Nutritional Dairy SDT SPRING CONFERENCE 2017

Cork | April 10 - 12

„Food concepts for specific target groups:

The role of dairy components and processing technologies”

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Ursprung des Bieres
PREMIUM BAVARICUM
SEIT
1040
BAYERISCHE STAATSBRAUEREI
Weihenstephan
ÄLTESTE BRAUEREI DER WELT

元代邹宏著《寿亲养老新书》中有关于牛奶的食疗方—“食治老人益气牛乳方”。

- 该书指出：“牛乳最宜老人，平补血脉、益心、长肌肉，令人身体康强、润泽、面目光悦，志不衰。”
- 书中强调指出:“故为人子者当须供之，以为常食，或为乳饼…，恒使恣意充足为度。此物胜肉远矣！

"Food with a value far above that of meat"
- Dairy products that can strengthen the body

During the Yuan-Dynasty (1206-1368) Zou Hong published a medical text book (which is still being referred to in modern China), entitled

"The new book about the theory, how to nurture your parents, for them to live a long life"

In this book a diet is proposed: The recipe to strengthen the body´s energy flow by means of bovine milk. It says:

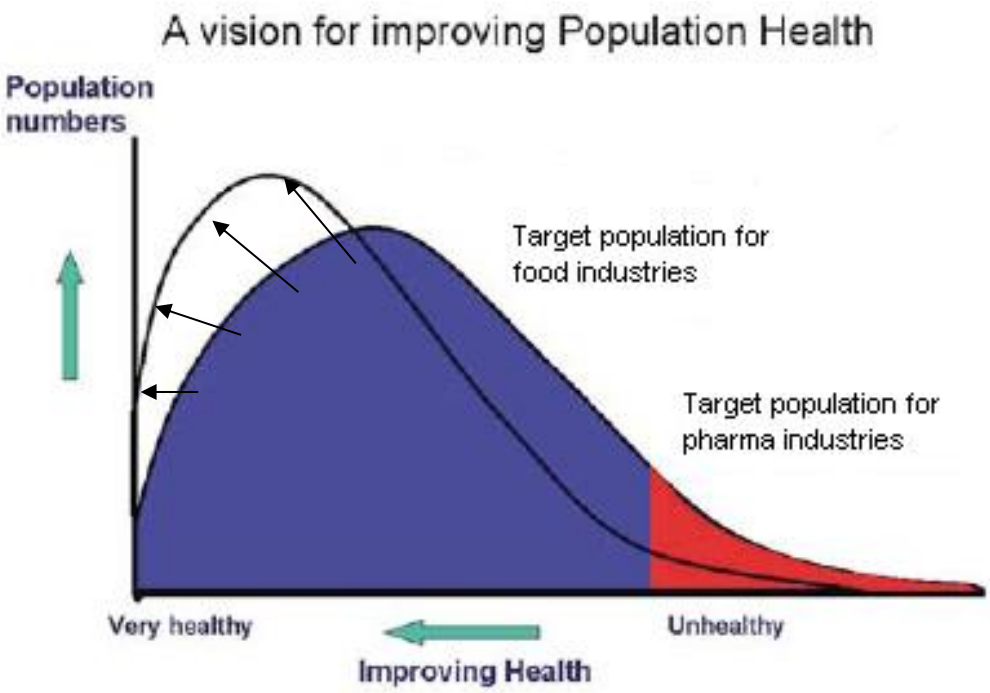
- "Cow milk is particularly good for the elderly. It can strengthen the blood circulation, it is good for the heart and build-up of muscles. Milk makes mentally and physically strong and yields a brilliant appearance."

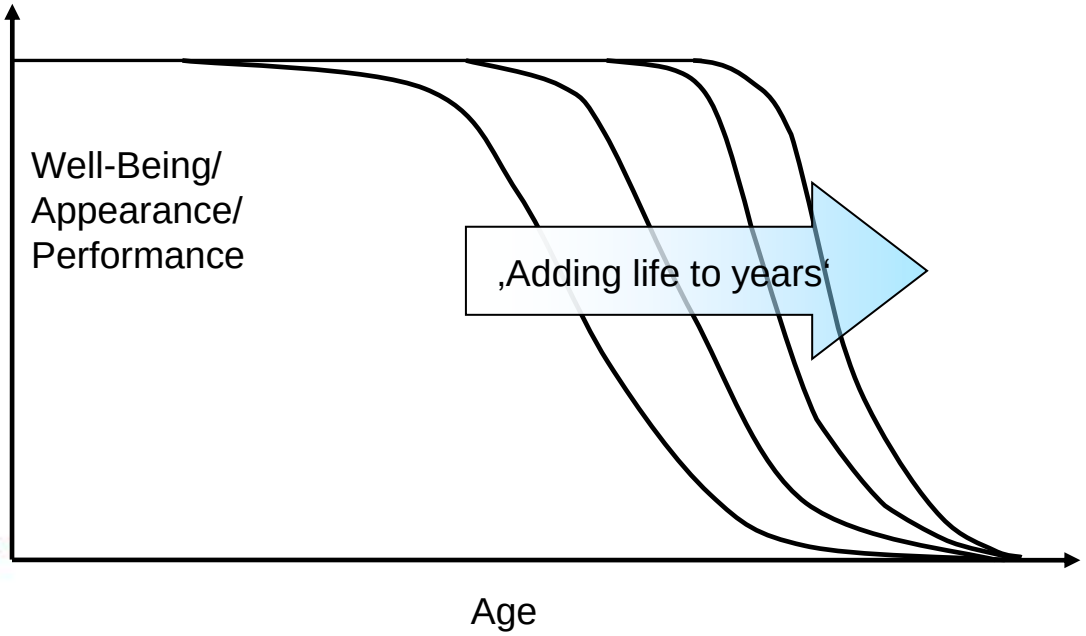
- „Therefore, one should prepare dishes for the parents using milk products, either a liquid milk or bakery products containing milk in sufficient amounts. Dairy products are much better then meat."

→ Milk is a preventively therapeutic substance as part of the Traditional Chinese Medicine (TCM)

Vision for foods for the developed world (EU strategy)

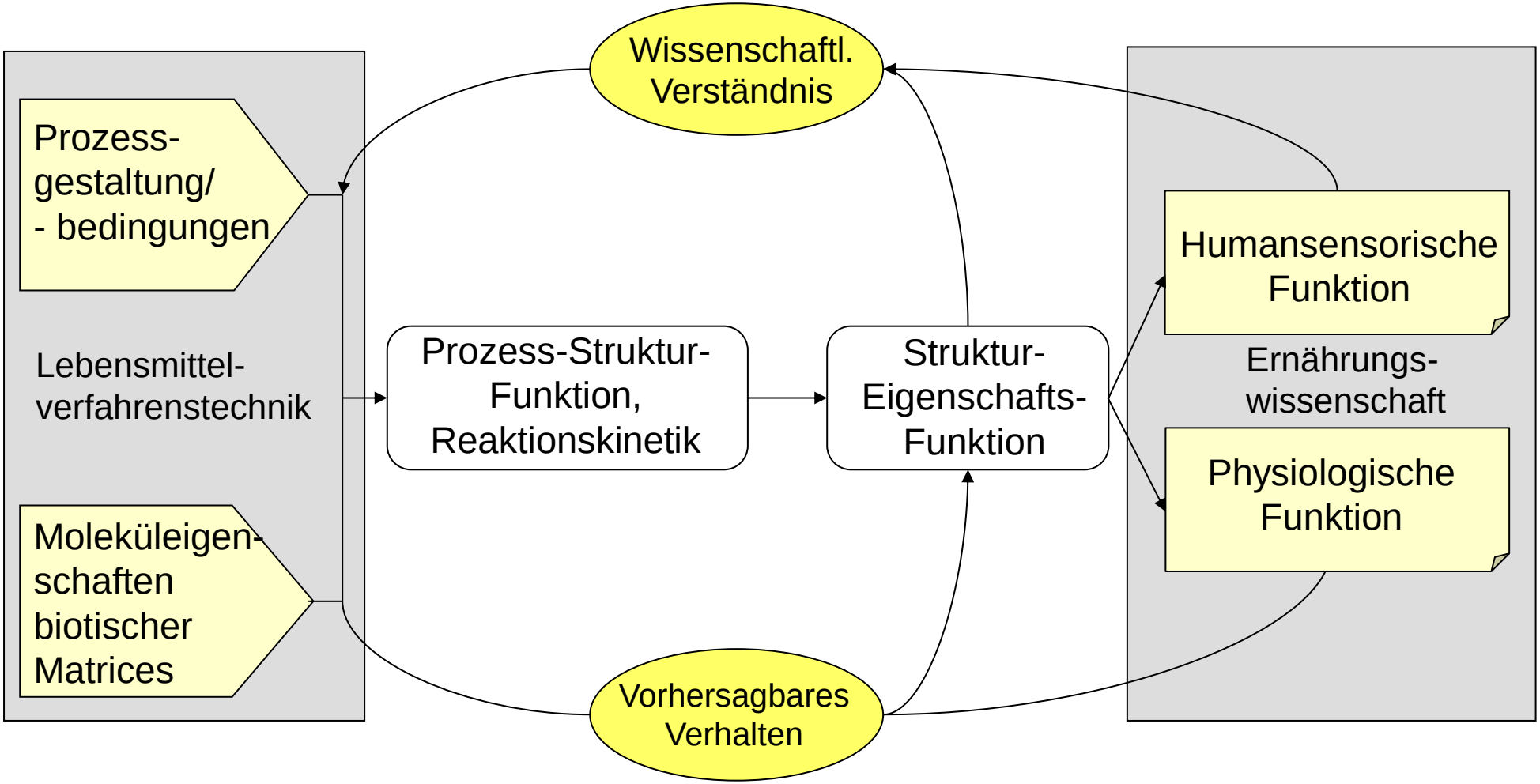
Target areas of the food and pharma industries in public health





Products with a positive effect on health and quality of life in the last quarter of life.

Arbeitsmethodik Life Science-Verfahrenstechnik
Verknüpfung von Substrat, Verfahrenstechnik und Analytik



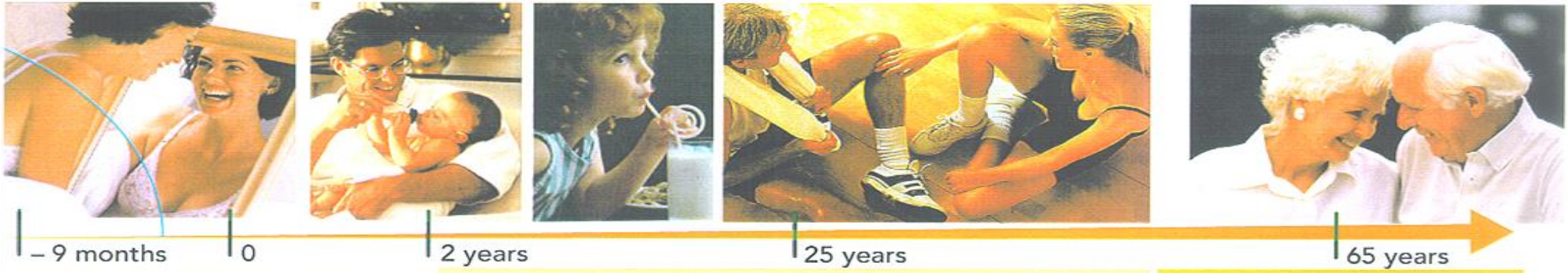
Molekulare Struktur; Interaktionspotenziale Funktionalitätsursachen → Prozesstechnische Stoffumwandlung → Biologischer Effekt

A non-dairy example: Healthy eggs concepts across age groups (1990ies)

Discover the new World of healthier eggs

belovo
6600 BASTOGNE
Tel. + 32 61/24.05.40
Fax + 32 61/24.05.41
E-mail : md@belovo.com

An egg is source of Life:
As an ingredient, it provides essential lipids and amino-acids to humans of all age.



Egg yolk phospholipids

ω6-PL-85

AA/DHA < 2.0

0,5 g ω6-PL-85 covers the amount of LC-PUFA present in 100 ml breast milk

Eggs with balanced fatty acid

Columbus

P/S = ω6/ω3 = 1/1

Human evolved for more than 4 million years on a balanced diet based on P/S and ω6/ω3 ratios of 1/1

Phosphatidylserine

ω3-PS-85

AA/DHA < 0.3

A source of highly bioavailable DHA and serine for the elderly

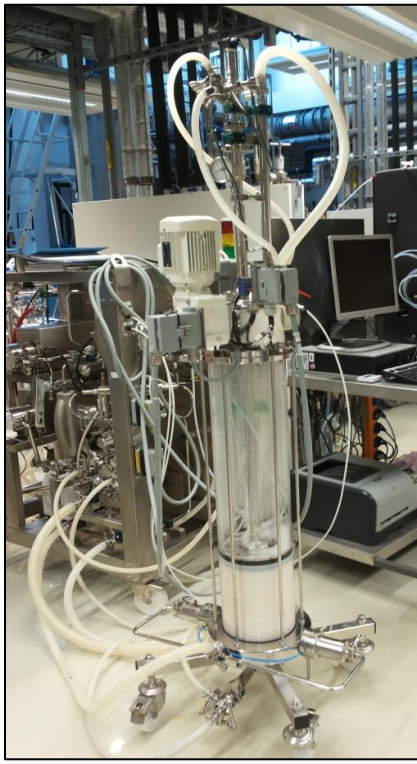
Belovo proposes a range of egg lipids and proteins whose nutritional properties cover specific needs of human at various stage of development and life time.

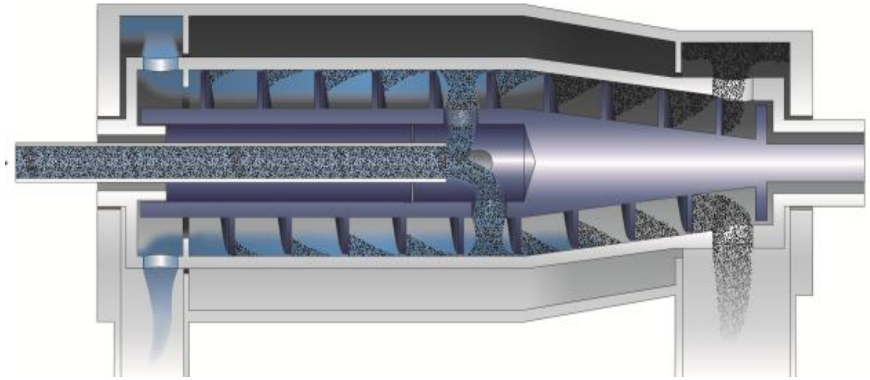
Target groups for personalized nutrition

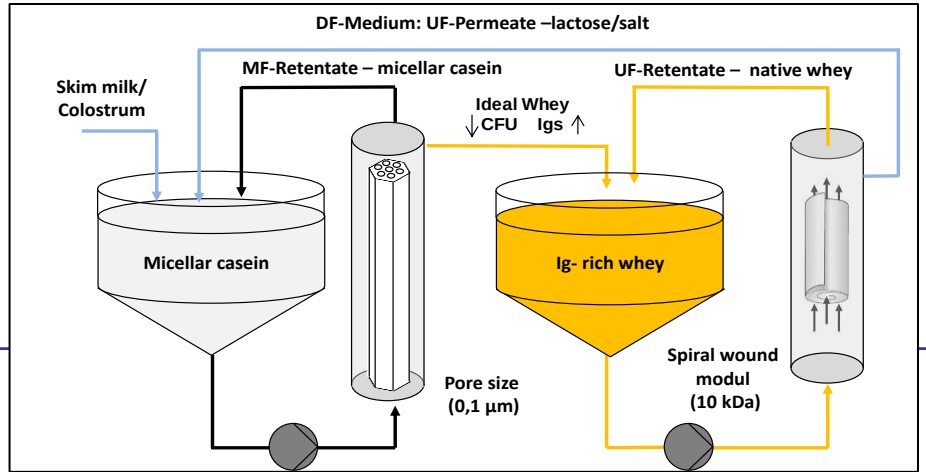
- Pregnant women
- Pre-term born infants
- Infants, Small children
- School children
- Sports people
- Genetically predisposed people (e.g. Phenylketonurie, PKU; Allergenicity)
- Reconvalescent patients
- Normal people (reducing the risk of developing certain diseases)
- Elderly people (Cognitive performance, Sarcopenia, Dysphagia)
- …

Methods for enrichment or isolation of single milk or whey fractions

- Classical coagulation/aggregation reactions
  - enzymatic
  - thermal
  - isoelectric
- Chromatography
- Ultrahigh pressure
- Centrifugation
- Membrane technologies
  - Mikrofiltration (0,1 – 2 µm)
  - Ultrafiltration (5 – 300 kDa)
  - Nanofiltration (200 – 5.000 Da)
- Combination methods/ Hybrid technologies







Method and process to obtain isolated α-La and microparticulated ß-Lg

WPI

↓

UF/Diafiltration
0,5-2,0% Protein

↓

Heat treatment
105°C/5 s; pH 7,5

↓

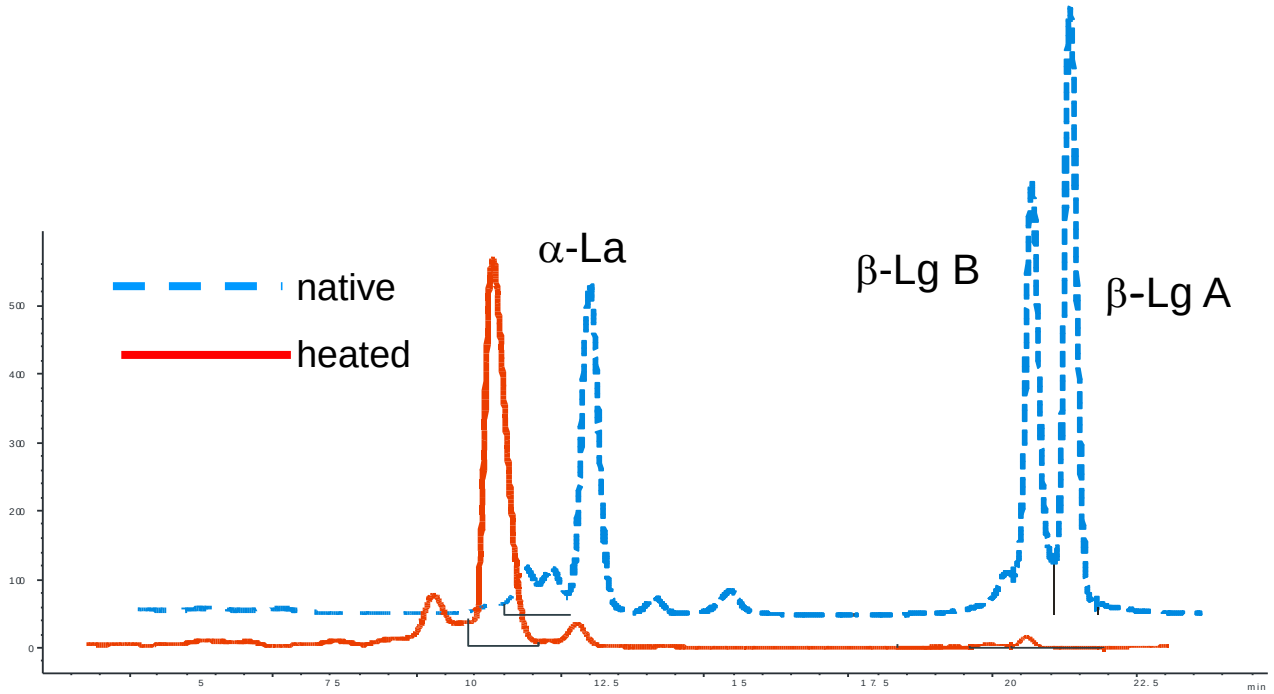
pH adjustment

↓

MF
0,1 µm

→ Retentate aggregated β-Lg

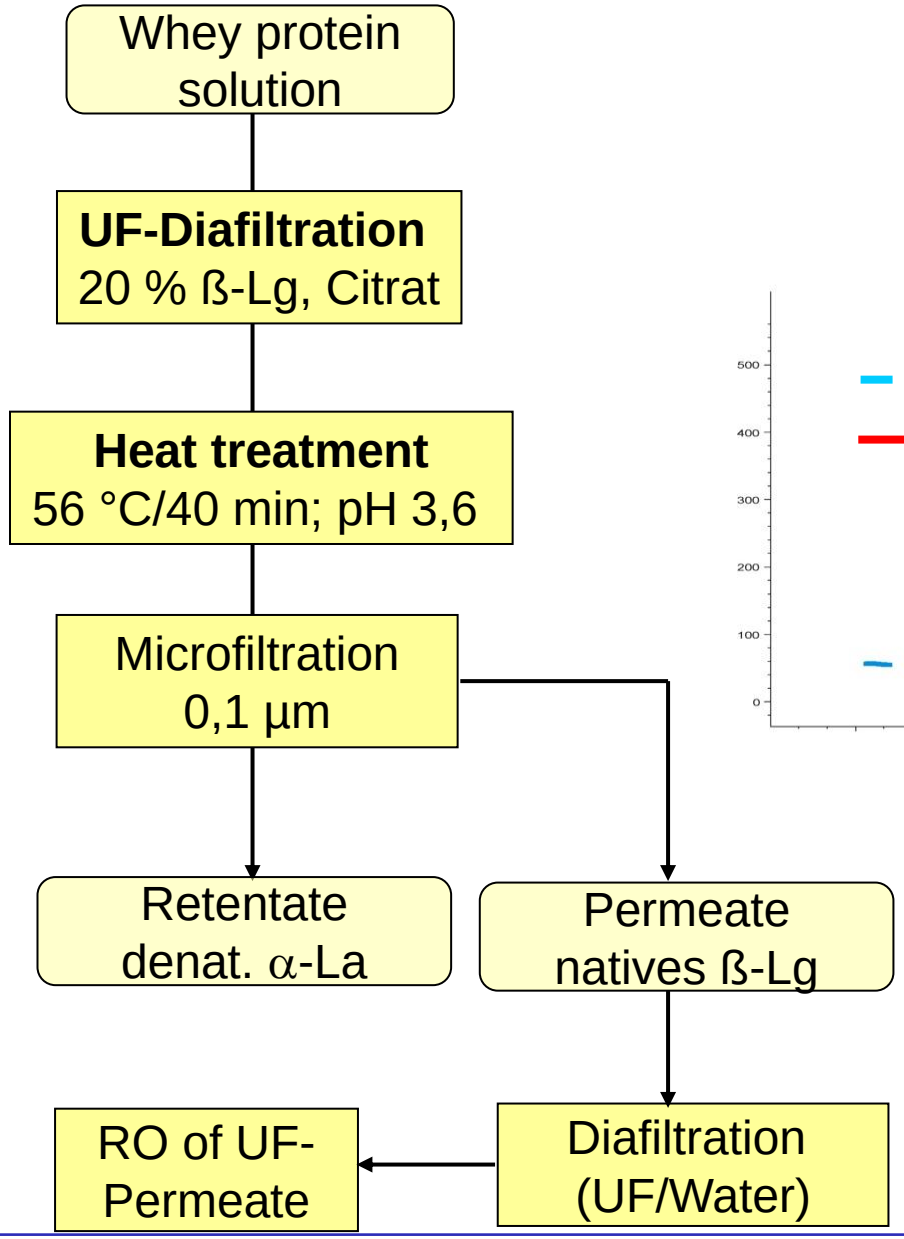
→ Permeate natives α-La

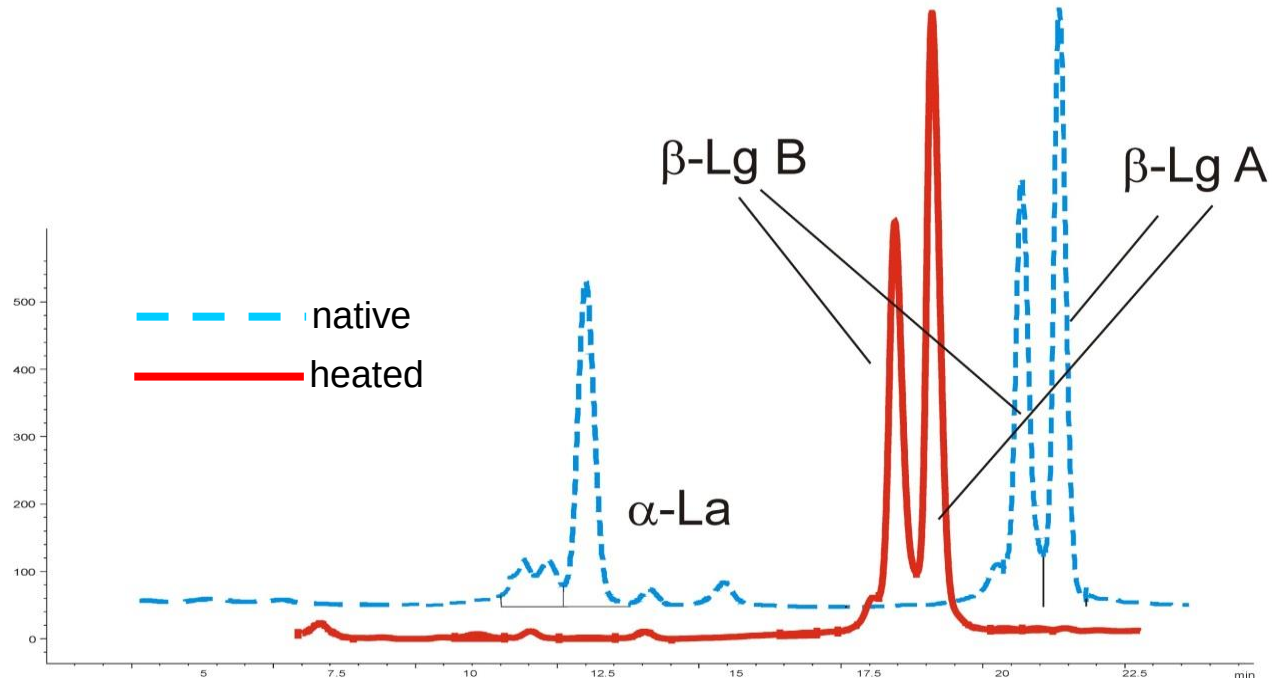


Potential medical applications of α-Lactalbumin

- Induction of apoptose of tumor cells („HAMLET/BAMLET“)
- Treatment of skin papilloma
- Increased serotonin release and induction of anxiolytic effects; Good mood under stress
- Ca-/Zn-/Fe-binding properties

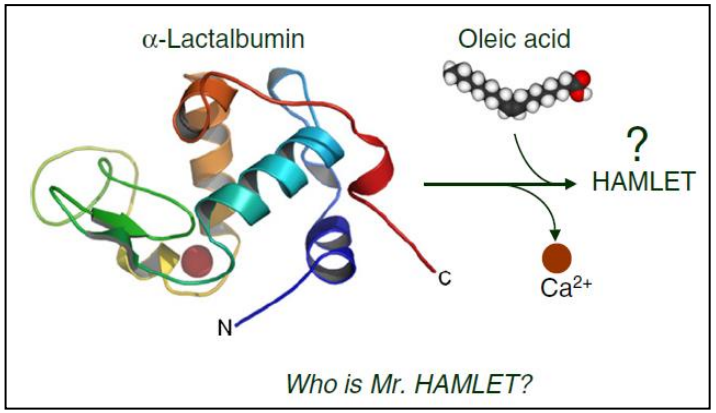
Method and process to obtain isolated ß-Lg and microparticulated a-La

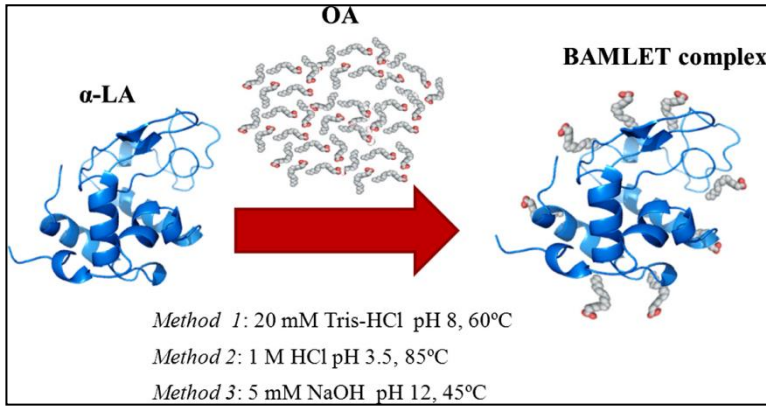


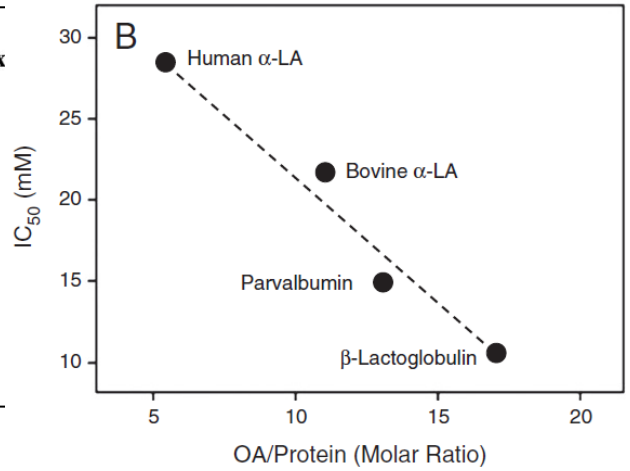


Effect of HAMLET/BAMLET on human skin papillomas

HAMLET/BAMLET“: **H**uman/**B**ovine **A**lpha-Lactalbumin **M**ade **LE**thal to **T**umor cells







Mechanism: Protein acts as emulsifier to expose oleic acid to the cell membrane, where it disrupts or disturbs the cell wall structure of the tumor cell.

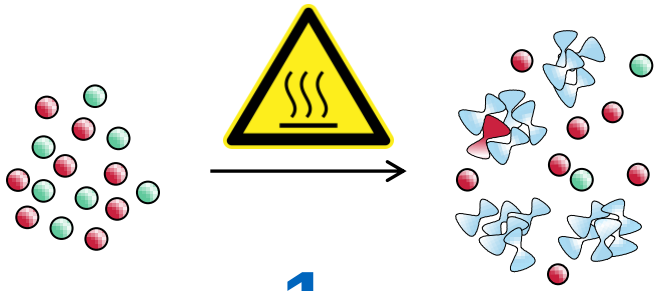
Before treatment **After 3 wks of treatment** **Two-year follow up**

Gustafsson et al (2005). J. Nutrition 135, 1299-1303
Delgado et al. (2015): FEBS Open Bio 5, 397-404
Hoque et al. (2015) Biochimica Biophysica Acta 1850, 1729–39

β-Lactoglobulin: Process for removal from whey for the manufacture of humanized infant food products

Reason: β-Lg does not exist in human milk. It is considered the main allergen in milk.

Selective thermal aggregation → **Centrifugal separation** → **Chromatographic purification/ isolation**



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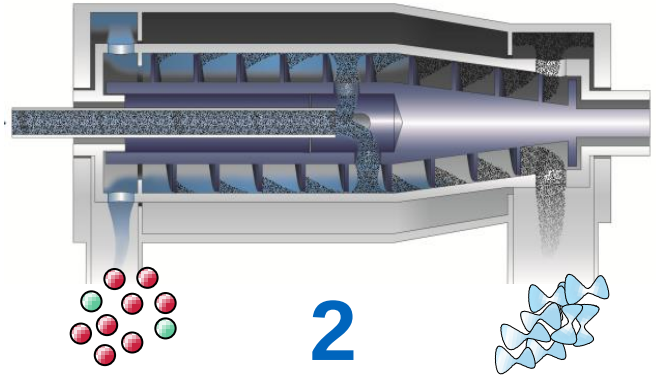

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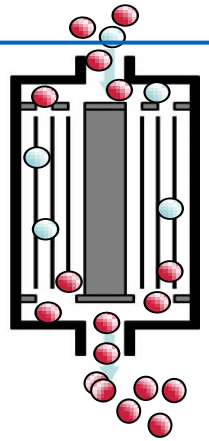

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85 °C/ 15 s/180 L·h^{-1}

2500 g/10 rpm/15 L·h^{-1}

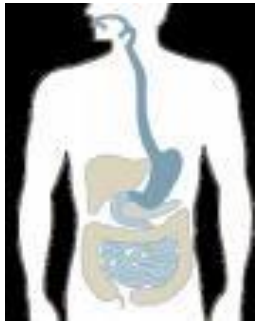
pH 7/50 °C/10 CV·min^{-1}

Yield (α -La) = 89%
Removal (β-Lg) = 95%

Yield (α -La) = 83%
Removal (β-Lg) = 98%

Examples of some natural bioactive substances in milk and their reported functions

Some bioactive peptides/proteins native to milk and colostrum



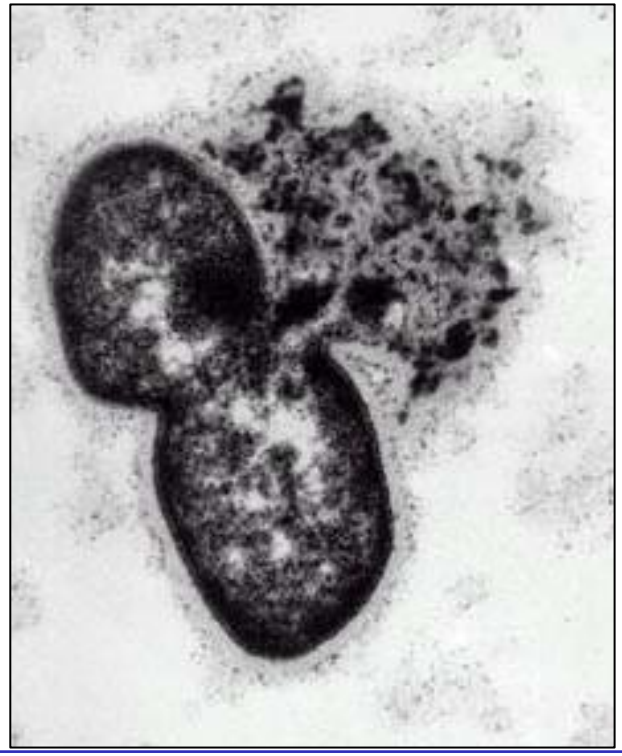
Intestinal or Plasma-Enzymes

Microbial enzymes

- Casein macropeptide
- Lactoferrin
- Immunoglobulins
- Growth factors
- Insulin
- Cytokines
- Milk fat globule membrane proteins
- β-Casomorphins
- Somatostatin
- Prolactin
- Delta-sleep peptides

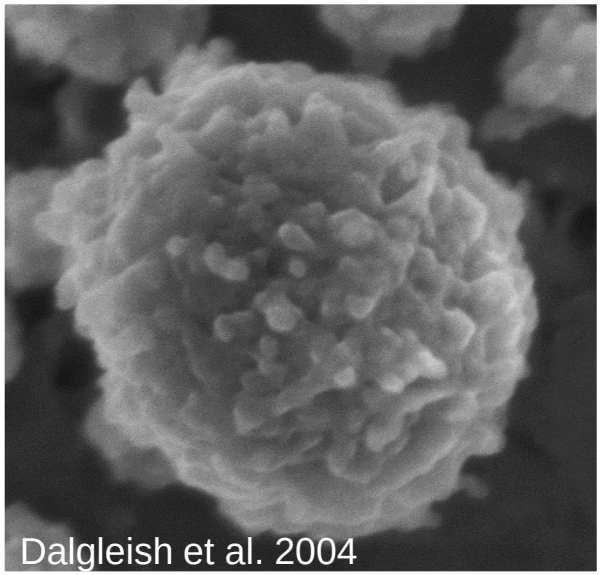
Functions

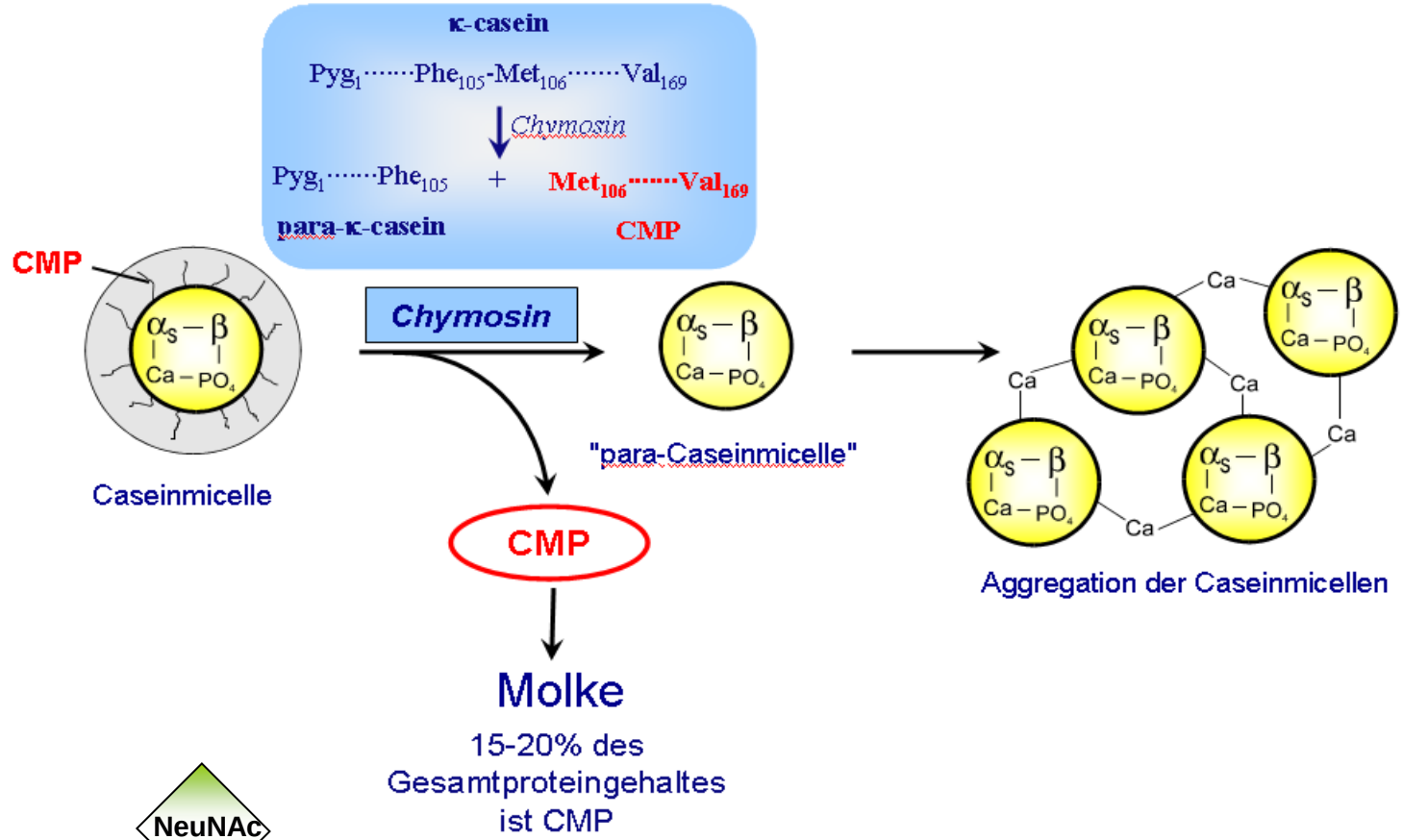
- Mineral binding
- Hypertensive
- Immuno peptides
- Antimicrobial
- Anti-inflammatory



The big question: Are the native bioactive peptides in milk enough to benefit the consumer?

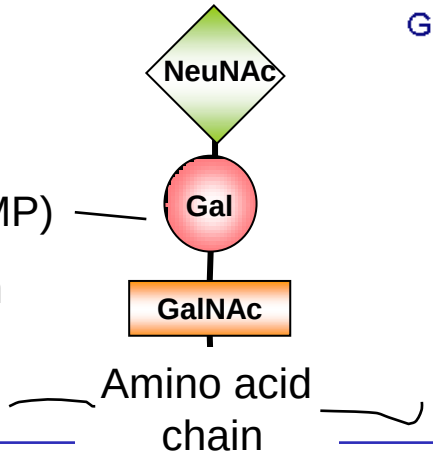
Release of caseinomacropeptide during renneting





CMP properties

- 50 % of CMP unglycosylated
- 50 % of CMP glycosylated (GMP)
- Very heat stable in comparison to whey proteins



- High amount of *essential* amino acids
- High amount of *branched-chain* amino acids
- Absence of all aromatic amino acids (Phe, Tyr, Trp)

Physiological and biological properties of CMP

Phenylketonuria (PKU)

- Metabolic disease: Phe can not be metabolised
- CMP contains no Phe ➔ tolerable natural protein source

➡ Enrichment of CMP in dietetic products

Gut microbiota

- CMP acts as prebiotic substance promoting Bifidobacteria
- Defence against pathogens

Other Properties

- CMP acts as anticariogenic substance
- Anti-inflammatory properties
- Positively supporting treatments of certain liver diseases

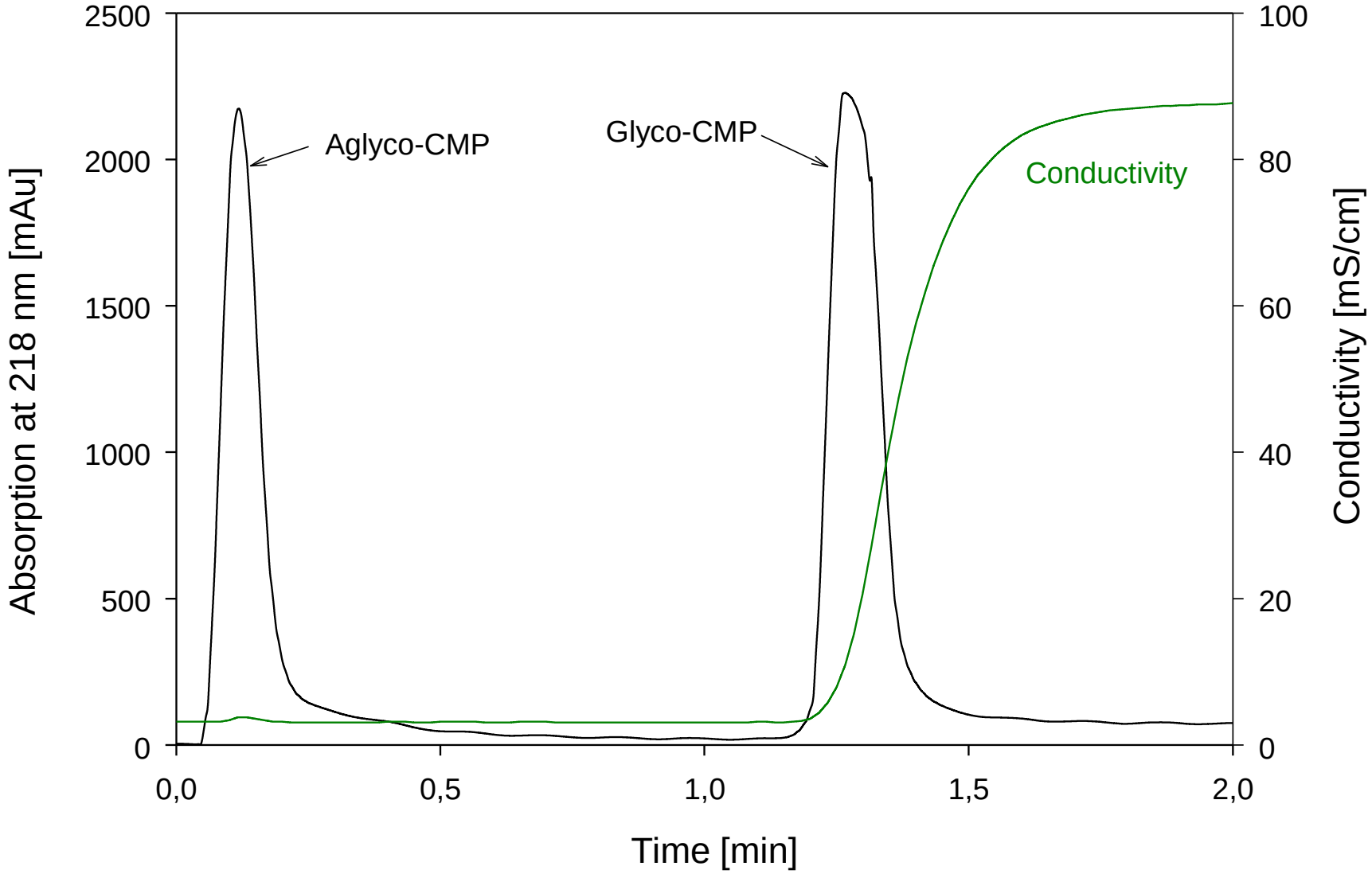
Hyperthreoninemia

- High content of Threonin ➔ Amino acid imbalance in infant milk formulas

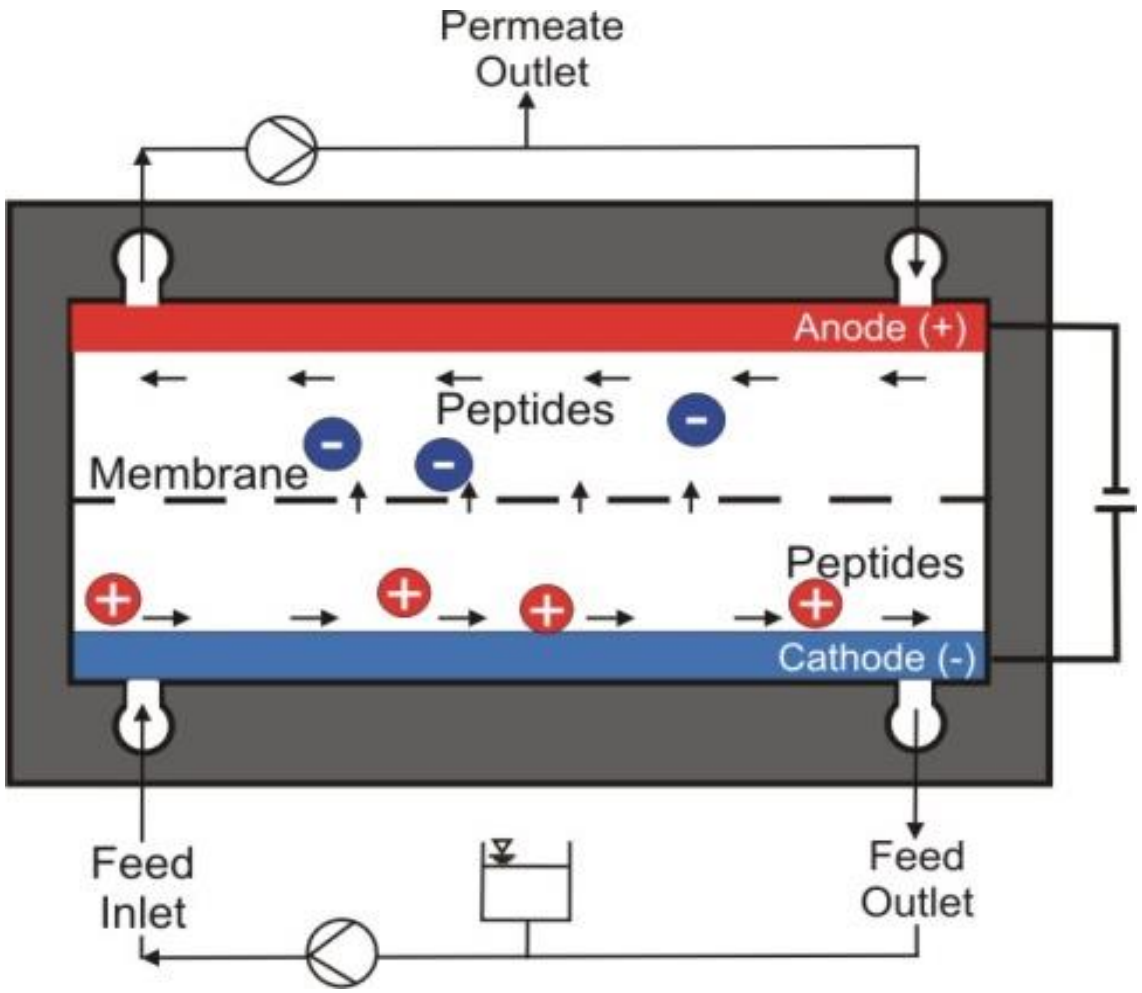
➡ Absence of CMP in infant milk formulas

Recio et al. (2009): Glycosylated dairy components- Their roles in nature … and functionality in dairy products. In. Dairy-based ingredients, CRC Press

Fractionation of CMP/GMP by IEX-Chromatography



Separation principle of Cross-Flow Electro membrane filtration (CFEMF)



Variables:

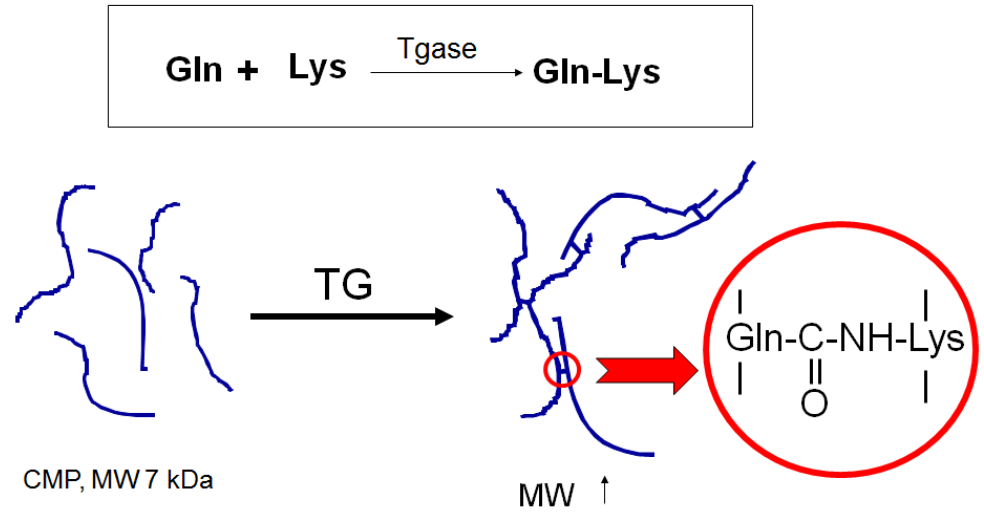
- Position of the electrodes
- Voltage
- pH
- Temperature
- Crossflow/pressure conditions

Diss. Holder, Univ. of Hohenheim, 2014

18

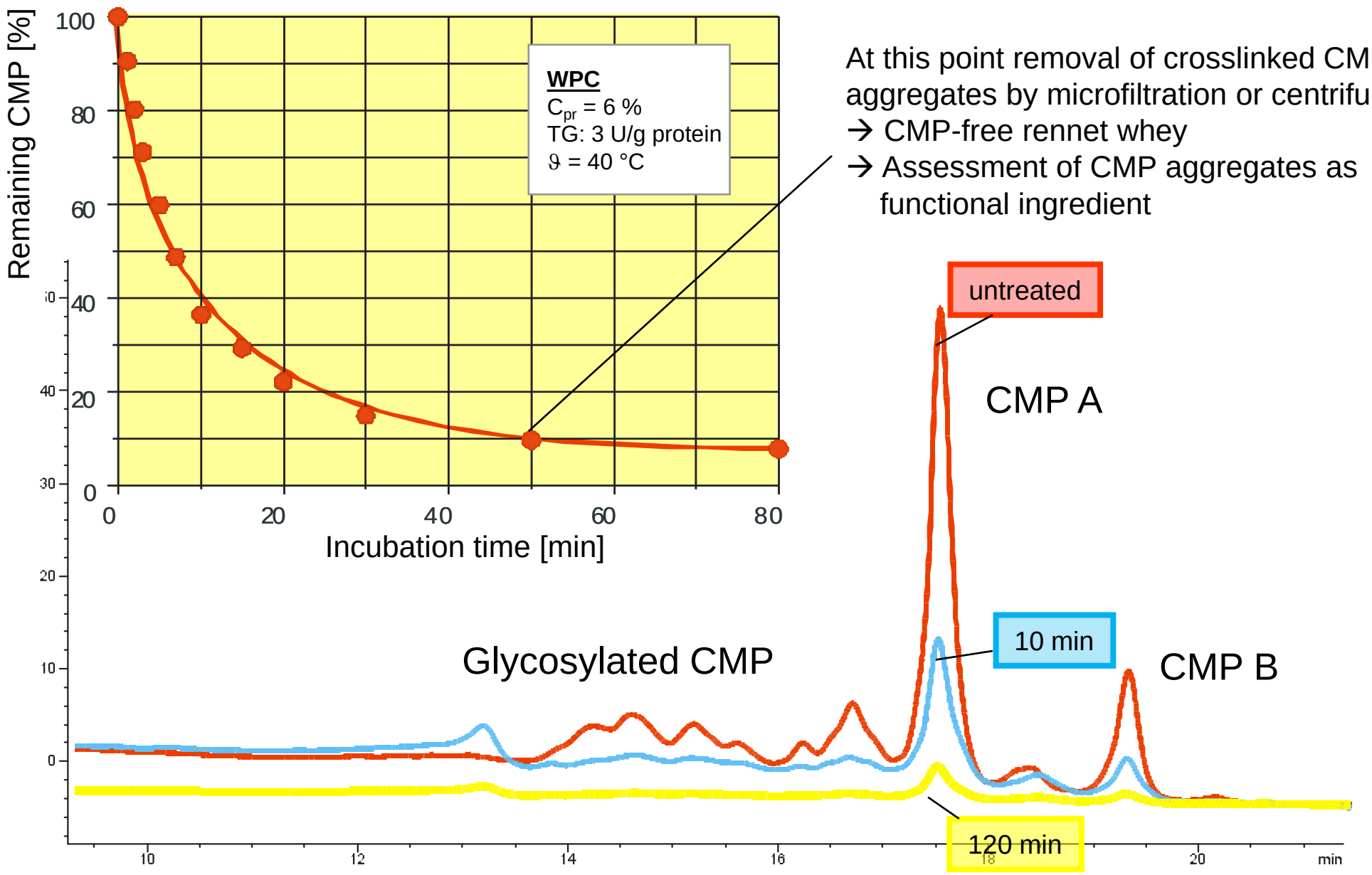
Enzymatic pre-treatment and fractionation of CMP from whey proteins

- Nominal MW 7 kDa
  (too close to other whey proteins to be separated by size)
- No aromatic amino acids
- High level of the amino acid threonin
- Contains Glutamin and Lysin → Crosslinking by enzyme Transglutaminase possible



Met - Ala - Ile - Pro - Pro - Lys - Lys - Asn - Gln - Asp - Lys - Thr - Glu - Ile - Pro - Thr - Ile - Asn - Thr - Ile - Ala - Ser - Gly - Glu - Pro - Thr - Ser - Thr - Pro - Thr - Ile - Glu - Ala - Val - Glu - Ser - Thr - Val - Ala - Thr - Leu - Glu - Ala - Ser - Pro - Glu - Val - Ile - Glu - Ser - Pro - Pro - Glu - Ile - Asn - Thr - Val - Gln - Val - Thr - Ser - Thr - Ala - Val - OH

Crosslinking of CMP by the enzyme Transglutaminase

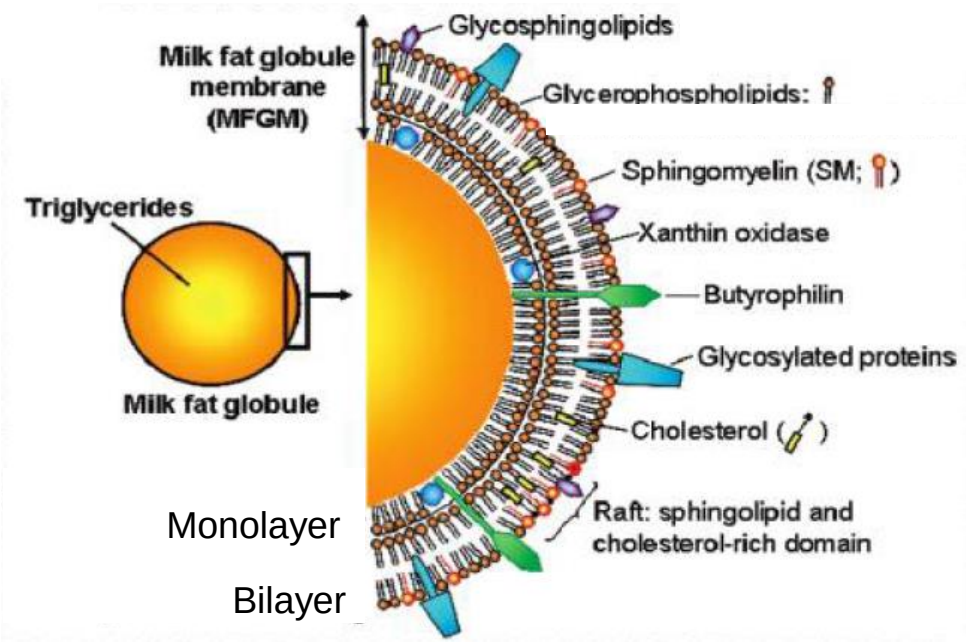


At this point removal of crosslinked CMP-aggregates by microfiltration or centrifugation

→ CMP-free rennet whey

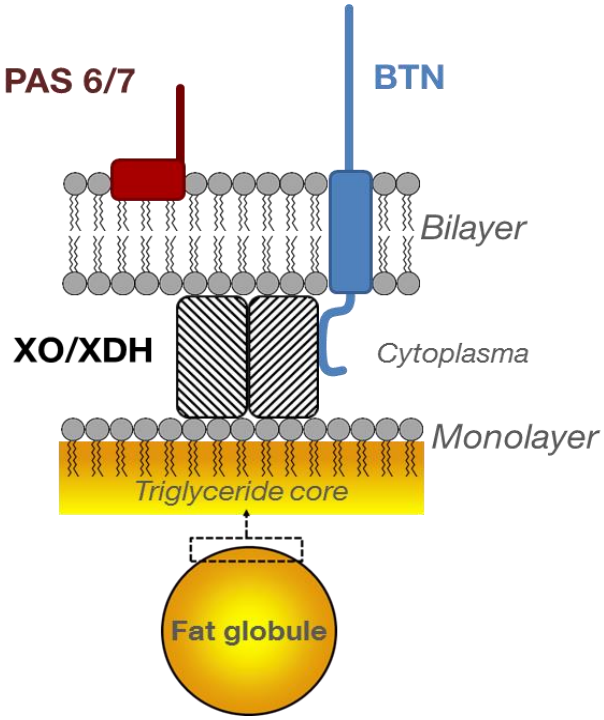
→ Assessment of CMP aggregates as functional ingredient

Origin, structure and composition of the Milk Fat Globule Membrane (MFGM)



Components	MFGM-DM
Proteins	70%
Phospholipids	25%

(Walstra et al., 2006)



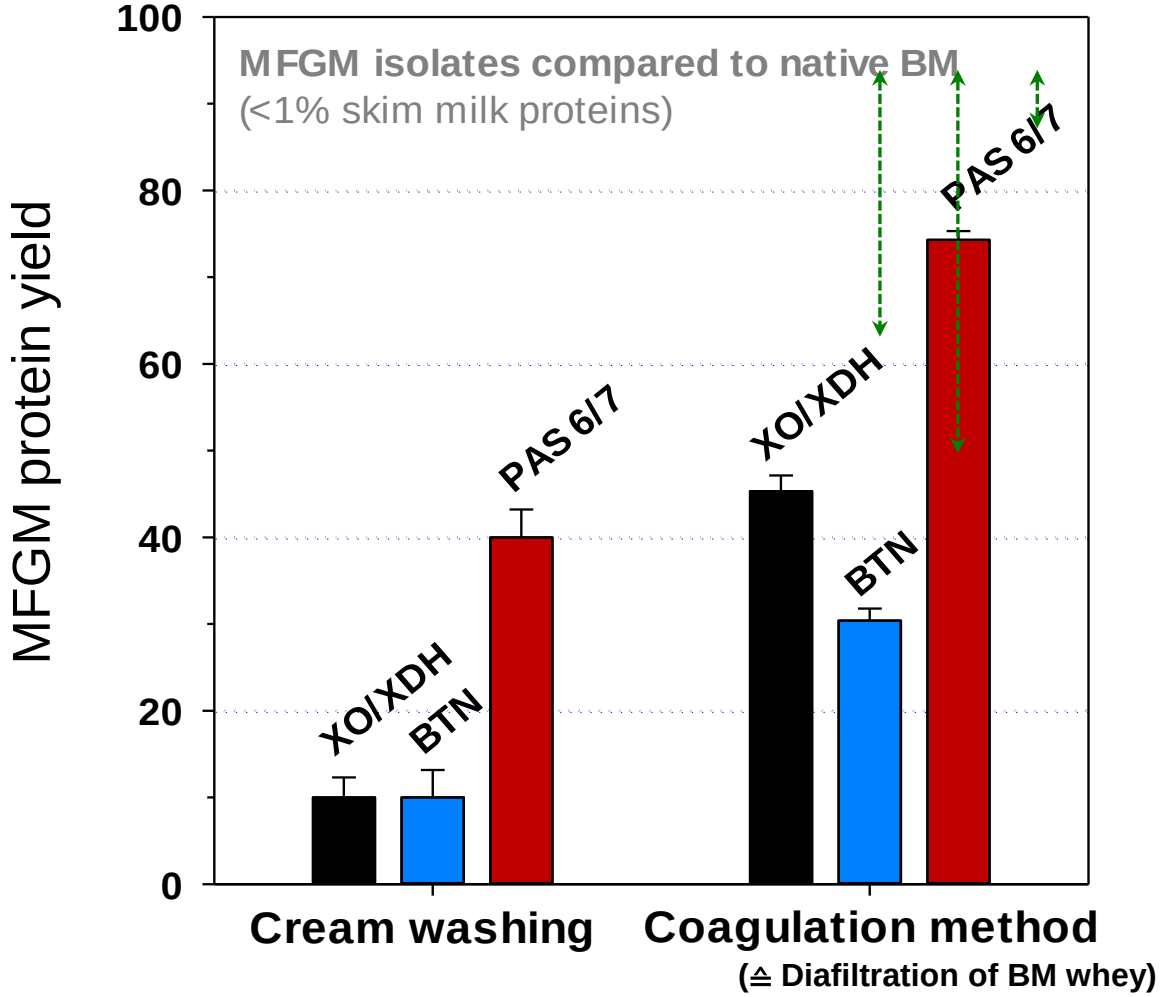
(Lopez et al., 2008)

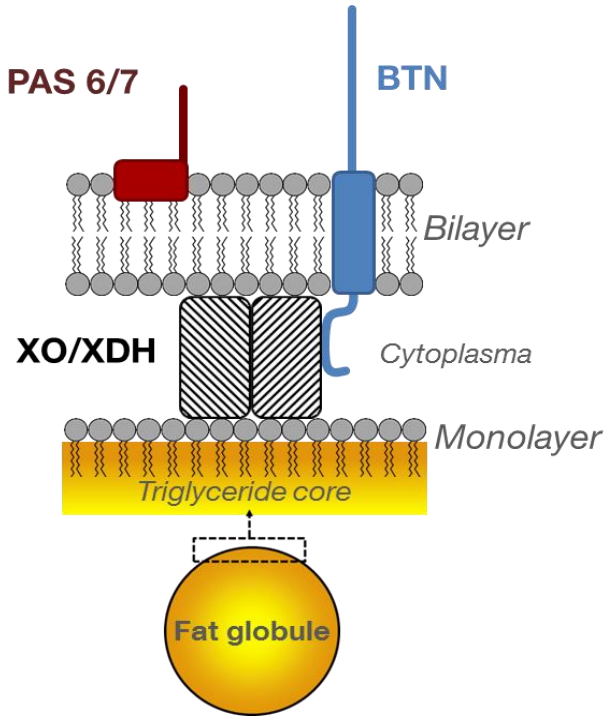
J. Dairy Sci. 97:2017–2026
http://dx.doi.org/10.3168/jds.2013-7563

Modulation of immune function by milk fat globule membrane isolates

R. Zanabria,* A. M. Tellez,*[1] M. Griffiths,* S. Sharif,† and M. Corredig*[2]

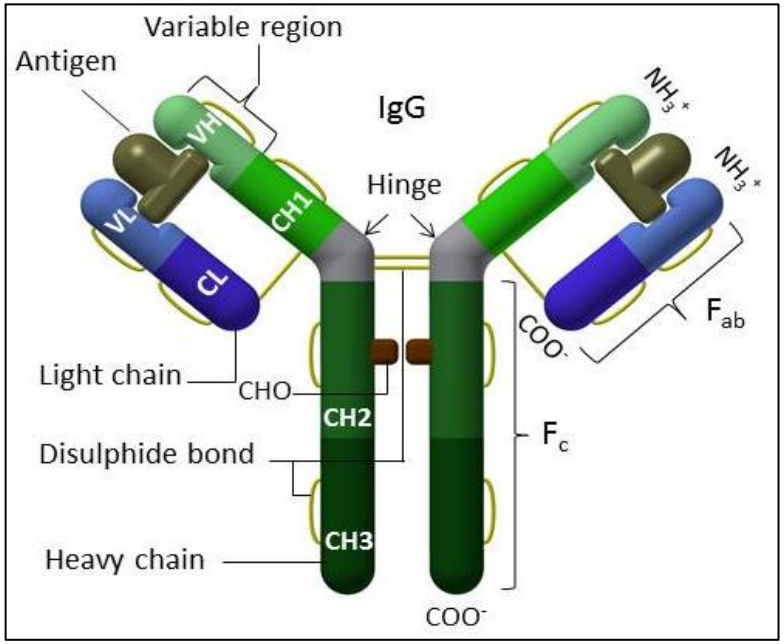
Increase of MFGM protein yield by optimizing the isolation method: Evaluation of washing method and coagulation method

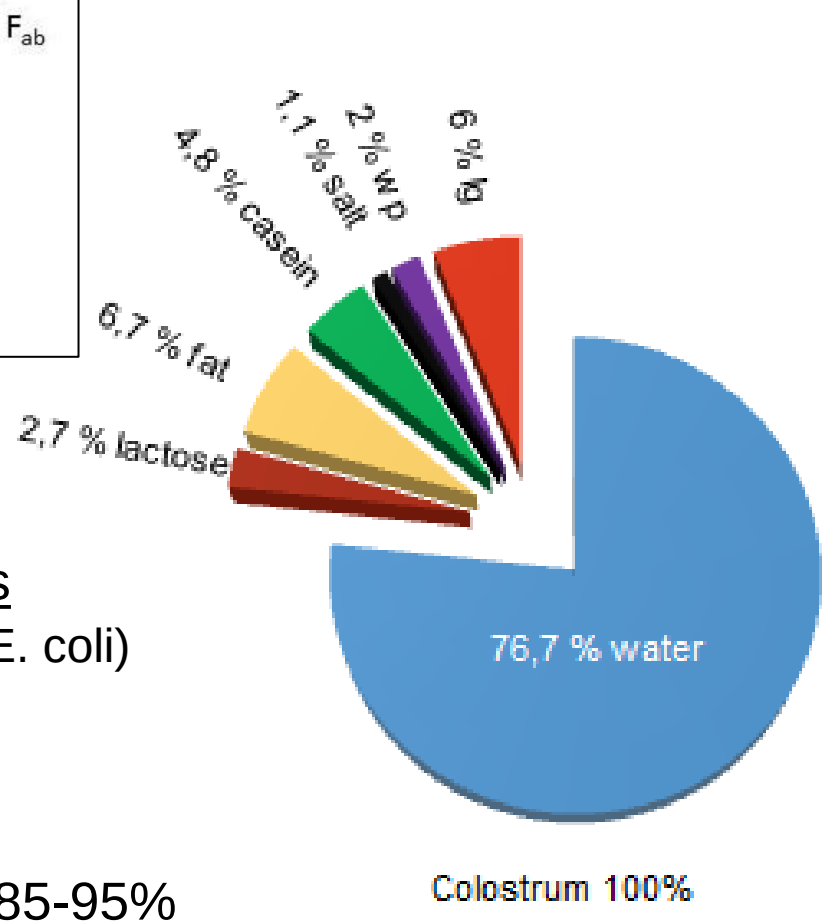


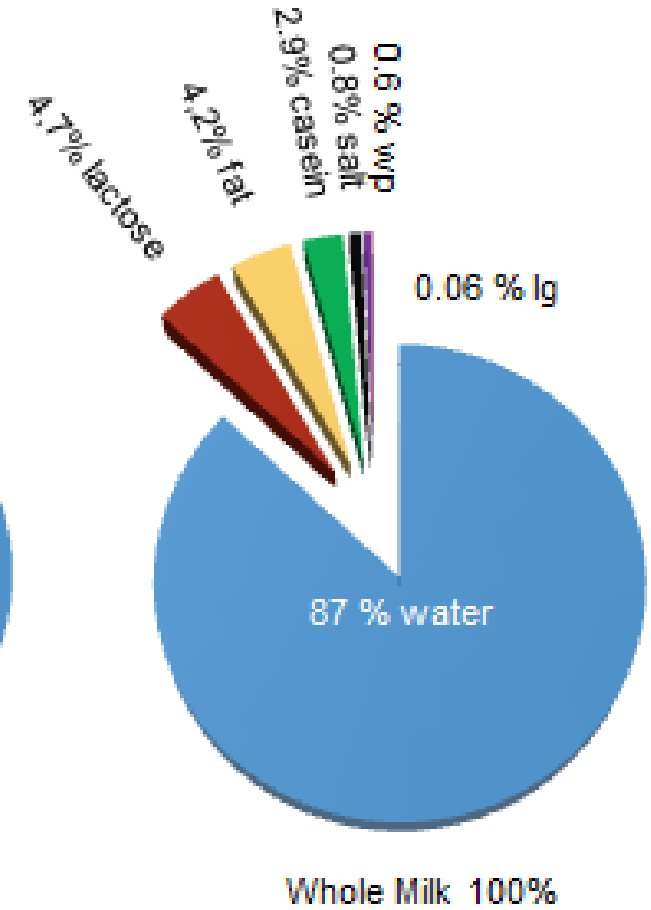


- Significantly higher protein yield using the coagulation method by avoiding losses of the outer double layer.

Immunoglobulins in colostrum and milk





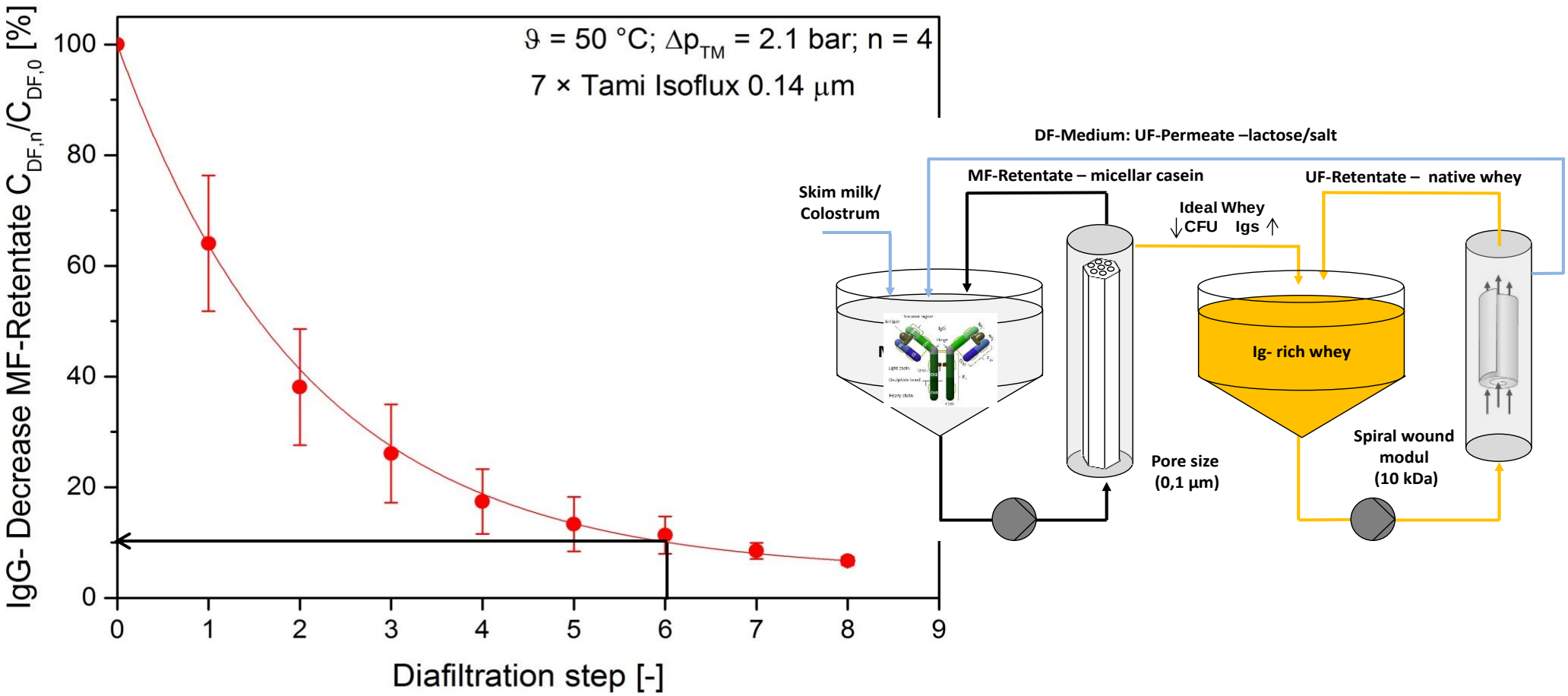


<u>Reported medical treatments</u>

- ETEC (14 strains enterotox. E. coli)
- HIV-1 gp140
- Clostridium difficile
- Influenza virus

with success rates between 85-95%

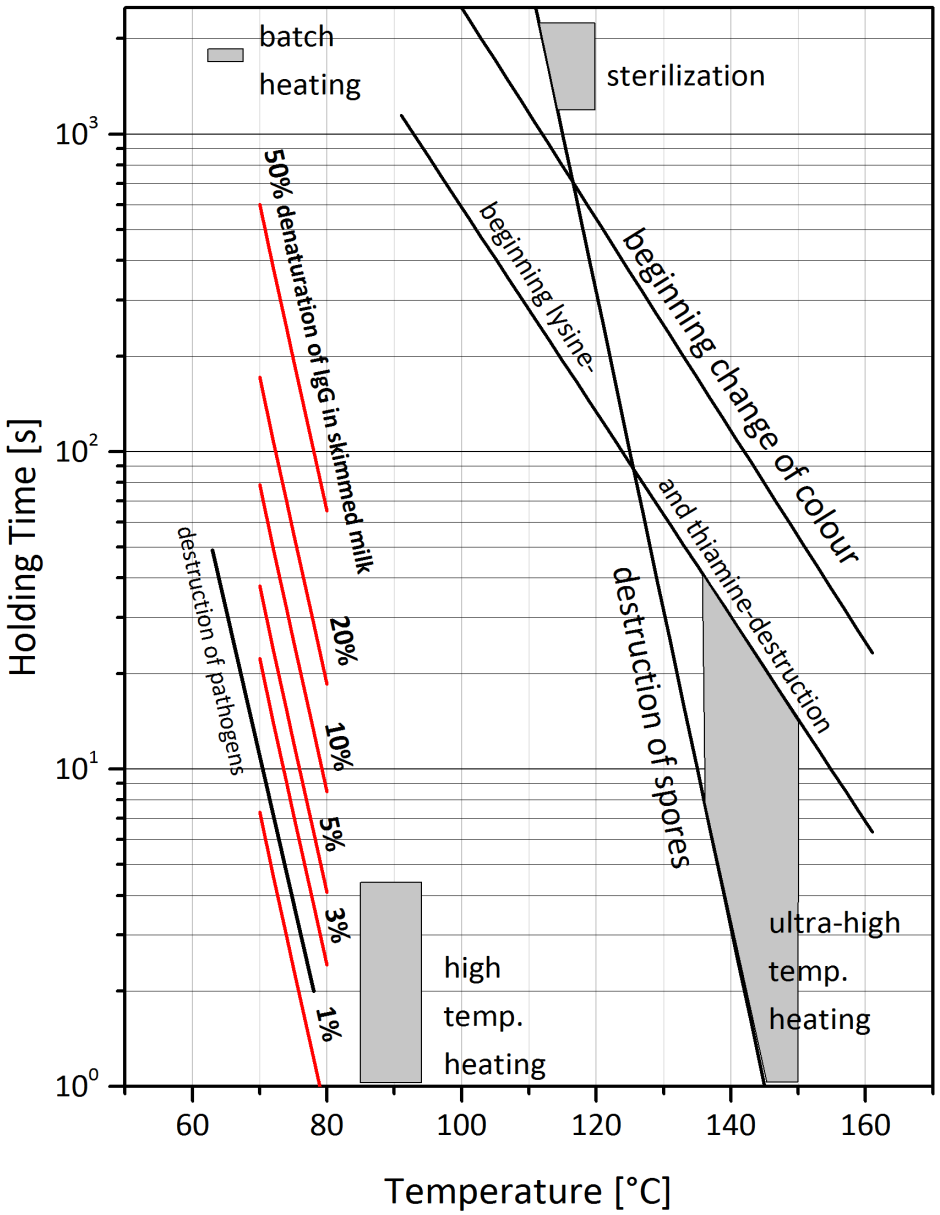
Fractionation of immunoglobulins from milk

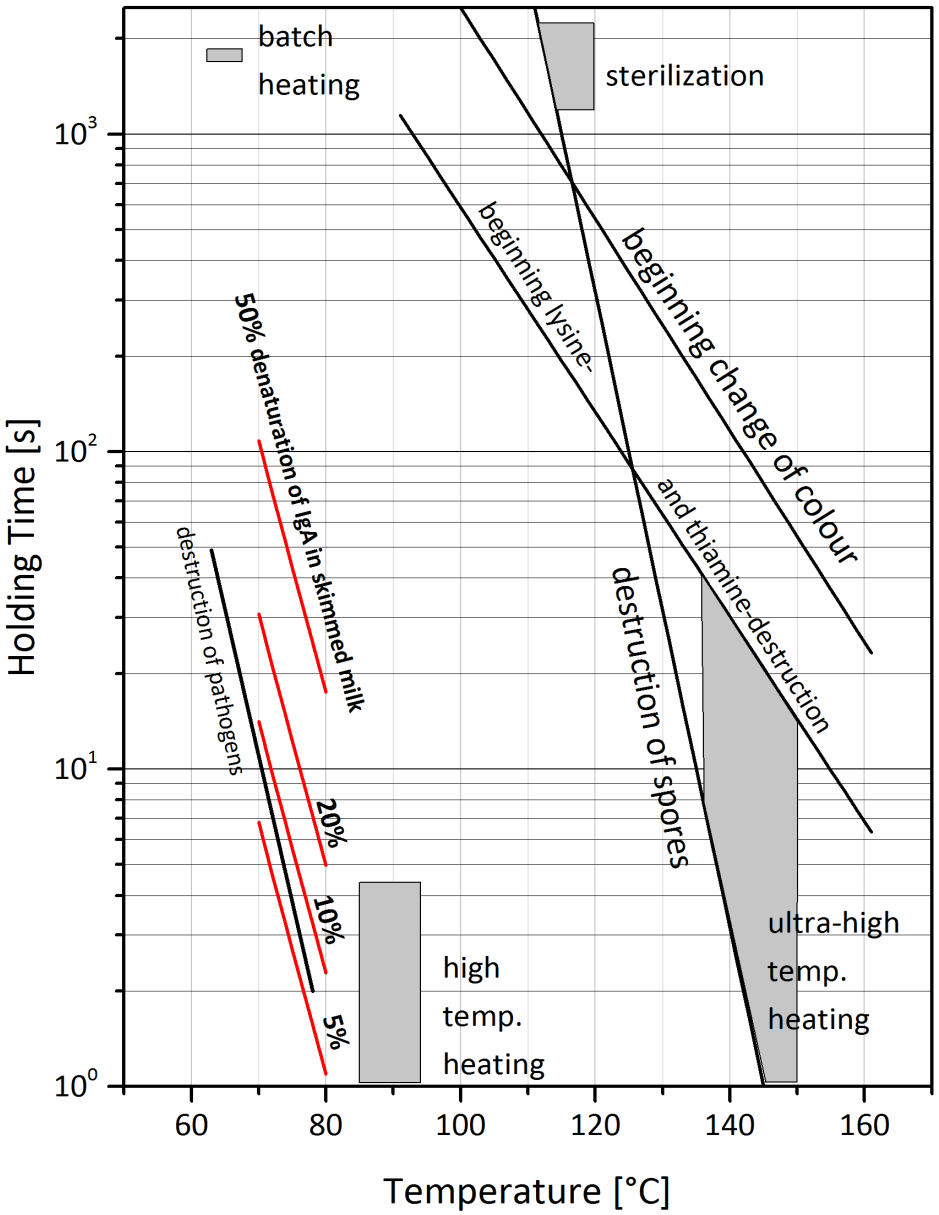


- After 6 diafiltration steps IgG is below 10% on the MF-retentate side

→ Combination of MF and UF operated in diafiltration mode opens the opportunity to produce Ig-rich WPC at industrial scale

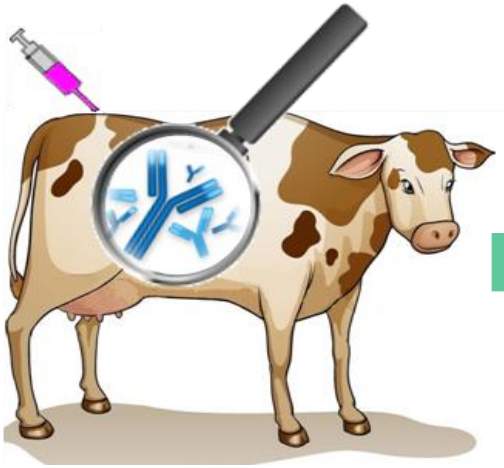
Heat sensitivity of immunoglobulins: Iso-effect lines for IgG and IgA in milk



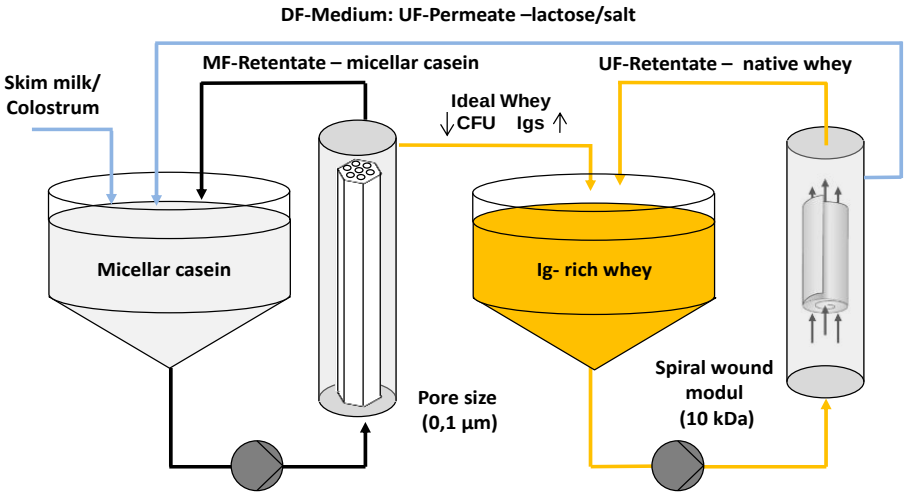


Immunoglobulins from vaccinated cows: Basic Idea

→ Need for alternative methods for the treatment of antibiotic resistant, pathogenic organisms



Immunizing the cow to obtain high titers against hospital germs



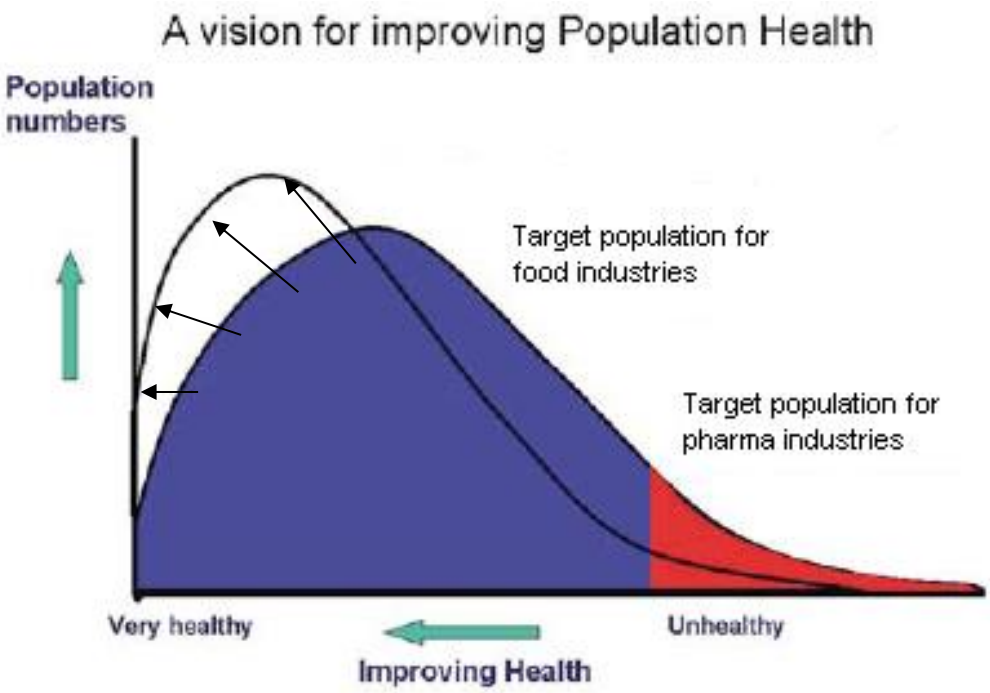
Membrane fractionation (MF/DF)

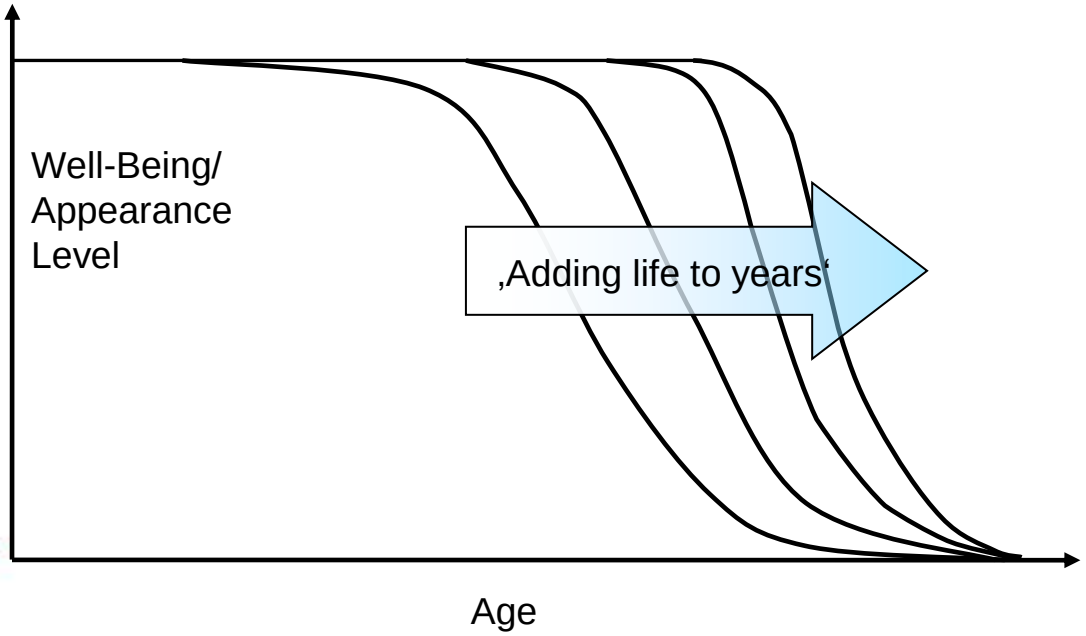
Mixed mode chromatography

Use for the treatment of human health issues resulting from MRS

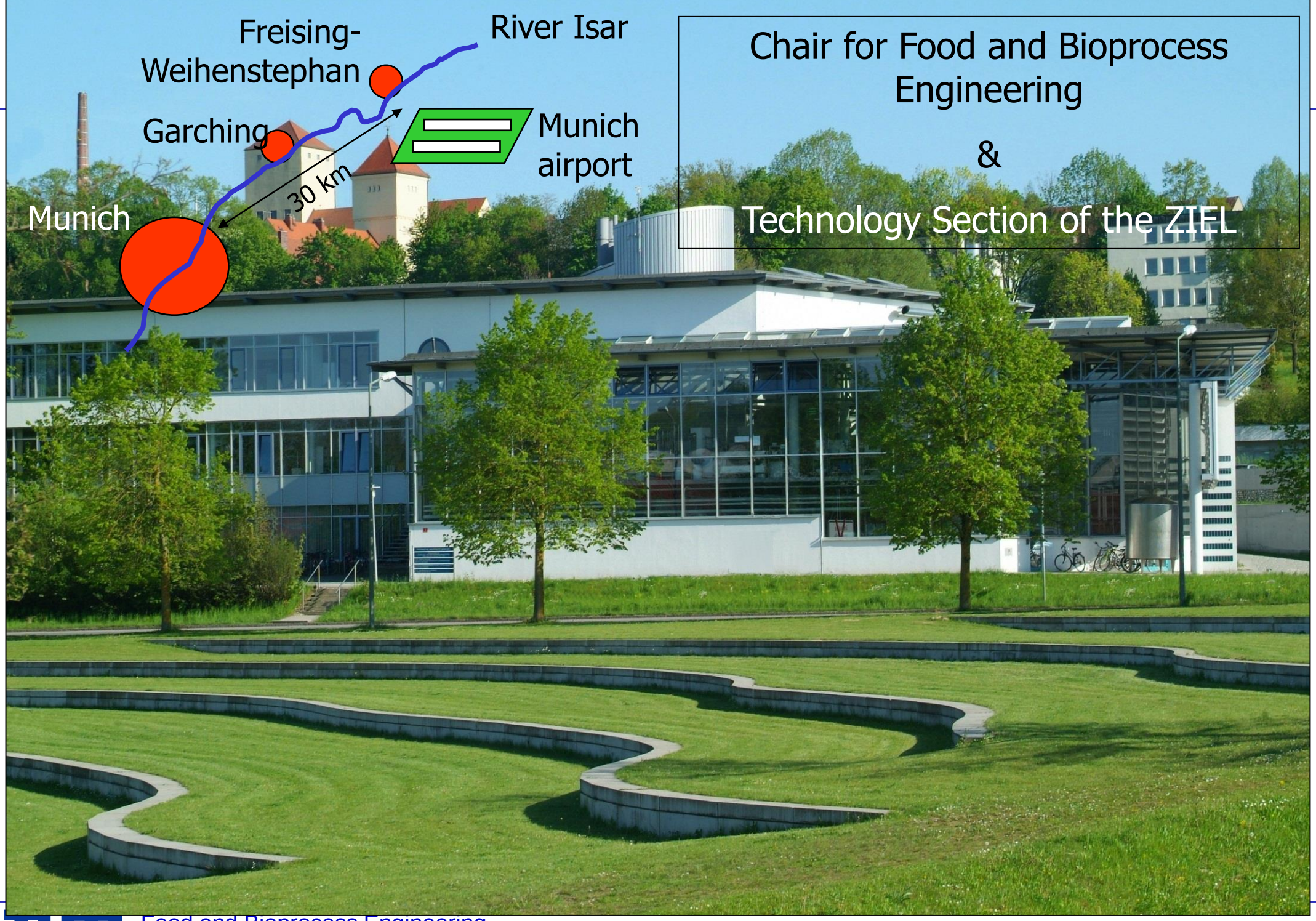
Vision for foods for the developed world (EU strategy)

Target areas of the food and pharma industries in public health





Products with a positive effect on health and quality of life in the last quarter of life.



Chair for Food and Bioprocess Engineering

&

Technology Section of the ZIEL

Conclusions

- Milk is a healthy juice and a good base for new product concepts also outside of conventional dairy product segments
- Basic insights into nutritional and physiological effects are there and appear to be reliable.
- Screening methods for more indepth assessment of physiological effects are established
- Controlled studies, preferably human clinical studies, should be conducted following the rules and principles of „Good Clinical Study Practice“
- Nutritional supplements and product concepts for special target groups are extendable market segments, even outside the food sector.
- So, what is missing? → Prospective, double blind, controlled in vivo studies?
- Who wants to go this way? → Positioning in the market place in/out of food segment?

Categories from conventional foods to functional foods and prescription drugs

	Food				Pharmaceuticals	
	Conventional Foods	Functional Foods				
Category	Conventional food	Natural & Organic	Free-from & Enriched	Techno-functional and nutritional supplements	OTC[a]	Prescription[a]
Example	Conventional foods and drinks, snacks	Unprocessed fruits, vegetables, meat, organic dairy	Lactose-free, gluten-free, added vitamins, added minerals, high-protein, low-fat, contains probiotics	Phytostanols/sterols, hydroxypropyl methylcellulose, DHA, CLA, sports nutrition	Painkillers, cough relief, stomach protectors	Antibiotics, antidepressants, biologics
Authorised claims	Health Claims				Disease claims	
Claims made	Mainly taste	Naturalness, lack of processing, sustainability	Removal of negative substances, addition of beneficial substances	Scientific support, innovation, health benefits	Disease claims (softer)	Disease claims (stronger)
Science & technology support	Less → More					
Regulation	Less stringent → More stringent					

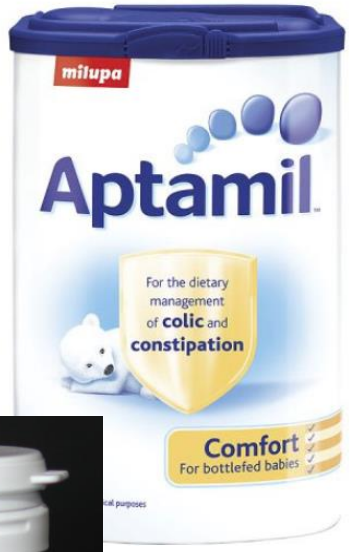
Frei verkäufliche Rezeptpflichtige Arzneimittel

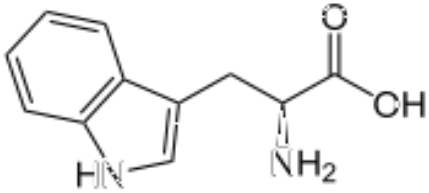
(Calvo et. al. 2016, Development of novel functional ingredients: … Trends in Food Sci.&Technol. 54, 197-203

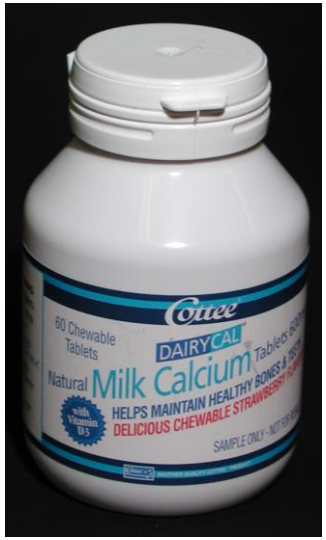
Zielprodukte für Fraktionen von Molkenproteinen

Abreicherung von β-Lactoglobulin für Babynahrung:

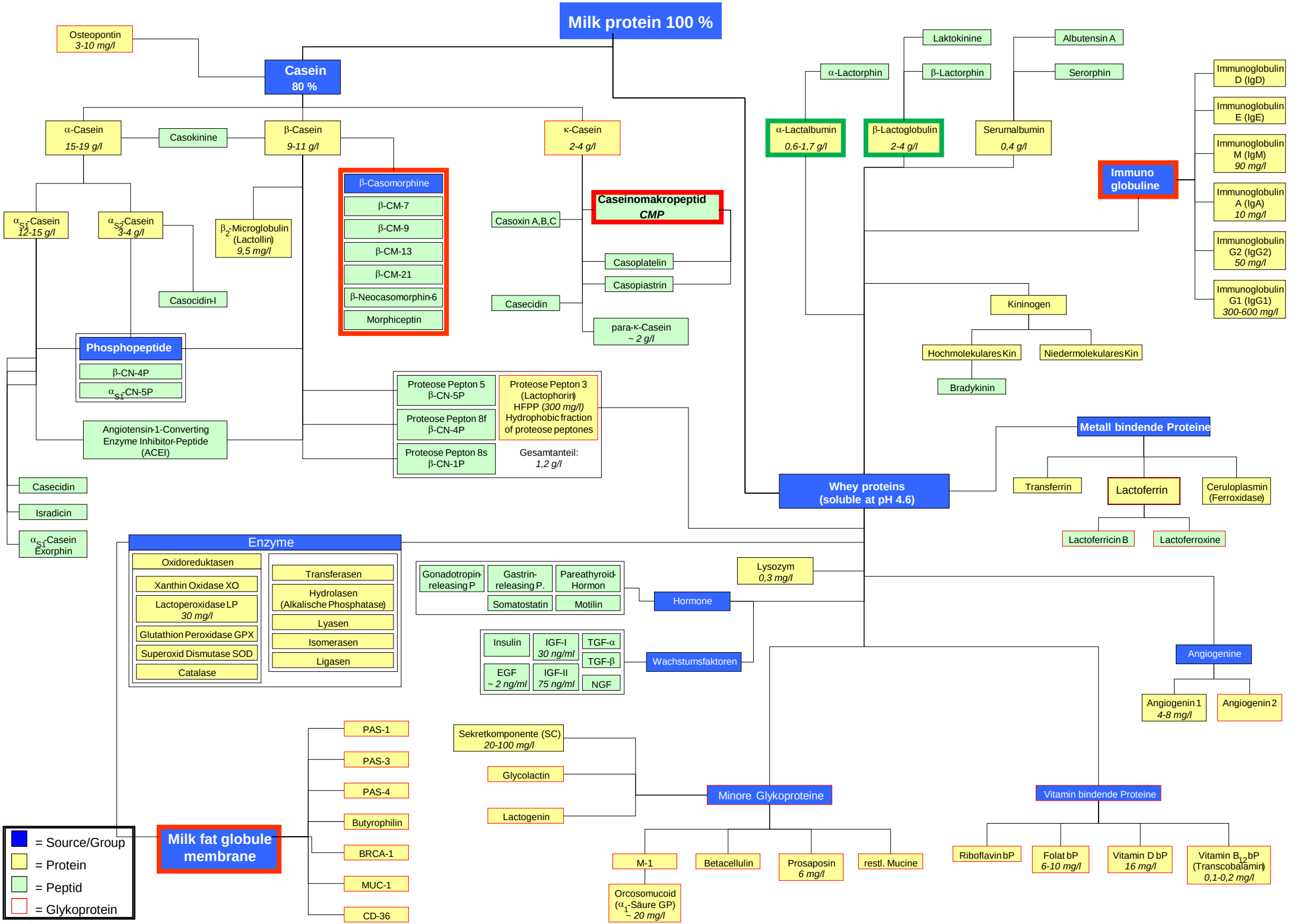
An Humanmilch angenäherte Protein-zusammensetzung mit erhöhtem Anteil essentieller Aminosäuren bei gleichem kalorischen Wert







Isolierung von Lactoferrin und anderen minoren Proteinen als *Functional Food* oder *Food for Special (Medical) Purposes*



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