

- Let's assume the following class prices and milk utilization:

Class I	$12.00/Cwt.	50%	=	$6.00
Class II	$11.00/Cwt.	10%	=	$1.10
Class III	$10.00/Cwt.	30%	=	$3.00
Class IV	$9.00/Cwt.	10%	=	$0.90

Weighted average price = $11.00
(blend price)

All milk handlers pay dairy producers at least this blend price of $11.00/Cwt. So all producers receive the same base blend price regardless of where they sell their milk.

Pooling - A Producer Settlement Fund

- Lets assume two handlers in the market, Handler A, a bottler, and Handler B, a cheese plant (supply plant)

Handler A has:

Class I	$12.00 X 90%	=	$10.80
Class II	$11.00 X 10%	=	$ 1.10
Class III	$10.00 X 0%	=	$ 0.00
Class IV	$9.00 X 0%	=	$ 0.00
Average milk value		=	$11.90

Handler A pays its producers the $11.00 blend price and pays INTO the pool the difference of $11.90 - $11.00 or $0.90/Cwt. on all milk handled .

Pooling - A Producer Settlement Fund

- **Handler B has:**

Class I	$12.00 X 10%	=	$ 1.20
Class II	$11.00 X 0%	=	$ 0.00
Class III	$10.00 X 90%	=	$ 9.00
Class IV	$9.00 X 0%	=	$ 0.00
Average milk value		=	$10.20

Handler B pays its dairy producers the $11.00 blend price a draws OUT of the pool the difference between $11.00 - $10.20 or $0.80

How Producers Are Paid Under Orders With Multiple Component Pricing

- All producers receive the following in their monthly milk check:

Butterfat price X pounds of butterfat marketed
\+ Protein price X pounds of protein marketed
\+ Other solids X pounds of other solids marketed
\+ Producer price differential X total hundredweight's of milk marketed
\+ Somatic cell adjustment X total hundredweight's of milk marketed
= **Federal order portion of the producer's milk check**

The component prices paid to producers are the Class III prices

Milk plants may pay dairy producers more than the federal order price.

The Producer Payment Differential (PPD)

- The PPD represents the value of total market utilization in Class I, Class II, and Class IV relative to Class III value.
- **Example:**

(Class I $15.00 - Class III $11.00) X 40% Class I = $1.60
(Class II $11.90 - Class III $11.00) X 10% Class II = $0.09
(Class IV $11.20 - Class III $11.00)X 15% Class IV = $0.02
PPD = $1.71

- **The PPD can also be easily calculated by Blend Price minus Class III price.**

Pooling: The Argument Over Who Gets To Share In Which Revenues

- The rapid growth of milk supplies in the West led to large quantities of milk that were in excess of fluid milk (Class I) needs.
- Producers shipping to manufacturing plants in areas dominated by Class III and Class IV usage would like to associated with a fluid milk market in order to share in the higher revenue associated with a Class I price.

Pooling: The Argument Over Who Gets To Share In Which Revenues

- When manufacturing plants associate their milk with a marketing order "pool," the average price received by the original pool producers usually declines.
- On occasions where Class III or Class IV prices are higher than the average pool price, pooling rules have allowed the manufacturing plants to de-pool their producers, again with the effect of lowering the pool price.

The Western Order

- The concerns of Utah producers regarding pooling and de-pooling of Idaho milk led to the dissolution of the Western Milk Marketing Order.
- As a result, more milk in the West is now "unregulated."
- Some of the milk previously regulated under the Western order is now associated with and regulated under another order.

Pricing Unregulated Milk

- The price paid by plants for unregulated milk will be determined by:
- Finished product yield and conversion costs.
- Local competitive milk supply/demand conditions.
- Impact of competition from nearby regulated markets.
- Most often, some combination of the above.

Pricing of Manufacturing Milk Or Components: Yield Formulas

- Milk or component prices are derived from finished product prices (butter, cheese, nonfat dry milk, whey).
- Manufacturing costs are explicitly or implicitly considered.
- Yield of finished products per pound of milk or milk component is factored into the formula.

Product Yield Pricing Formulas: Cheddar Cheese Example

- What saleable products are made in the cheese plant? Cheese, whey cream, nonfat whey solids.
- Basic Formula = (Product price - plant margin) x product yield.
- Value of the individual producer's milk will depend upon how much of each product is yielded from his unique milk.

Suppose Producer Milk Tests: 3.8% Fat, 3.3% Protein, 5.6% O.S.

- Cheese contribution: (Cheddar block price - plant margin) x cheese yield. ($1.50 per lb. - $0.15) x yield.
- Cheese Yield = ((fat x fat ret.%) + (protein x casein%)- casein loss)x 1.09/(1-moisture %). ((3.8 x 0.9) + (3.3 x 0.78)-.1) x 1.09/ (1-0.36) = 10.04
- Cheese contribution = ($1.50- $0.15) x 10.04 = $13.55 per cwt.
- Whey cream contribution = whey cream yield x (Grade B butter price – margin)
  - Whey cream yield = 3.8 x 0.1 = 0.38
  - Whey cream contribution = 0.38 x ($1.40 – 0.12) = $0.49 per cwt.
- Dry Whey contribution = (whey price – margin) x whey yield
  - Whey yield = 5.6 + (3.3 x 0.22) = 6.3
  - Whey contribution = ($0.23-$0.18) x 6.3 = $0.32 per cwt
- Milk Price = $13.55 + $0.49 + $0.32 = $14.36 per cwt.

Local Competitive Conditions

- Product yield formulas describe what plants ARE ABLE TO PAY, given finished product prices.
- Local competitive conditions determine what plants ARE WILLING TO PAY for milk.
  - When supplies of milk are tight, plants will accept narrower margins in order to stay wet.
  - When supplies are long, plants may take larger margins on their regular supply, and will only take on additional milk at a discount, which can be substantial.

Regulated Prices In Other Areas

- If producers can get a regulated price by shipping to another plant, that regulated price becomes the competitive standard for unregulated plants.
- In newly deregulated areas, producers may demand the old regulated price because it is familiar to them.
- Unregulated plants may have to compete for product sales with plants in regulated areas. For example, the California price for cheese milk may influence what plants in other areas can pay for milk.

What Price Will Prevail For Unregulated Manufacturing Milk?

- Depends upon the area, but cheese manufacturing is supplanting butter-powder production as the principal manufactured product in the West.
- Currently, the situation is in flux
  - some plants paying based on cheese yield (with whey factors)
  - Some plants paying the Class III price
  - Some plants making adjustments to the above to compete with other regulated areas (California).

What Price Will Prevail For Unregulated Manufacturing Milk?

- As opportunities to draw revenues from federal order pools wane, manufacturing plants may have to accept narrower margins to keep their milk supply viable.

What About Unregulated Fluid Milk (Class I) Prices

- Competition will determine what price level will prevail.
- Currently, negotiations between producers and Class I plants have set the price in Utah (reportedly at about the same level as under the Western Order).
- Competitive pressures could come from Class 1 plants with lower raw product costs in Montana or unregulated areas.
- Competition could also come from bulk milk originating in Idaho.
  - Is the cheese yield price plus transportation less than the Salt Lake City Class I price?

Will We Face More Or Less Regulation Of Prices In The Future?

- For Class I, it is difficult for unregulated milk supplies to maintain price levels without protection from the regulated price structure. The Western Order will probably return.
- If pooling rules limit the opportunity of manufacturing plants to jump in and out of the pool, we may see more milk, rather than less, subject to regulated pricing. Plants will benefit from the pool draw over the long haul.

Will We Face More Or Less Regulation Of Prices In The Future?

- Plants in areas where there is little opportunity to pool their milk will continue to be the most innovative with regard to adopting pricing systems that are responsive to economic forces.

6

Western Dairy Center

Utah State University

Utah State University
16th Biennial
Cheese Industry Conference

Flavor Development in Accelerated Ripened Cheddar Cheese

Carl Brothersen
Associate Director, Western Dairy Center
Utah State University

16th Biennial Cheese Industry Conference

Flavor Development in Accelerated Ripened Cheddar Cheese

Carl Brothersen

Objective:

Develop a signature cheese for USU

- Unique flavor
  - Helveticus CNRZ 32
- Decrease the ripening time

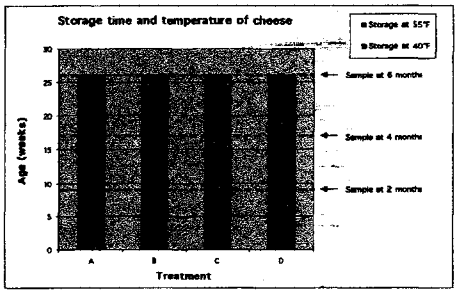
Experimental design:

Two ripening temperatures, 40°F and 55°F

Cheese evaluated at 2, 4, and 6 months of age
- Trained flavor panel - 19 panelists
- Trained texture panel - 11 panelists

Cheese from one vat divided into 4 treatments

Repeated three times



Composition of cheese

	Rep 1	Rep 2	Rep 3
pH	5.07	5.09	5.00
Moisture	34.8	37.48	35.23
FDB	49.86	53.58	51.03
Salt	2.00	1.79	1.67

Flavor scale*

Spectrum ™ method
- 0-15 scale
- universal for all foods
- cheese range = 0-7

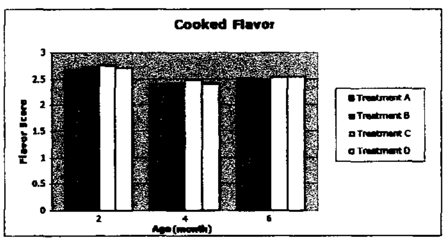
Example

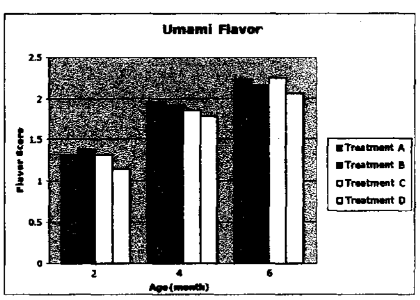
Food	Sweet	Salt	Sour	Bitter
Chocolate bar	10	0	5	4
Grape juice	6	0	7	2
NaCl 0.2%	0	2.5	0	0
Sucrose 2.0 %	2	0	0	0
Ritz cracker	4	8	0	0
Grapefruit juice	3.5	0	13	2
Cheese	1.8	3.5	3.3	0.02

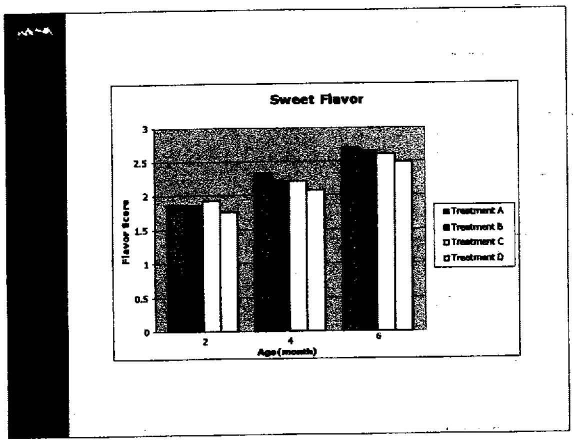
* Drake MA, JFS 66:1422

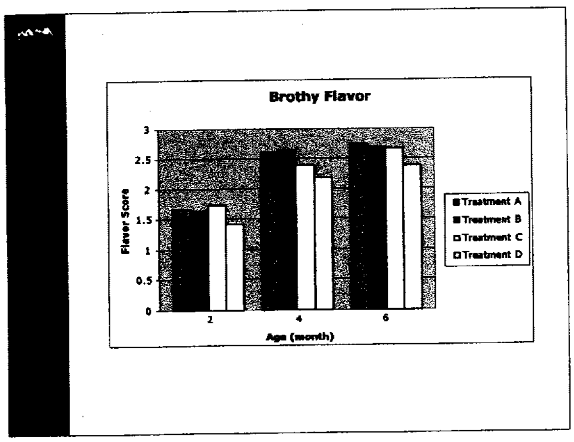
Flavor descriptors

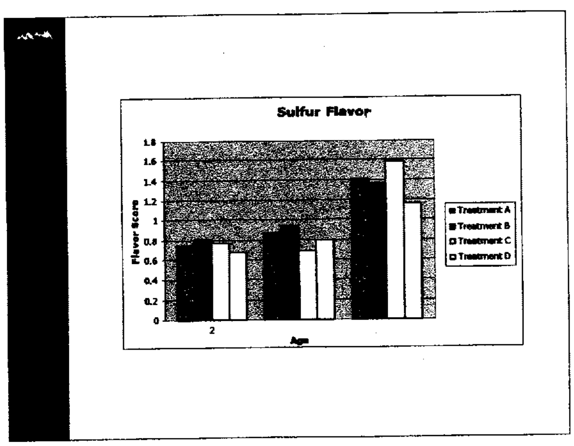
- Cooked
- Whey
- Diacetyl
- Milkfat
- Fruity
- Sulfur
- Free fatty acid
- Brothy
- Nutty
- Catty
- Sour
- Bitter
- Salty
- Sweet
- Umami

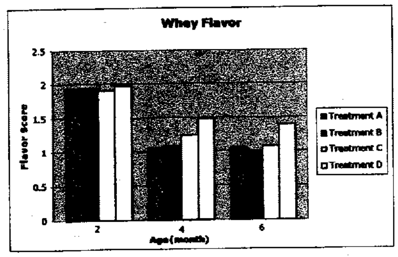





Sweet Flavor
Flavor Score
3
2.5
2
1.5
1
0.5
0
2
4
6
Age(month)
Treatment A
Treatment B
Treatment C
Treatment D


Brothy Flavor
Flavor Score
3
2.5
2
1.5
1
0.5
0
2
4
6
Age (month)
Treatment A
Treatment B
Treatment C
Treatment D


Sulfur Flavor
Flavor Score
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
Age
Treatment A
Treatment B
Treatment C
Treatment D



Flavor with scores less than 0.2

- Catty
- Free fatty acid
- Diacetyl
- Bitter

Flavor with scores between 0.2 and 1

- Fruity
- Nutty
- Sulfur

Flavors which increased with storage temperate and age:

- Ummi
- Sweet
- Sulfur
- Brothy

Flavors which decreased with storage temperature and age:

- Whey

Flavors which did not change with storage temperature or age:

- Cooked

Texture scale

0-15 scale
Product specific
Reference points
- Parmesan
- Feta
- Velveeta
- Sharp Cheddar
- Muenster

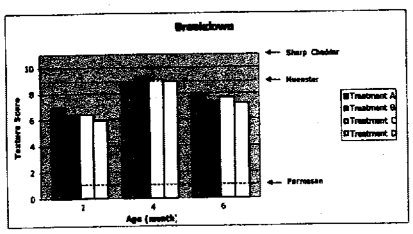
Texture descriptors

Hand evaluation
- Firmness
- Springiness
- Rate of recovery

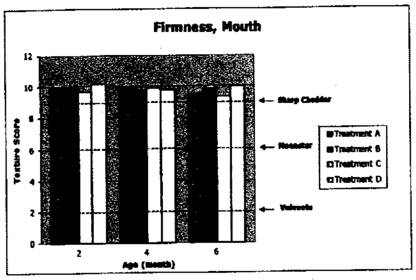
Mouth evaluation
- Firmness
- Fracturability

Mouth evaluation - chew down
- Degree of breakdown
- Cohesiveness
- Adhesiveness
- Smoothness of mass

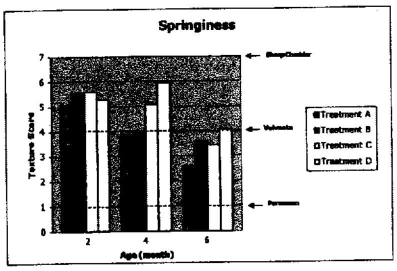
Mouth evaluation -residual
- Smoothness of mouth coating



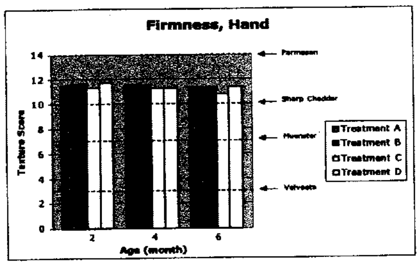
Evaluate how much the sample breaks down during mastication. (Formerly Meltability-rate the amount of "melting" or "dissolvability" in the sample.)



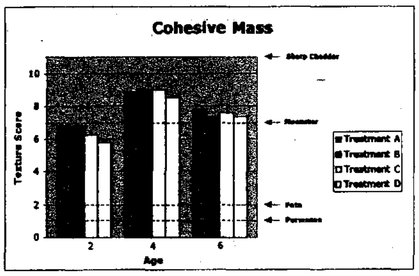
Evaluate the amount of force that is required to completely bite through the sample.



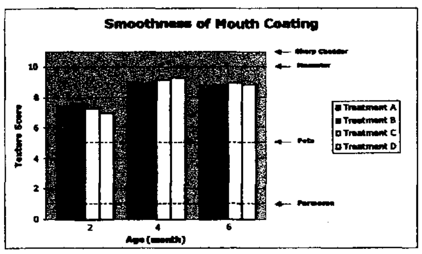
Depress the sample between your fingers until it is depressed 30%. (If you cannot depress the samples 30%, depress it as much as possible.) Evaluate the rate of recovery (I.E. how long it takes to recover to the original shape. Note: If the samples fractures as it is depressed, the sample does not recover.



Press your fingers completely through the sample. Evaluate the amount of force required to completely compress the sample.



Evaluate how well the mass sticks together.



Evaluate the degree of smoothness felt in your mouth after expectorating.

Textures which improved with storage temperate and age:

- Adhesiveness

Textures which improved with age but not with treatment

Fracturability
Breakdown
Cohesivmess
Smooth Mass
Smooth Mouth Feel

Textures which did not change with storage temperature or age:

- Firmness, hand
- Firmness, mouth

Textures which worsened with storage temperature and age:

- Springiness
- Recovery

Fatty acid + Alcohol $\underset{\text{High moisture}}{\overset{\text{Low moisture}}{\rightleftharpoons}}$ Esters

Acknowledgement

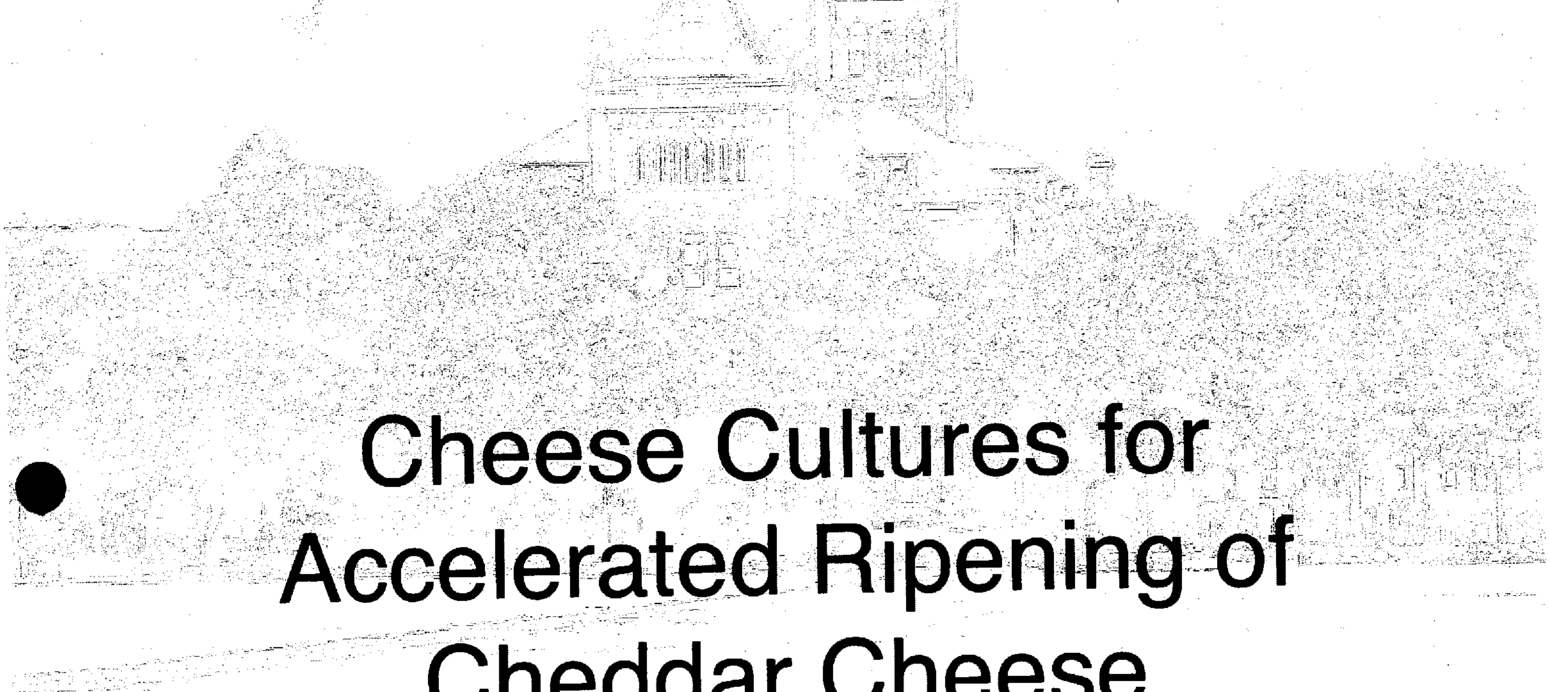
- Don McMahon - Project Leader
- Jeff Broadbent - Microbiology
- MaryAnne Drake - Sensory Analysis
- Steve Larsen - Cheesemaker
- Carl Brothersen - Oxidation/Reduction
- Agricultural Experiment Station - Funding

7

Western Dairy Center

Utah State University

Utah State University
16th Biennial
Cheese Industry Conference



Cheese Cultures for Accelerated Ripening of Cheddar Cheese

David McCoy
Principle Scientist
Chr. Hansen, Inc.

CHR. HANSEN

Cultures for Accelerated Ripening of Cheddar Cheese

16th Biennial
Cheese Industry Conference
August 11, 2004

David McCoy, PhD.
Principal Scientist

Agenda

CHR. HANSEN

- Cultures for Accelerated Ripening of Cheddar Cheese
  - Culture Selection - Historical -> Current
  - Protein Breakdown to Aroma and Flavor
  - Currently Available Culture Selection

2

Historical Selection Pre - 1975

CHR. HANSEN

- Culture Selection Based On:
  - From a Plant That Made Good Cheese
  - Met the Activity Criteria
    - Phosphated Media
    - Cheesemake
    - Flavor (Cheesy vs Bland, Bitter, Malty)
  - Resistant to Phages in a Whey Collection
  - Gas Production

3

Historical Selection Post-1980

CHR. HANSEN

- **Strain Selection Based On:**
  - Parent Culture Made Good Cheese
  - Met the Activity Criteria
    - Phosphated Media
    - Cheesemake
    - Flavor
    - Work Well on Combinations
  - Resistant to Isolated / Purified Phages
  - Species Identification (Gas Production)

Historical Selection Post-1980

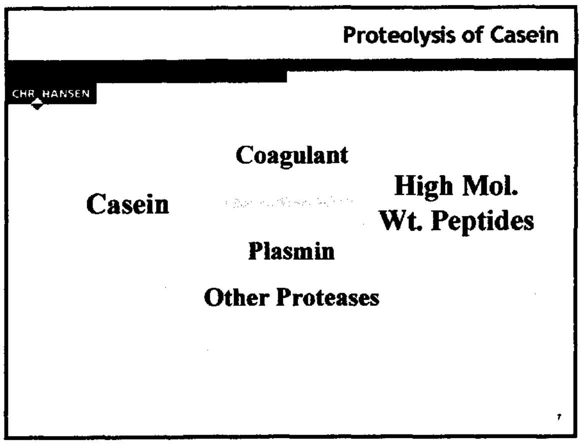
CHR. HANSEN

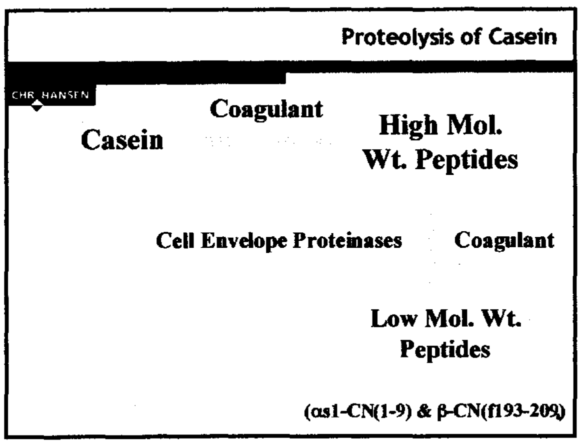
- **Culture Selection Based On:**
  - Species
    - Primarily *Lactococcus*
    - Occasionally *S. thermophilus*
  - Met Activity Criteria
    - Cheesemake - Decreasing Make Time
    - Salt Tolerance
    - Flavor (Lab and Trial)
    - "Proteolysis"
  - "Unique" Phage Pattern

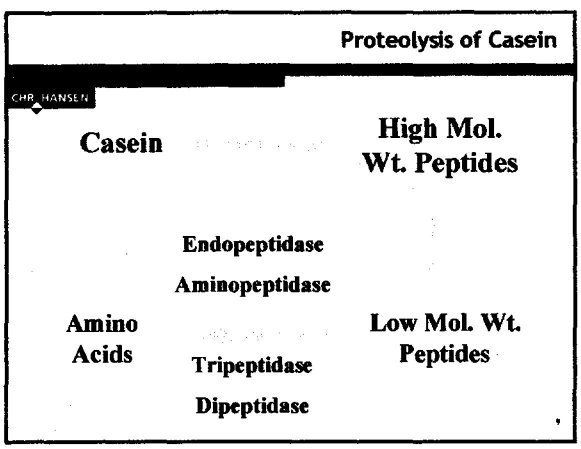
Current Selection Criteria

CHR. HANSEN

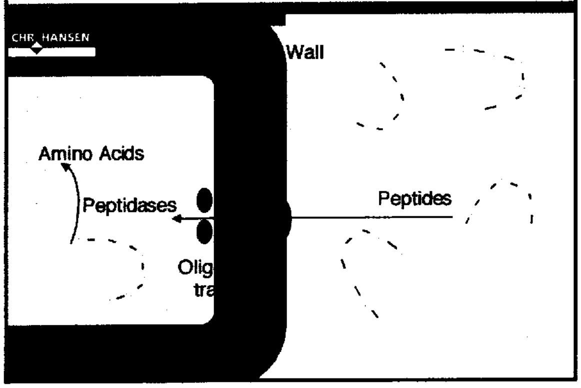
- **Primary Culture**
  - Rate of Acid Formation In Plant Procedures
  - Phage Resistance
- **Adjunct Culture Selection**
  - Uniform Flavor Quality of Cheese
  - Unique Functionality of Cheese -
  - Yield - Moisture Control


Proteolysis of Casein
CHR. HANSEN
Casein
Coagulant
Plasmin
Other Proteases
High Mol. Wt. Peptides

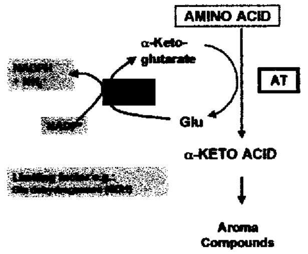

Proteolysis of Casein
CHR. HANSEN
Casein
Coagulant
High Mol. Wt. Peptides
Cell Envelope Proteinases
Coagulant
Low Mol. Wt. Peptides
(αs1-CN(1-9) & β-CN(f193-209)


Proteolysis of Casein
CHR. HANSEN
Casein
High Mol. Wt. Peptides
Endopeptidase
Aminopeptidase
Amino Acids
Tripeptidase
Dipeptidase
Low Mol. Wt. Peptides

Proteolytic System of LAB



Aminotransferases (AT), E.C.2.6.1



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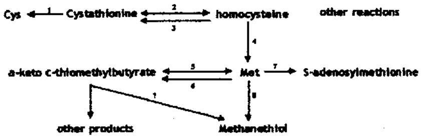
Methionine to Thiols - Sulfury Flavors



Enzyme 1 is cystathionine c-lyase. Enzyme 2 is cystathionine b-lyase. Enzyme 3 is cystathionine b-synthase. Enzyme 4 is homocysteine methltransferase. Enzyme 5 is arometic aminotransferase (tyrB) or transaminase B (ilvE). Enzyme 6 is amino acid oxidase. Enzyme 7 is Met adenosyltransferase and enzyme 8 is Met c-lyase

Seitz, 2003

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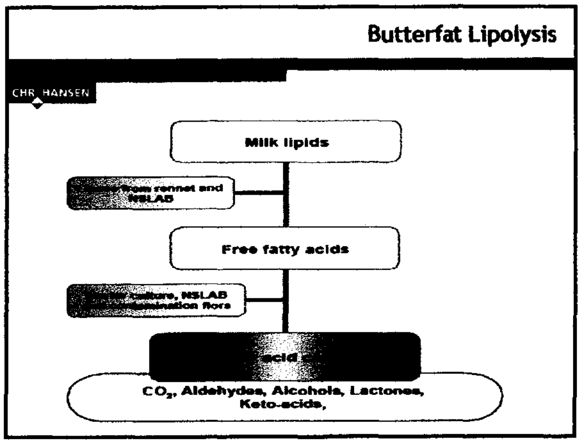
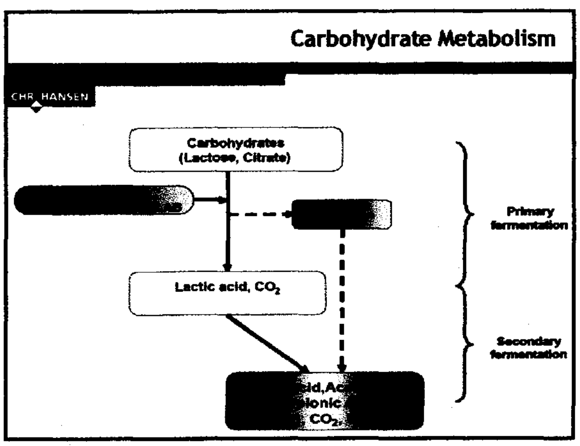
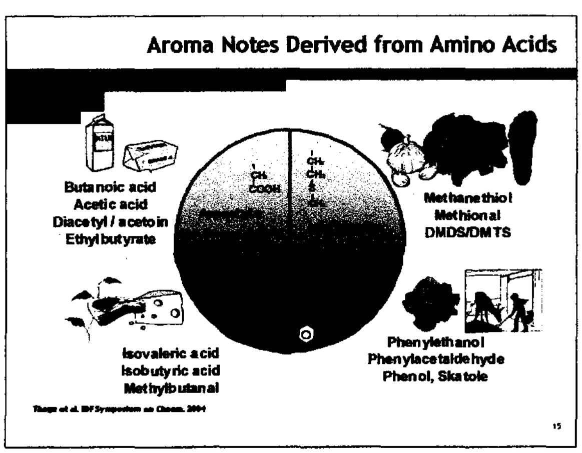

Butterfat Lipolysis
CHR. HANSEN
Milk lipids
Free fatty acids
CO_2, Aldehydes, Alcohols, Lactones, Keto-acids,


Carbohydrate Metabolism
CHR. HANSEN
Carbohydrates (Lactose, Citrate)
Lactic acid, CO_2
Primary fermentation
Secondary fermentation


Aroma Notes Derived from Amino Acids
Butanoic acid
Acetic acid
Diacetyl / acetoin
Ethylbutyrate
Methanethiol
Methional
DMDS/DMTS
Isovaleric acid
Isobutyric acid
Methylbutanal
Phenylethanol
Phenylacetaldehyde
Phenol, Skatole

Cheese Flavor Formation by Amino Acid Catabolism

CHR. HANSEN

Amino acids	Aldehydes	Alcohols	Carboxylic acids	Other Derivatives
Leucine	3-Methylbutanal or isovaleraldehyde	3-Methylbutanol	3-Methylbutanoic acid or isovaleric acid	
Isoleucine	2-Methylbutanal	2-Methylbutanol	2-Methylbutanoic acid	
Valine	2-Methylpropanal or isobutyraldehyde	2-Methylbutanol	2-Methylpropanoic acid or isobutyric acid	

Source: M. Yvon and L. Rijnen, (2001) International Dairy Journal 11:185-201

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Cheese Flavor Formation by Amino Acid

CHR. HANSEN

Amino acids	Aldehydes	Alcohols	Carboxylic acids	Other Derivatives
Phenylalanine	Phenylacetaldehyde, benzaldehyde (-2C)	Phenylethanol	Phenylacetic acid	
Tyrosine	OH-Phenylacetaldehyde, OH-benzaldehyde (-2C)	OH-Phenylethanol	OH-Phenylacetic acid	q-Cresol, phenol
Tryptophane	Indol-3-acetaldehyde, indol-3-aldehyde	Tryptophol	Indol-3acetic acid	Skatole, indole
Methionine	3-Methylthiopropanal, or Methional	3-Methylthiopropanol	3-Methylthiopropionic acid	Methanethiol

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The Flavor / Aroma Challenge

CHR. HANSEN

- Singh, Drake and Cadwaller
  - >110 Volatile Compounds in Cheddar Cheese
  - + Non-volatiles
    - Amino Acids
    - Peptides
    - Fatty Acids and Derivatives
  - Unknown Flavor Components (Harper)
- Interactions Between Flavor Components and the Matrix
- Which Compounds Create Which Flavors?
- Which Flavors Do Which Customers Want?

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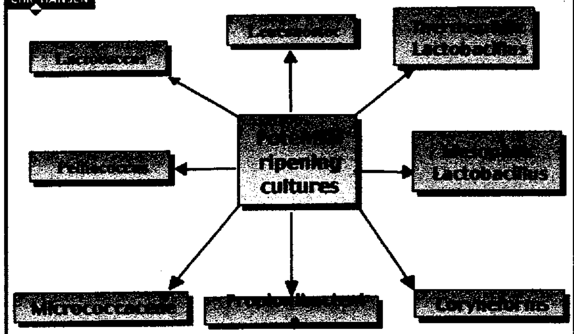
Potential ripening cultures

CHR. HANSEN



Ripening Cultures

CHR. HANSEN

- **Important Properties of adjunct NSLAB Cultures for Cheddar Cheese**
  - Grows well at 10°C and as low as 7°C ???
  - Not sensitive to salt-in-moisture of 5 to 6.5% ???
  - Grows well at pH 4.9 to 6.2
  - Produces no flavor or body defects (CO_2)
  - Should not interfere with normal cheese manufacturing

Source: Crow, V. et al. International Dairy Journal 11; 275-283 (2001)

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Ripening Cultures

CHR. HANSEN

- **Important Biochemical Properties of Adjunct NSLAB Cultures for Cheddar Cheese**
  - Should not racemise L+ lactic acid
  - Should not decrease glutamic acid during ripening
  - Produces succinate from citrate
  - Produce low levels of CO_2, acetic acid, formic acid and acetoin
  - Produce lipases, proteases and peptidase
  - Provide other enzymatic activities

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Ripening Cultures

CHR. HANSEN

- Important Properties of Adjunct Cultures for Cheddar Cheese
  - Economically Effective
  - Follows a "Normal" Ripening Progression
  - Insensitive to Normal Make Variations
  - Limits Impact of Cheese Plant Flora

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Types of Adjuncts

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- Autolysis
- Bacteriocins
- Selected Strains

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Starter Cell Lysis - Autolysis

CHR. HANSEN

- Mode of Action
  - Strains Selected By Sensitivity to:
  - Salt
  - Temperature
- Method of Use
  - Selected Combination of Strains, Some are Sensitive to Autolysis.
- Disadvantages
  - Bitterness
  - Regeneration of Cofactors
  - Concentration of Enzymes & Substrate

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Starter Cell Lysis - Bacteriocin

CHR. HANSEN

- Bacteriocin
  - Lacticin
  - Lactococcin
  - Nisin
- Mode of Action
  - Interferes with cell membrane
    - Leakage of Cell Material
    - Lysis
- Method of Use
  - Selected Combination of Acid-Formers, Bacteriocin Producers, Target Cells.

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Bacteriocins

CHR. HANSEN

- Advantages
  - Low Cost
  - Control Non-Starter Bacteria
  - Clean Label
- Disadvantages
  - Balance of Strains
  - Number of Strains Available (Non GMO)
  - Robustness
  - Cost of Purified Nisin

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Selected Strains

CHR. HANSEN

- *Lactococcus* or *Lactobacillus*
  - Slow Acidifying Species
  - Lactose Negative
  - Protease Negative
- Mode of Action
  - Increases the Amount of Desirable Enzymes
- Method of Use
  - Selected Strains or Combinations

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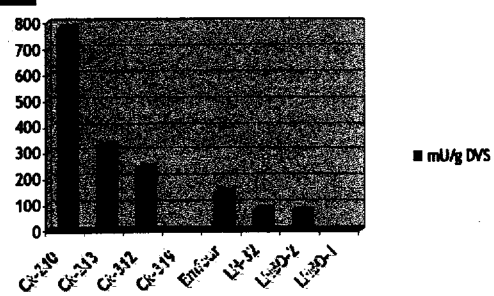
Selected Strains

CHR HANSEN

- Advantages
  - Low Cost
  - Clean Label
- Disadvantages
  - Balance of Strains
  - Number of Strains Available (Non GMO)
  - Narrowly Impact Flavor (Primarily Debitter & Generally Increase Flavor)
  - Phage ?

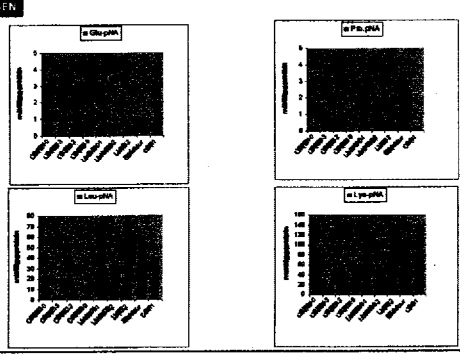
Proteolytic Activity of Selected Cultures

CHR HANSEN



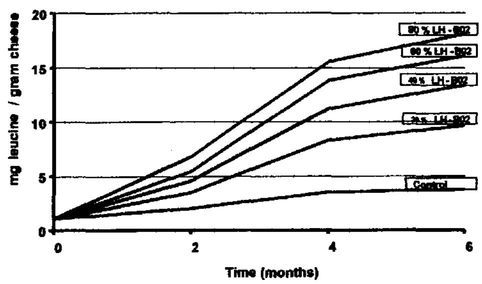
Aminopeptidase Activity

CHR HANSEN



Amino Acids in Cheddar Cheese

CHR. HANSEN



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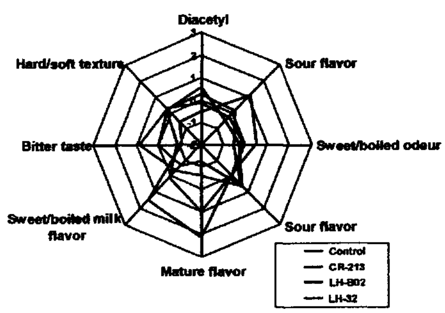
Sensory evaluation of Cheddar Cheese

CHR. HANSEN



O-culture + CR-213, LH-B02 or LH-32

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Typical Recommendation

CHR. HANSEN

- Standard Lactococcus Culture
  - 5000 grams per 50,000 lbs. milk
  - Moisture 36-37%, pH 5.0-5.1, salt 1.7-1.8%
- Selected Lactobacillus helveticus Culture
  - 250 grams per 50,000 lbs. milk
  - Higher levels provide more nutty / parm notes
- Selected Lactococcus Culture (lac-)
  - 500 grams per 50,000 lbs. milk
- Ripening
  - 40 F = typical, well balanced flavor
  - 50 F = New York cheddar flavors

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Observations

CHR. HANSEN

The Amount of Knowledge in Genetics, Bacterial Physiology, Cheese Chemistry, Analytical Chemisty & Flavor Recognition Has Increase Dramatically in the Last 5 Years.

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Observations

CHR. HANSEN

We still need to know how each reaction is affected by:

- Temperature
- pH
- Redox Potential (O2 Concentration)
- Moisture (Aw)
- Substrate & Cofactor Concentrations
- Product Concentration
- NaCl Concentration
- Solubility and Partitioning
- Interaction of Chemical on Flavor Perception

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Current Selection Criteria

CHR. HANSEN

- Primary Culture Selection Based On:
  - *Lactococcus* & / or *S. thermophilus*
  - Cheesemake Time
  - Salt Tolerance
  - Phage Sensitivity
- Secondary Culture Selection Based On:
  - Flavor (Lab and Trial)
  - Experience

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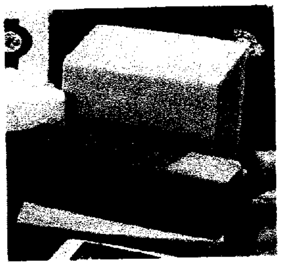
CHR. HANSEN

Questions?

Thank You



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Western Dairy Center

UtahState UNIVERSITY

Utah State University
16th Biennial
Cheese Industry Conference

Application of Microbial Genomics to Cheese Technology

Jeffery Broadbent
Professor
Utah State University

Application of Microbial Genomics to Cheese Technology

Jeff R. Broadbent, Professor
Department of Nutrition and Food Sciences
Utah State University, Logan

INTRODUCTION

Human civilizations place great value on technologies that improve the keeping qualities and flavor of foods, and one of the most ancient of these practices involves fermentation by lactic acid bacteria (LAB). The most important types of LAB in the manufacture of cheese and fermented milks include species of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus*. Because these types of LAB are common constituents of raw milk, it is likely that cheese and other fermented milk foods have been part of the human diet since milk was first collected in crude containers. Over the centuries, these "accidental" fermentations were slowly molded into the more than 1000 unique cheeses, yogurts, and fermented milks that are available in modern times. Because these products were developed long before the emergence of microbiological science, manufacturing processes for all varieties initially relied upon spontaneous acidification of milk caused, of course, by naturally occurring LAB in milk. It was not until discovery of the lactic acid fermentation by Pasteur in 1857, and development of pure LAB dairy starter cultures later that century, that the door to industrialized cheese and milk fermentations was finally opened. Since then, economic value of fermented milks foods and especially cheese has demonstrated dramatic and sustained growth. Cheese production in the US alone, for example, has increased more than 200% in the last quarter century, and total worldwide production now equals approximately 13 million tons per year.

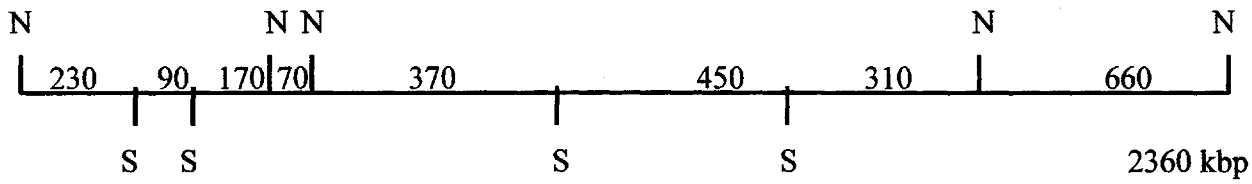
To sustain such high productivity, the dairy industry has become a leader in fermentation technology and starter microbiology. Decades of experience have proved that large-scale industrial production of uniform, high quality cheese is facilitated by the use of well-characterized starter cultures. Thus, even though some traditional cheese fermentations still rely on the natural souring of raw milk, virtually all industrialized processes utilize starter cultures. Since the economic vitality of the cheese industry depends on starter cultures with known, predictable, and stable characteristics, great resources and efforts have been directed toward understanding the physiology and genetics of dairy LAB. Research during the last quarter century was primarily focused on cellular biochemistry and the development of genetics tools, with commercial application in key areas such as bacteriophage resistance and flavor production. With genome sequence information now available for several LAB species, research in the coming decades is expected to provide refinements in starter technology that enhance product quality and consistency, promote consumer health and well being, and reduce manufacturing losses and safety concerns.

THE GENOMIC REVOLUTION

Because genes for all of the essential housekeeping, catabolic, and biosynthetic activities of the cell are located in the chromosome, knowledge of chromosome structure and organization in starter cultures has great fundamental and applied value to the dairy industry. Efforts to characterize chromosomes of LAB were begun in the early 1970s and 1980s by researchers who sought to estimate the genome size of these bacteria. The most useful method for this purpose was pulsed-electric field gel electrophoresis (PFGE), which allows one to purify relatively intact bacterial chromosomes, cut them with rare-cutting restriction endonuclease enzymes, then resolve the large molecular-weight DNA products by electrophoresis. If appropriate size standards are included in the gel, summation of individual restriction fragments after PFGE provides a rapid and relatively accurate means to estimate genome size. Using this approach, size estimates have been collected for chromosomes from strains representing more than 15 species of LAB, and researchers have shown that LAB have a relatively small chromosome (range = 1.8 to 3.4 million (mega) base pairs). One of the practical observations to come from this work was that restriction fragment polymorphisms are common in the PFGE profiles from different strains of the same LAB species, and this finding has allowed industry to use PFGE as a DNA fingerprinting tool for strain identification.

Another important outcome of PFGE technology has been its use, in combination with other procedures, to assemble modest physical and genetic maps of LAB chromosomes (Fig. 1). This strategy has been used to assemble maps of the chromosomes from several industrially important LAB, and those maps have confirmed that individual species and even strains may differ in genome size and organization, and they also show that all LAB characterized to date possess a single and circular chromosome. Although PFGE analysis is still a component of LAB genome research, the most exciting and innovative work in this field is now being fueled by DNA sequence analysis of complete genomes.

Figure 1. Physical map of the *Lactobacillus helveticus* CNRZ 32 chromosome. The map was derived from data collected after pulsed-field gel electrophoresis with the restriction enzymes *Not*I (N) and *Sfi*I (S). Numbers represent fragment sizes in kilobase pairs (kbp).



The compilation and annotation (computer-assisted identification of genes and gene products) of entire genome sequences has revolutionized bacteriology and microbial genetics, and has created great opportunities to study bacterial evolution, genetics, physiology, and metabolism. As such, genome sequence information for lactobacilli and other dairy LAB will endow industry and academia with unprecedented power to determine the means by which LAB have evolved in, interact with, and respond to, the microenvironments of cheese and milk. With respect to the relationship between LAB physiology and cheese flavor development, research efforts should be focused on strains that 1) possess established and desirable flavor-producing capabilities; 2) are genetically pliable; and 3) are characterized at the genome sequence level.

In 2001, *Lactococcus lactis* IL1403 became the first publicly accessible genome sequence for a starter LAB. Since then, genome sequences for several other important dairy LAB have become available, and sequencing projects are underway for additional LAB as well as several other species of bacteria that are significant to the dairy fermentation industry (Table 1). Because of their industrial significance, many of these projects are still being mined for intellectual property and so have not yet been released to the general scientific community. Still, 6 of the 13 genomes listed in Table 1 are in the public domain, and 4 of those 6 sequences were contributed by the Department of Energy's Joint Genome Institute (JGI) in collaboration with the Lactic Acid Bacterial Genomics Consortium (LABGC). The LABGC is a group of 11 US scientists representing 8 US Universities. Its mission is to advance academic and industrial research on LAB through the creation of publicly accessible genome sequence information, and foster research collaborations that will further US industry leadership in LAB-based food and agricultural processes.

Table 1. Current genome sequencing projects for dairy-related lactic acid bacteria and other species

Species	Strain	Genome size (Mbp)	Project sponsor[1]	Public access?
Lactobacillus acidophilus	ATCC700396	2.0	Dairy Management, Inc. and Rhodia, Inc.	no
Lactobacillus brevis	ATCC 367	2.0	JGI-LABGC	yes
Lactobacillus casei	ATCC 334	2.9	JGI-LABGC	yes
Lactobacillus casei	BL23	2.6	INRA, FR	no
Lactobacillus delbrueckii subsp. *bulgaricus*	ATCCBAA-365	2.3	JGI-LABGC	yes
Lactobacillus delbrueckii subsp. *bulgaricus*	ATCC11842	2.3	INRA and Genoscope, FR	no
Lactobacillus delbrueckii subsp. *bulgaricus*	DN-100107	2.1	Danone Vitapole, FR	no
Lactobacillus gasseri	ATCC 33323	2.0	JGI-LABGC	yes
Lactobacillus helveticus	CNRZ32	2.4	Dairy Management, Inc. and Chr. Hansen, Inc.	no
Lactobacillus helveticus	DPC 4571	??	University College, Cork, Ireland	no
Lactobacillus johnsonii	NCC533	2.0	Nestlé, Switzerlerland	yes
Lactobacillus plantarum	WCFS1	3.3	Wageningen Centre for Food Sciences, NL	yes
Lactobacillus rhamnosus	HN001	2.4	Fonterra Research Center, NZ	no
Lactococcus lactis subsp. *cremoris*	SK11	2.3	JGI-LABGC	yes
Lactococcus lactis subsp. *cremoris*	MG1363	2.6	Univ. Groningen, NL, and INRA, FR	no
Lactococcus lactis subsp. *lactis*	IL1403	2.3	INRA and Genoscope, FR	yes
Leuconostoc mesenteroides	ATCC 8293	2.0	JGI-LABGC	yes
Pediococcus pentosaceus	ATCC 25745	2.0	JGI-LABGC	yes
Streptococcus thermophilus	LMD-9	1.8	JGI-LABGC	yes
Streptococcus thermophilus	LMG18311	1.9	Univ. Catholique de Louvain, Belgium	no
Streptococcus thermophilus	CNRZ1066	1.8	INRA, FR	no
Non-LAB:				
Bifidobacterium longum	NCC2705	2.3	Nestlé, Switzerlerland	yes
Bifidobacterium longum	DJ010A	2.1	JGI-LABGC	yes
Bifidobacterium breve	NCIMB8807	2.4	University College, Cork, Ireland	
Brevibqacterium linens	ATCC9174	3.0	JGI-LABGC	yes
Propionibacterium freundenreichii	ATCC6207	2.6	DSM Food Specialties, NL	no

[1]JGI-LABGC, Department of Energy Joint Genome Institute and lactic acid bacteria genomics consortium.