Dairy Australia/NCDE Webinar

Fermented milk as functional food

21st June 2016

Cardiff Metropolitan University
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Milk & Fermented Dairy Products

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Publisher - Wiley Blackwell 2014
Presentation Topics

I. Brief Introduction
   Public Health – Minimising illnesses
   Functional Foods

II. Fermented Milk & dairy 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 inflammation, 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 – **Nutraeuticals**

**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, athero- sclerosis & 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)

Fig 1: Control – No hydrolysis

Fig 2: 4 hours hydrolysis

Fig 3: 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)

ACE Inhibitory Activity of hydrolysate

RP-HPLC of Fraction 2
BAP – Antioxidant Activity

Casein Hydrolysate - UF Fractionation

**Radical Scavenging activity of 10 kDa permeate**

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<th>Activity</th>
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**Radical Scavenging activity of 1 kDa permeate**

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<tr>
<td>1</td>
<td>15.50%</td>
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Hypercholesterolemia

Cholesterol reduction by casein hydrolysate – UF permeates

Casein Hydrolysate – UF 10 kDa permeate

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
  \[L. \text{ acidophilus}, L. \text{ casei}, L. \text{ reuteri}, L. \text{ rhamnosus}\]

• Bifidobacteria - 9 species predominate
  \[B. \text{ longum}, B. \text{ bifidum}, B. \text{ breve}, B. \text{ infantis}, B. \text{ lactis}\]

• Yeast  \[\text{Saccharomyces boulardii}\]
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\textsubscript{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*
Factors affecting Probiotics growth & Survival

• Temperature
Various temperatures 20, 30, 37 and 45°C
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 4°C 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-oligosaccharides (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?

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THANK YOU

Any Questions?