Dairy Australia/NCDE Webinar

Fermented milk as functional food

21st June 2016

Cardiff Metropolitan University
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Milk & Fermented Dairy Products

Ara Kanekanian PhD, FIFST

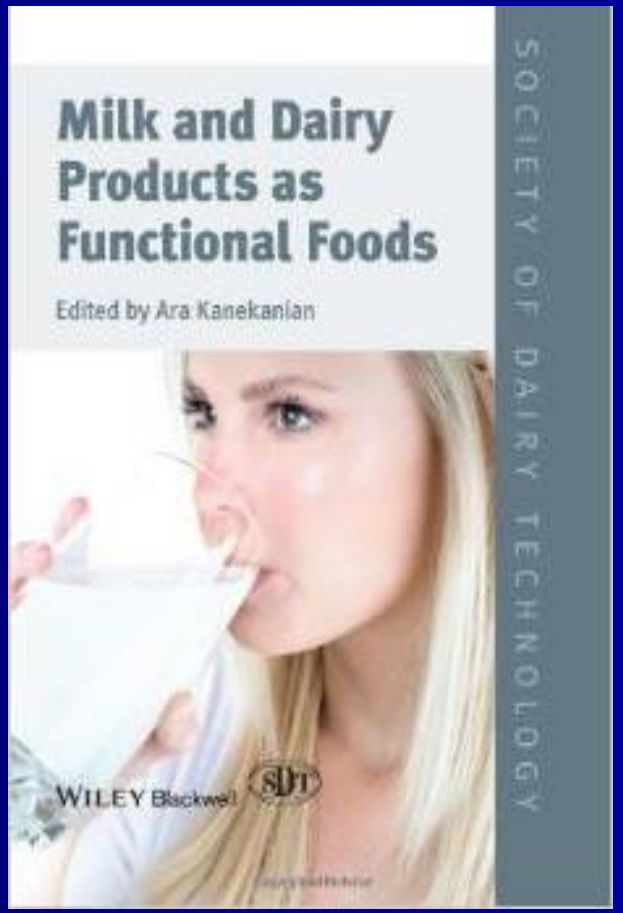
Cardiff Metropolitan University

Department of Healthcare & Food

Centre for Functional Foods

Western Avenue - Cardiff CF5 2YB

Email: akanekanian@cardiffmet.ac.uk



Publisher - Wiley Blackwell 2014

Presentation Topics

I. Brief Introduction

- Public Health – Minimising illnesses
- Functional Foods

II. Fermented Milk & dairy products products

- Yogurt & Yogurt Drinks
- Cultures used – Probiotics
- Factors affecting probiotics growth & Survival
- Prebiotics

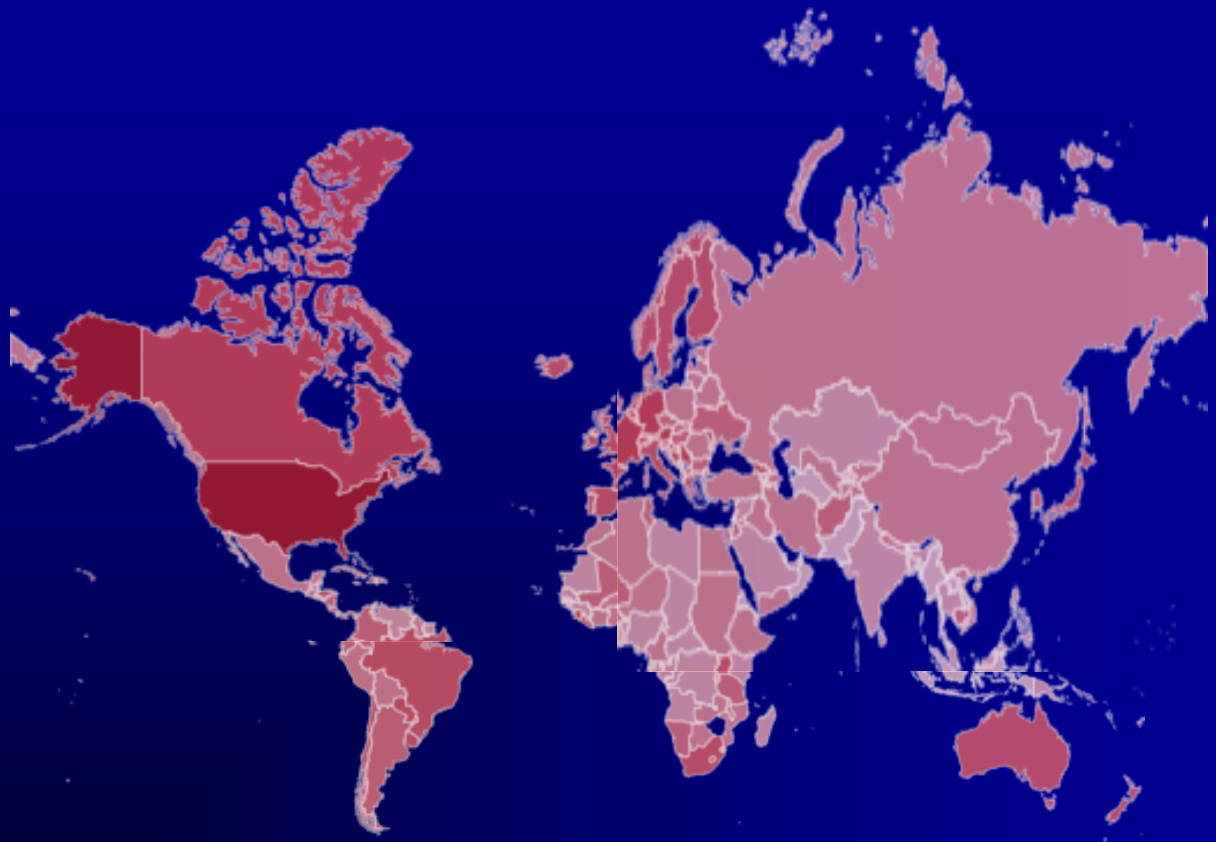
III. Future Research & Product Development

Public Health – Main Concerns & Strategy

- Mental Health
- Heart disease: e.g. CVD – Cardiovascular disease
- Type 2 Diabetes
- Obesity
- Cancer
- Others: Chronic inflamation, allergy, osteoporosis ...

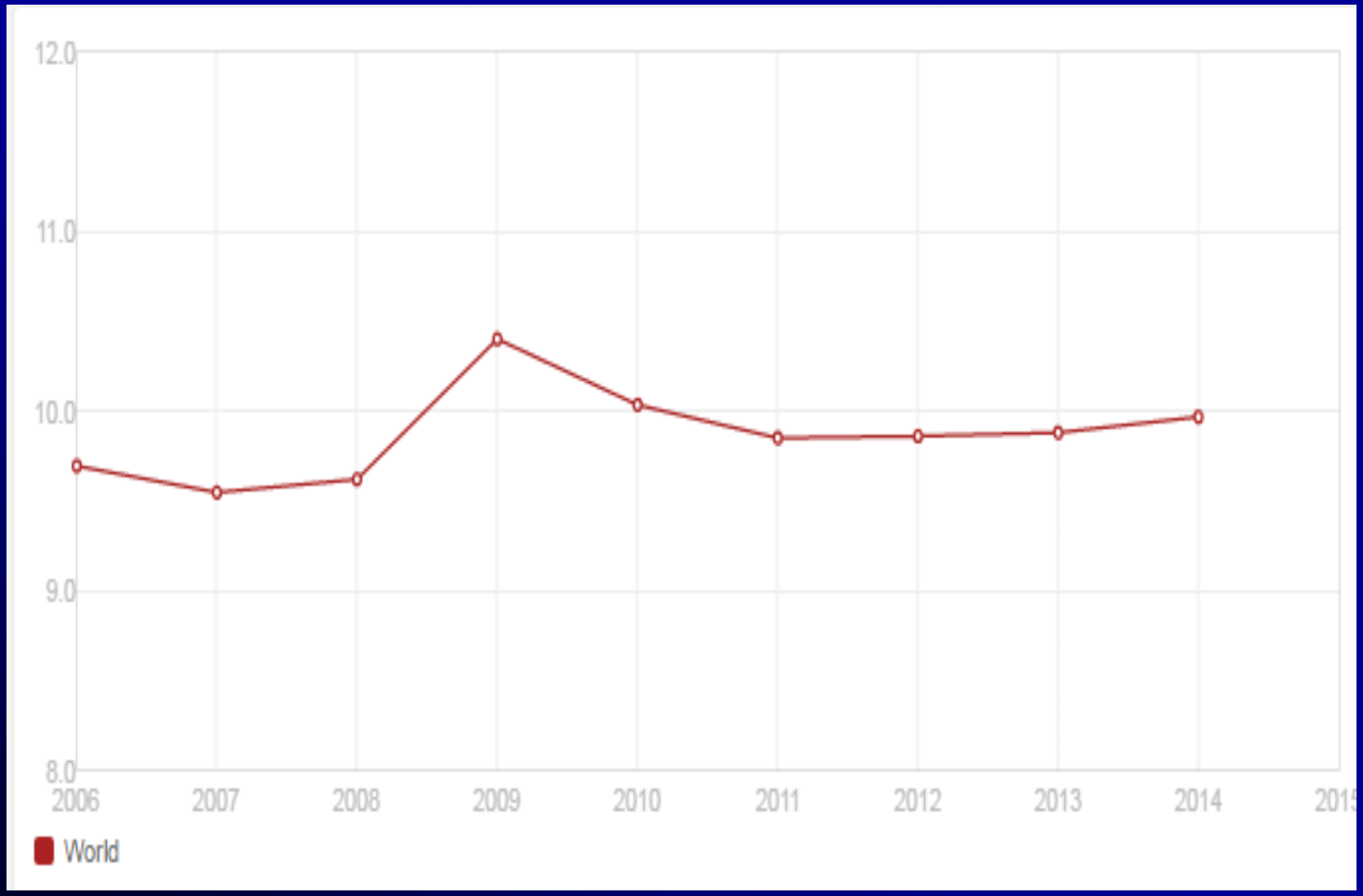
Is there a role for fermented food/milk to play an active part?

PUBLIC HEALTH – Cost of Health care – % of GDP



World Data Bank – World development Indicators 2015

PUBLIC HEALTH – Cost of Health as % of GDP



World Data Bank – World development Indicators 2015

II. Functional Foods

Non-nutrient food constituents with physiological function & Bioactivities which benefit human health.

Sources of Functional Foods

Animal origin	Milk & dairy products, oily fish, eggs …
Plant Origin	Consumed as whole: Cereals, fruits, vegetables, nuts …
	Extracted: Polyphenols (olive waster), stanols, prebiotics …
Others	Microbial – Probiotics

Bioactive compounds – Nutraceuticals

BAC – Bioactive compounds – Bioactives

Endogenous and/or exogenous compounds exhibiting specific biological / physiological activities with positive impact on human health

BAP – Bioactive Peptides

Specific peptides released from protein by microbial and/or enzymatic hydrolysis

Probiotics

Live micro-organisms offering direct or indirect health benefits to the host through manipulation of the gut microbiota

Prebiotics

Non-digestible food ingredient benefiting the health of the host by stimulating the growth of probiotics in the gut

Research into the health benefits of bioactive peptides

- Cholesterol reduction – Antihypercholesterolemic
- Antihypertensive – ACE-Inhibition
- Antioxidant & Anti-inflammatory
- Antiothrombotic
- Anticarcinogenic
- Immune modulation
- Dental erosion ...

BAP – Release Mechanism

- Hydrolysis by
  - Digestive enzymes
  - Microbial action by proteolytic micro-organisms
  - Any proteolytic enzymes – different sources
  - Combination – Microbial, digestive & others
- Synthesis

Lys-Arg-Glu-Ser	Reduce - inflammation, atheroseclerosis & LDL peroxidation
Lys-Glu-Arg-Ser	No bioactivities

Fermented milk – microbial hydrolysis of casein

Profile of casein hydrolysis by *Bifidobacterium animalis* subsp *lactis* (BB-12)

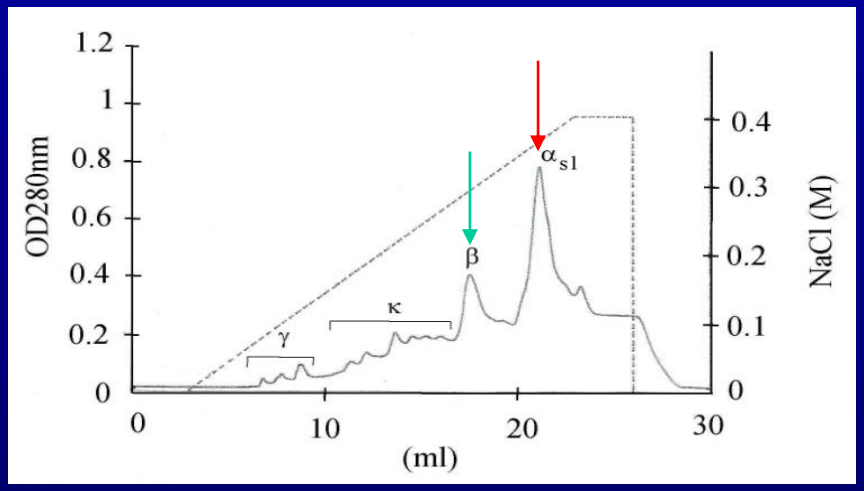


Fig1: Control – No hydrolysis

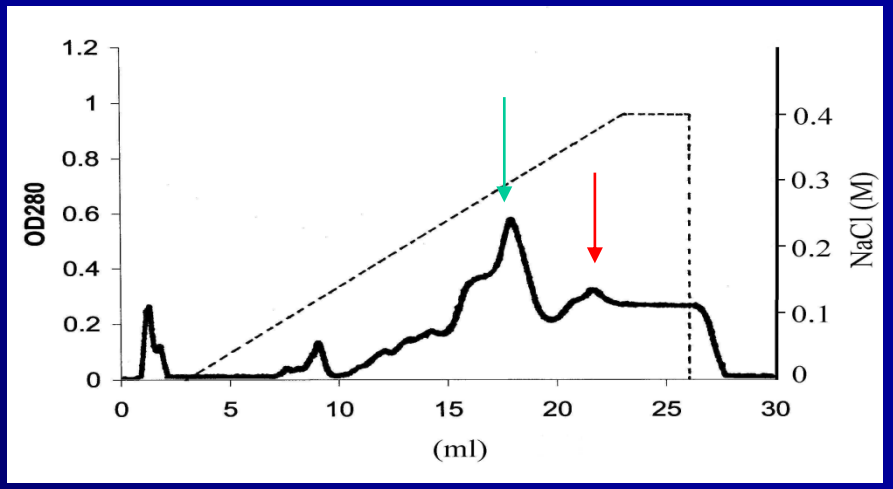


Fig 2 : 4 hours hydrolysis

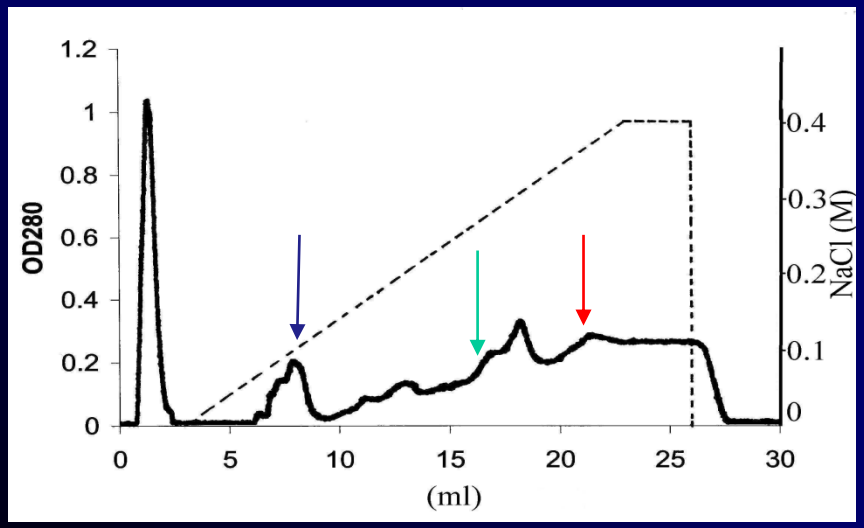


Fig3: 24 hours hydrolysis

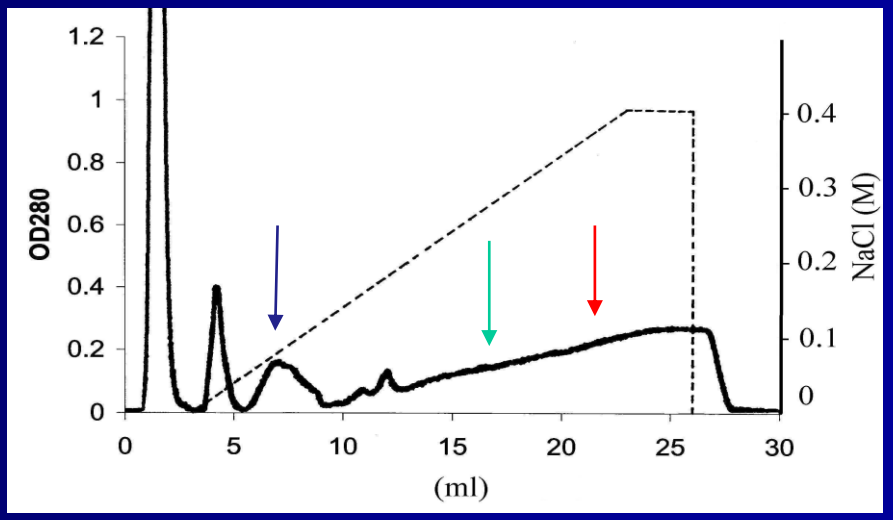


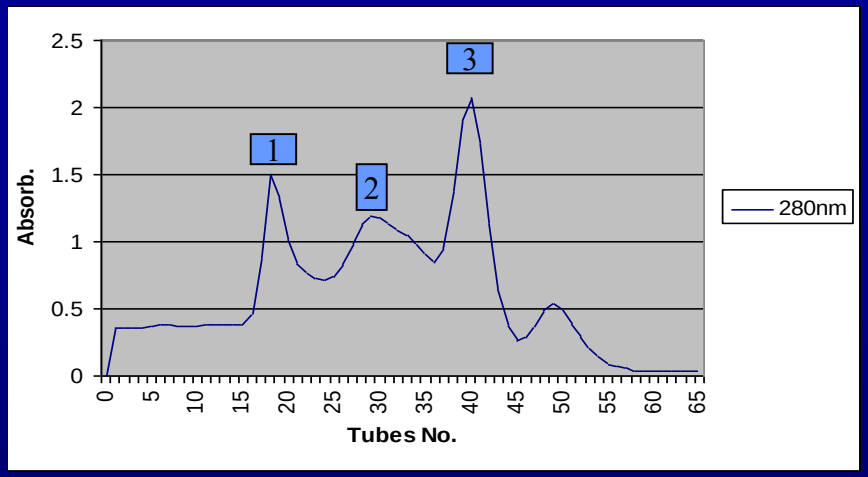
Fig 4: 48 hours hydrolysis

BAP – Fractionation & identification

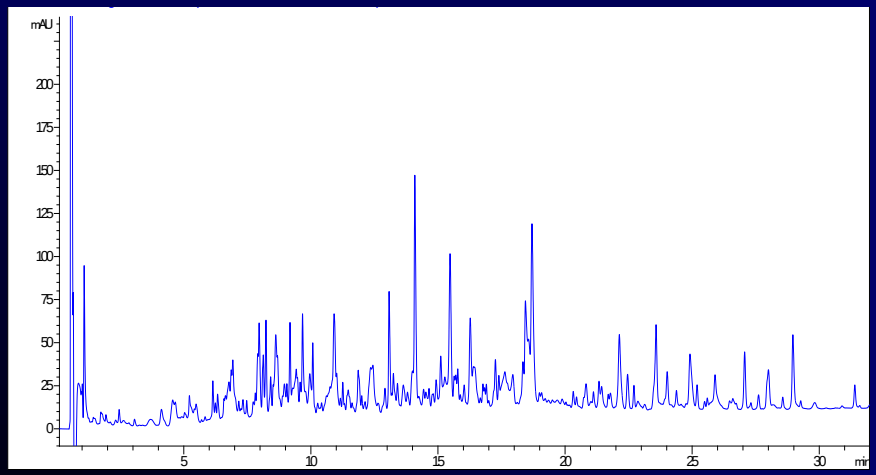
- **Chromatography**
  - Size exclusion
  - Ion exchange
  - RP-HPLC
- **Membrane separation**
  - UF – different MWCO (1-10 kDa)
- **Polarity based solvent extraction**

Identification & Isolation of BAP from tryptic casein hydrolysate

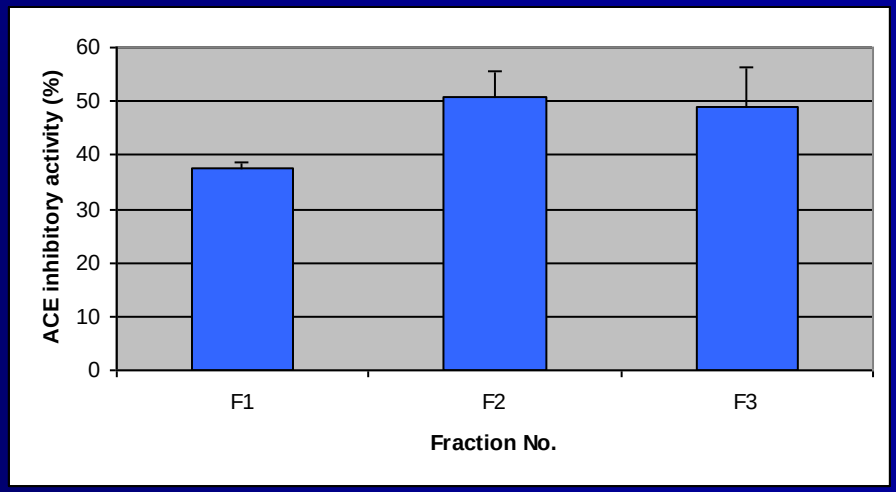
Activity of Angiotensin Converting Enzyme (ACE) Inhibitor



SEC-Casein hydrolysate (48h)



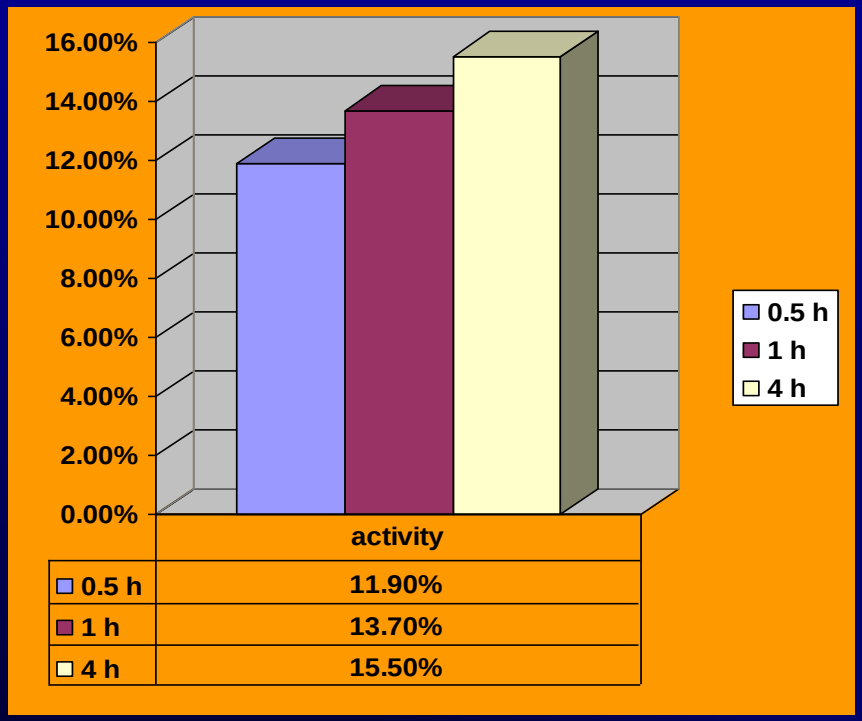
RP-HPLC of Fraction 2



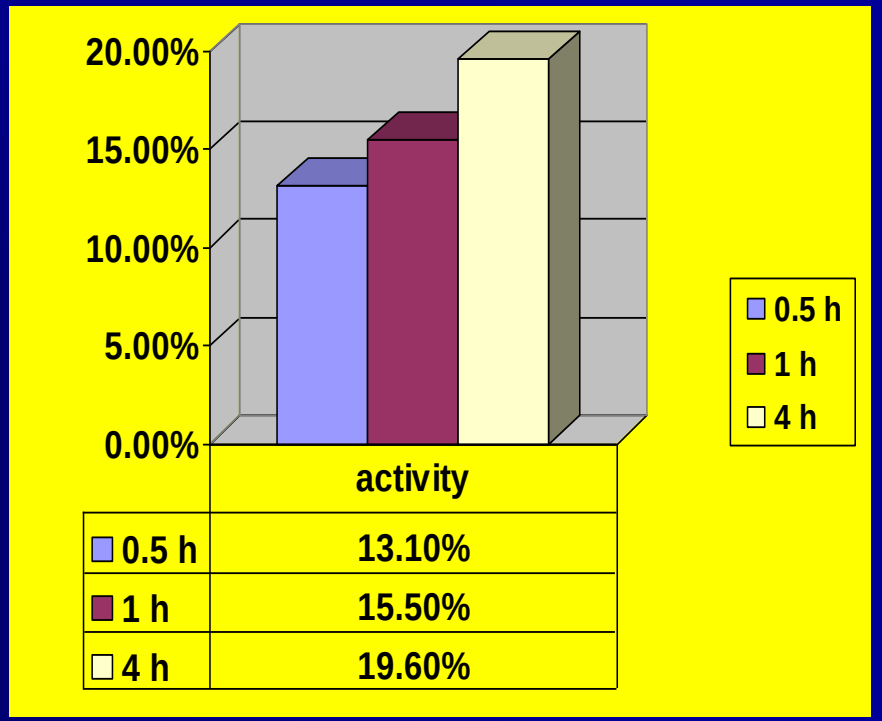
ACE Inhibitory Activity of hydrolysate

BAP – Antioxidant Activity

Casein Hydrolysate - UF Fractionation



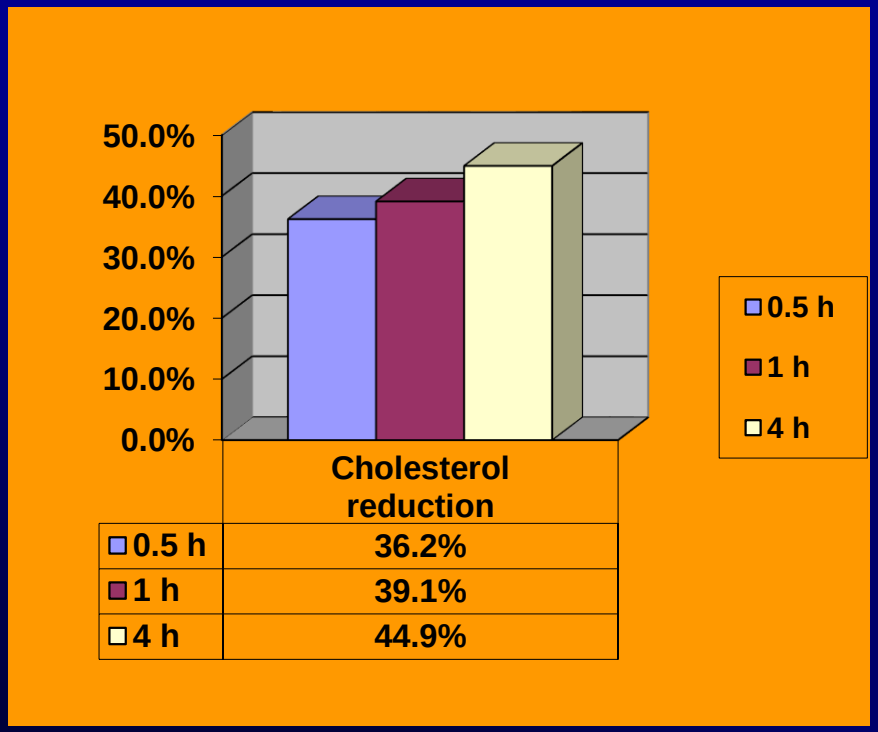
Radical Scavenging activity of 10 kDa permeate



Radical Scavenging activity of 1 kDa permeate

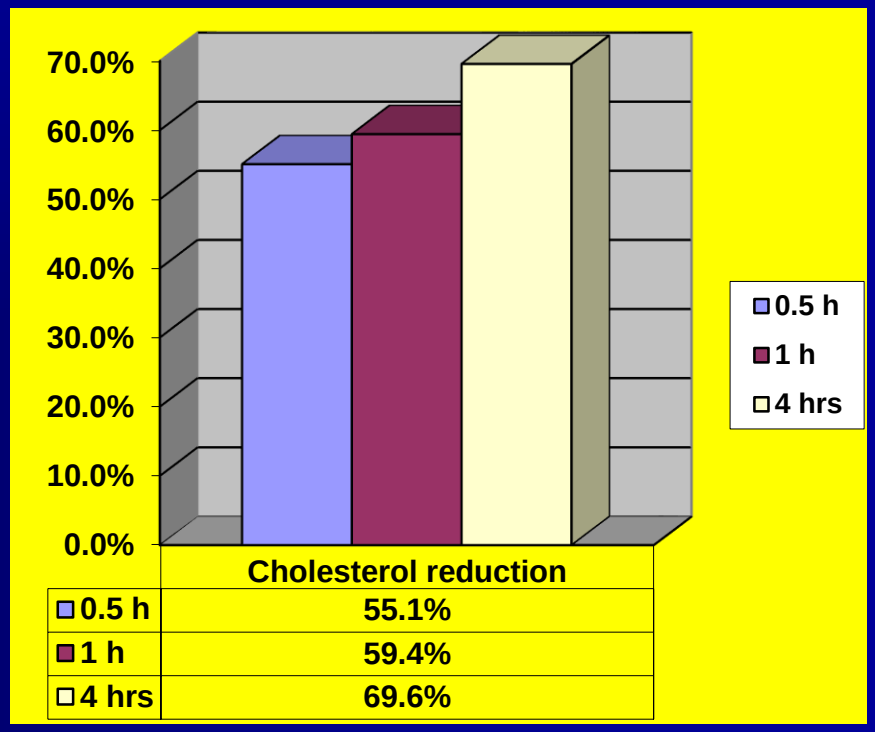
Hypercholesterolemia

Cholesterol reduction by casein hydrolysate – UF permeates



	Cholesterol reduction
0.5 h	36.2%
1 h	39.1%
4 h	44.9%

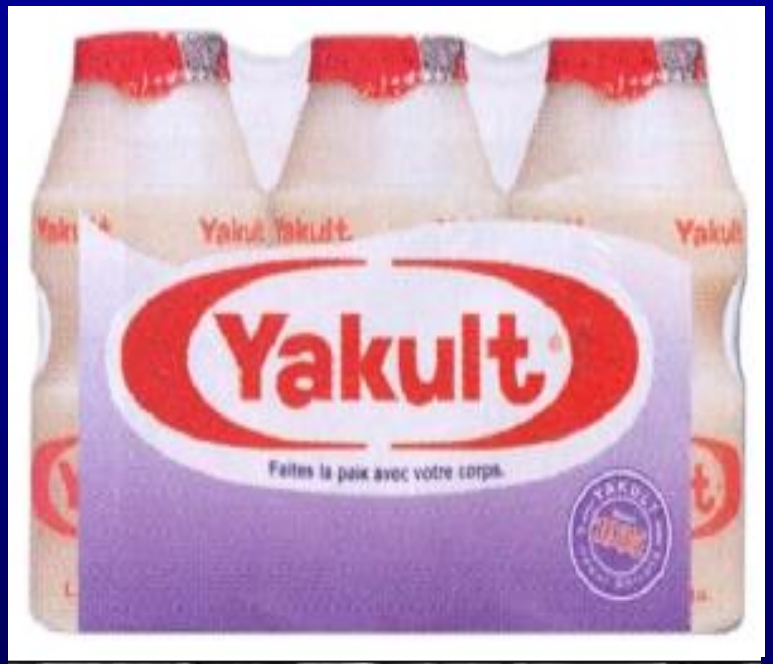
Casein Hydrolysate – UF 10 kDa permeate

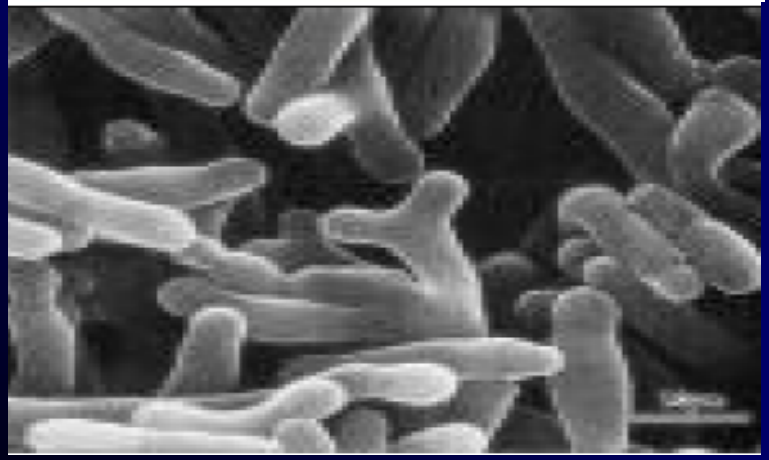


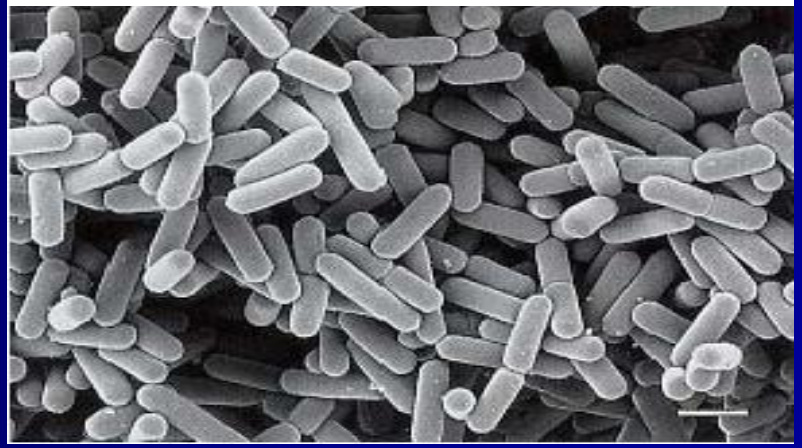
	Cholesterol reduction
0.5 h	55.1%
1 h	59.4%
4 hrs	69.6%

Casein Hydrolysate – UF 1 kDa permeate

PROBIOTICS & Human Health







Gastrointestinal commensal Microbiota

- Complex relationship with host
- Estimated between 500 – 1000 species mostly anaerobic/fermentative
- Over 50% non-culturable
- More microorganism than human cells in the body
- Mass of 1 – 2 kg: ‘a hidden organ’ in human body

Establishment of the microbiota in the human gut

Birth	Sterile gut
Breast fed	*Bifidobacterium* & *Lactobacillus*
Formula fed	Streptococci, Enterobacteriaceae
Weaning	Diversification of flora
Adult	Relatively stable Change with age, diet, health … *Bifidobacterium* spp. may form 25%

Probiotic Microorganisms

- Lactobacilli - six species predominate

  L. acidophilus, *L. casei*, *L. reuteri, L. rhamnosus*

- Bifidobacteria - 9 species predominate

  B. longum, *B. bifidum*, *B. breve*, *B. infantis , B. lactis*

- Yeast *Saccharomyces boulardii*

Fermented milk products

Different types of yogurt

- Culturing with 1:1 Lactobacilli : Streptococci
- Normal – Natural, Fruited, drinkable & probiotic products
- Concentrated – Greek style – Traditionally concentrated or by UF
- Powder – as food ingredient
- Frozen product

Kefir – Different from normal yogurt

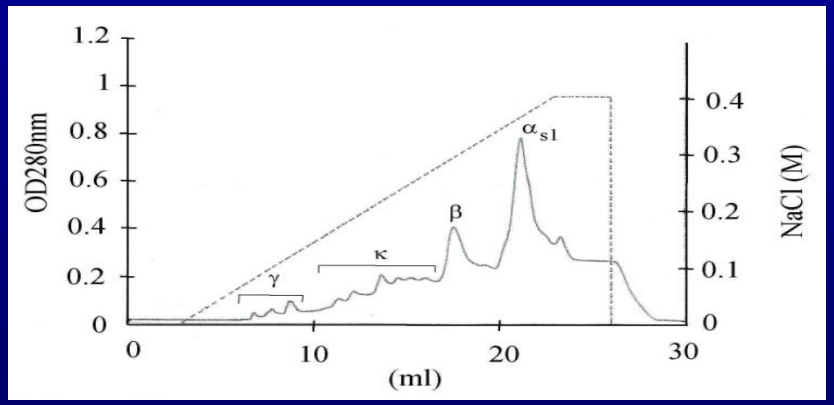
Fermented milk drink with mixed cultures of bacteria & yeast in symbiosis

Refreshing – The presence of CO_2, alcohol and other organic compounds

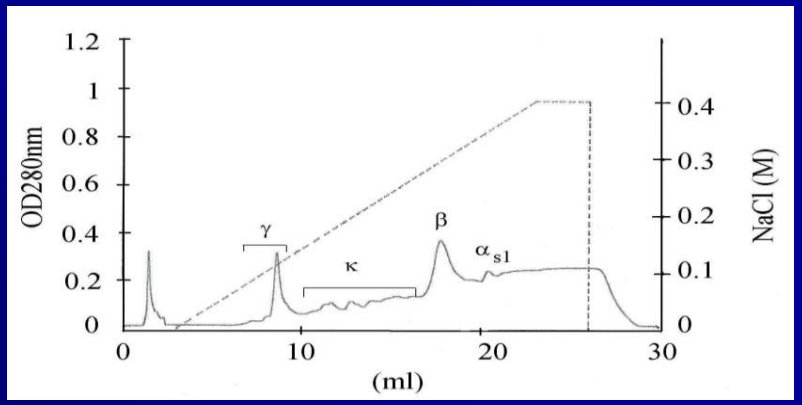
Culture – Using Kefir grains or lyophilised/freeze dried – each with limitations

Casein profile of commercially available probiotic drinks

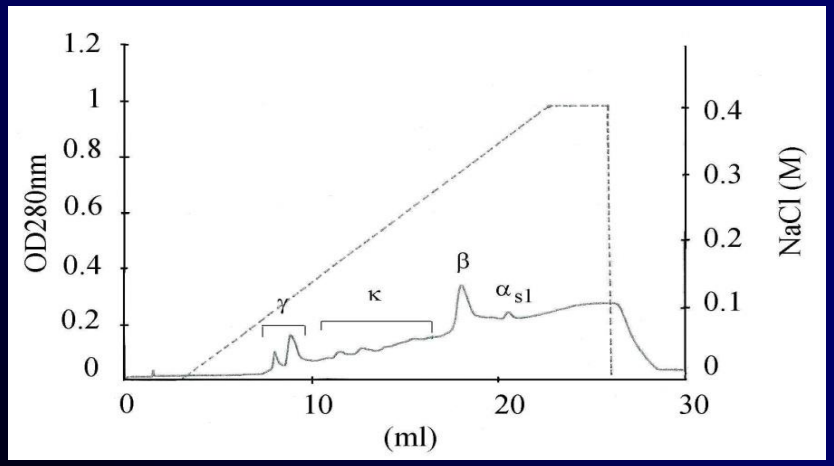
- Profile of casein hydrolysis by different probiotics
- Different commercial drinks containing *L.Casei, L. acidophilus & B. longum*



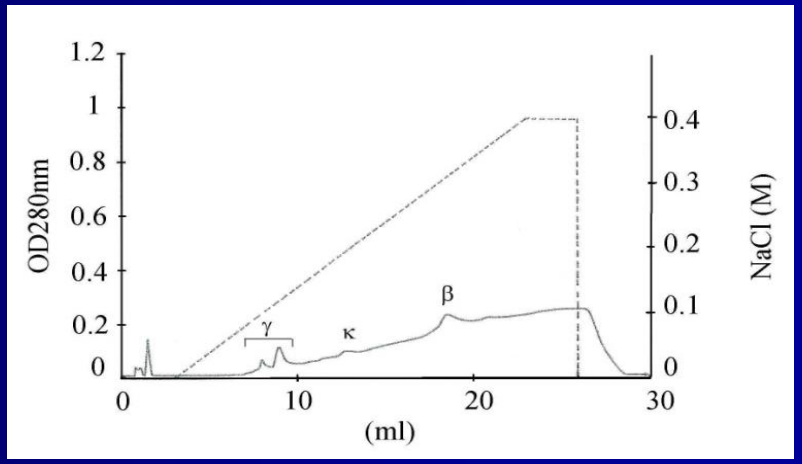
Casein Control – No hydrolysis



Commercial drink Sample A



Commercial drink Sample B



Commercial drink Sample C

Factors affecting Probiotics growth & Survival

- Temperature

Various temperatures 20, 30, 37 and 45C

Affecting the growth – log cfu/mL

Different pattern of metabolites (organic acids, volatiles, CO2 …)

Probiotics & Conventional yogurt cultures grow at different optimum temp.

- Oxygen

Probiotics are microaerophilic and anaerobic

Processing introduces more oxygen

Minimising the effect

Microencapsulation

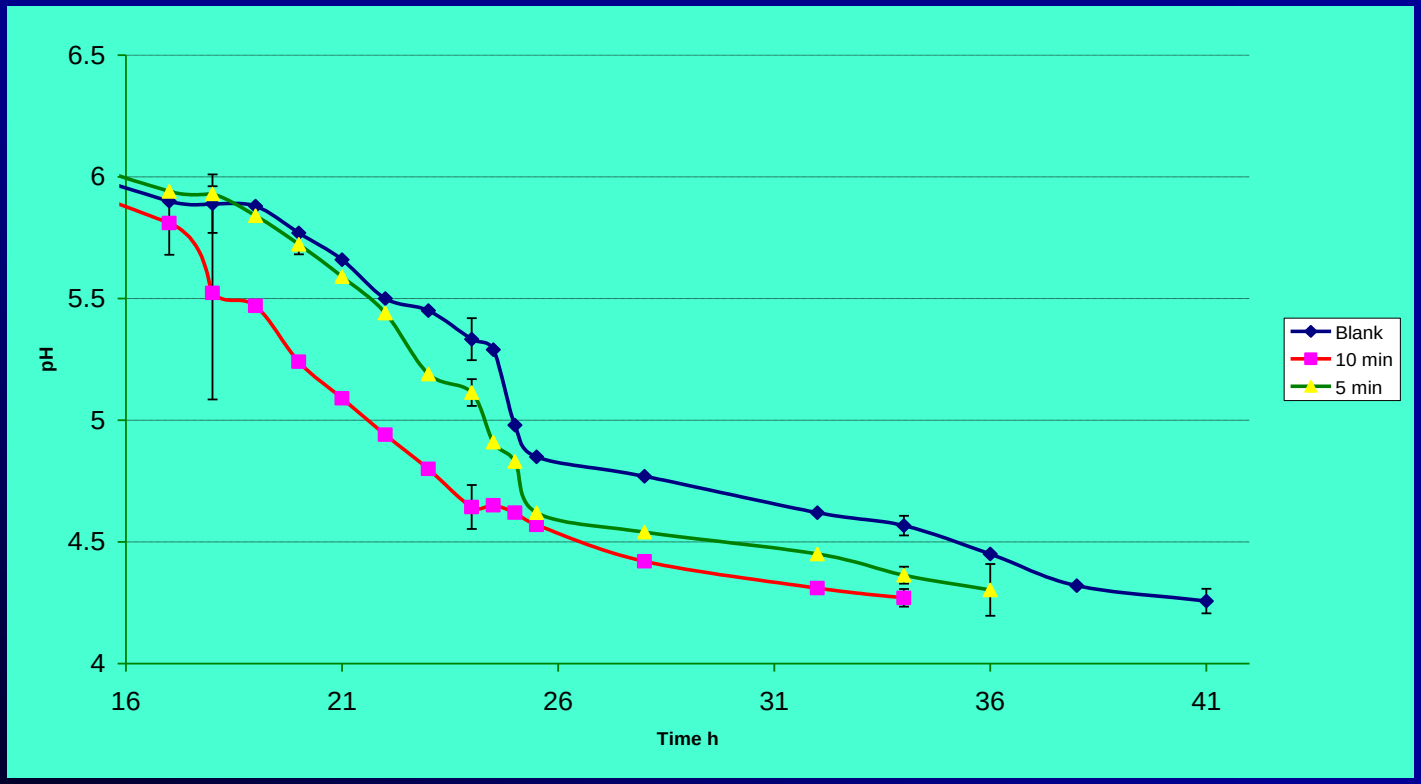
Using oxygen scavengers? VC, Polyphenols - Catechin or any antioxidants

Less susceptible strains, mixed cultures

Packaging

Factors affecting Probiotics growth & Survival

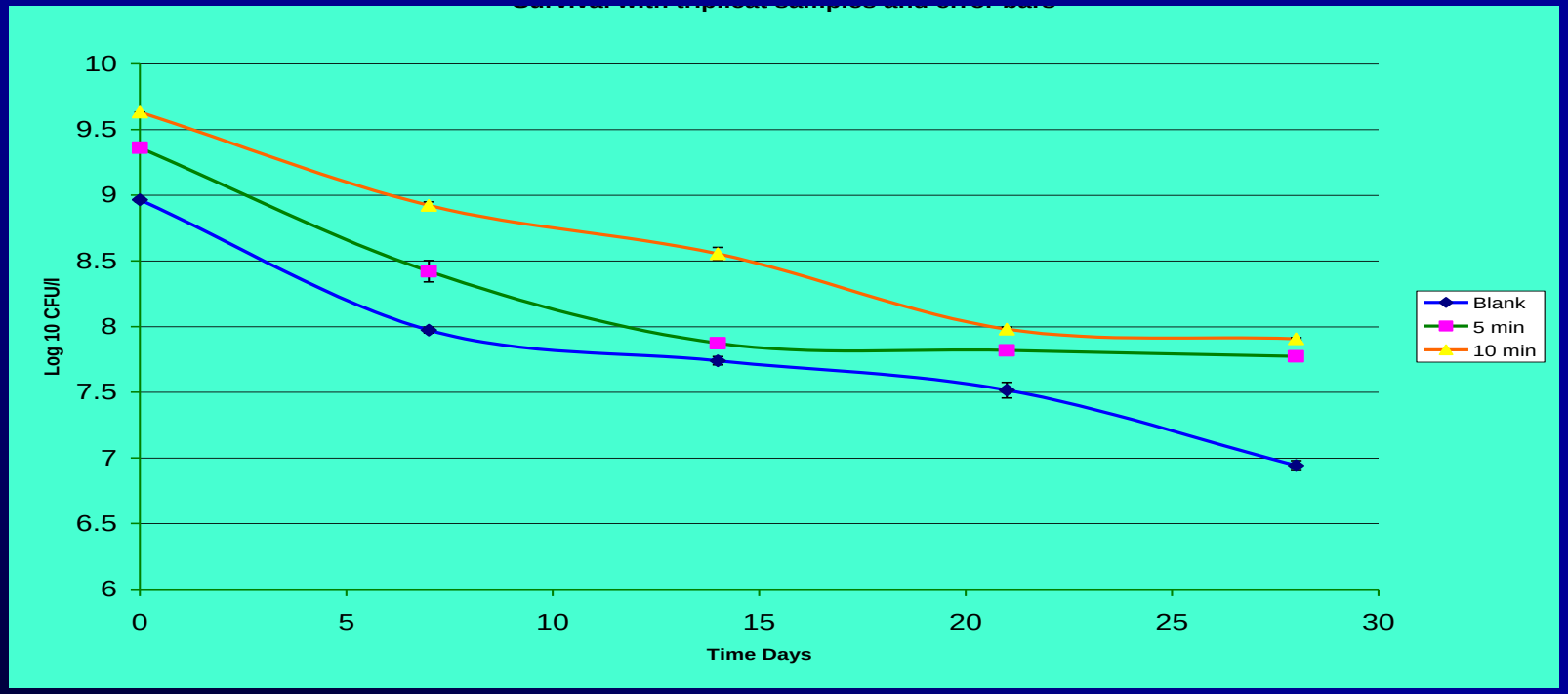
Partial protein hydrolysis



***Bifidobacterium animalis* subsp *lactis* (Bb12) growth in tryptic hydrolysate of skim milk after 5 and 10 minutes**

Factors affecting Probiotics Survival

Storage temperature of partially hydrolysed protein



***Bifidobacterium animalis* subsp *lactis* (Bb12) survival at 4C for 28 days in tryptic hydrolysate skim milk after 5 and 10 minutes**

Probiotics – Health Benefits

- Therapeutic manipulation of gut microflora
- Reduction of blood cholesterol - LDL
- Reduction in blood pressure – ACE inhibition*
- Vitamins synthesis & production of SCFA - acetate, propionate, butyrate
- Fermentation and salvation of energy
- Immunomodulatory effects
- Metabolic conversion of pro-drugs and carcinogens
- Adhesion to gut membrane through ‘Adhesin’
- Increase uptake of Ca and Mg
- Production of bacteriocin – Nisin
- Many others …

Prebiotics

- Non-digestible food ingredient that preferentially utilised by probiotics
- Not hydrolysed or absorbed in upper GIT
- Improve the balance of microbiota
- Breast milk contains 10 - 12 g/L oligosaccharides
- Inulin and fructo-oligosaccharides (FOS)
- Galacto-ligosaccharides (GOS)
- Others – Arabimogalactan, xylo-oligosaccharides, Rafinose, lactulose, isomalto-oligosaccharides , sorbitol, xylitol, soy oligo-ssachrides

INNOVATION – R & D

- New cultures
  - Probiotics
  - Growth & survival
  - Other physical & organoleptic properties
- Micro- and Nano-encapsulation
- Enrichment and Supplements market
- Functional yogurt containing BAC, PP, WP
- New Product Development – Infants & the elderly

How effective are the current assessment models and methods for determining the functionality of bioactive compounds?

Drinking Yogurt Production – Wales, UK

Courtesy of Health Food products Ltd,Wales – A.D. Kanekanian

THANK YOU

Any Questions?