

Biological and technological application of kefiran, an EPS from kefir grains



CIDCA

Analía G Abraham

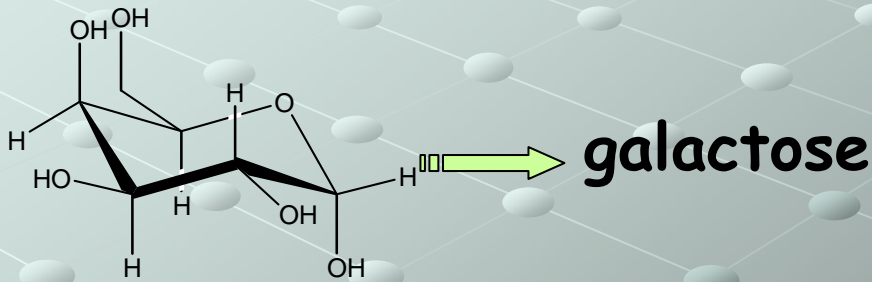
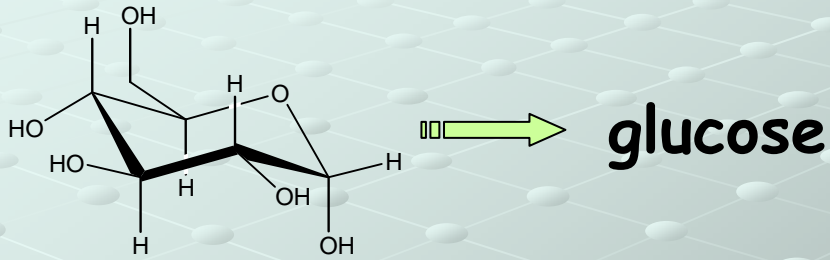
**Centro de Investigación y Desarrollo
en Criotecnología de Alimentos**



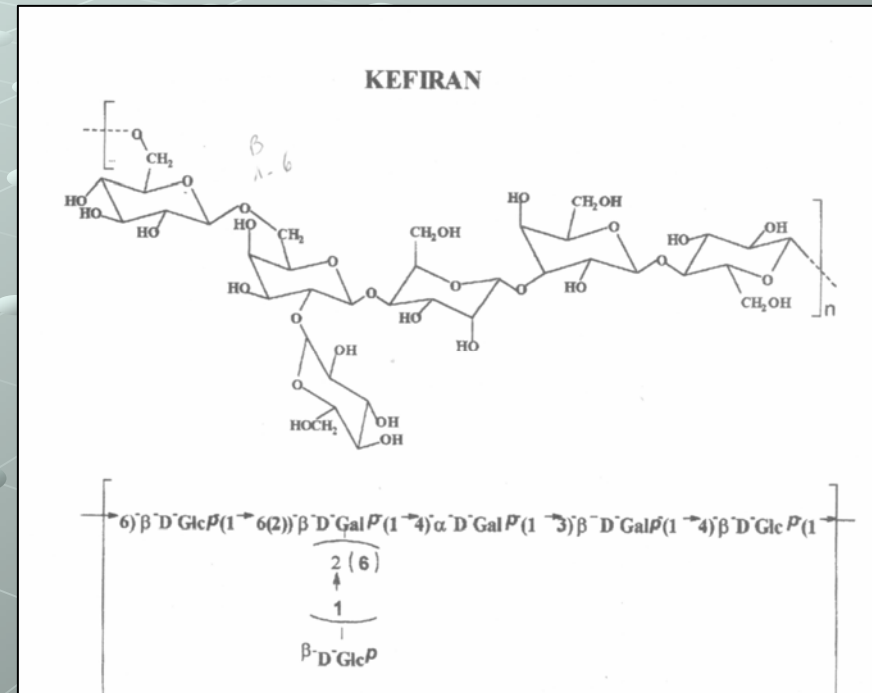
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Kefiran

Non-digerible polysaccharide composed of glucose and galactose



Produced by lactic acid bacteria (GRAS) included into kefir grains

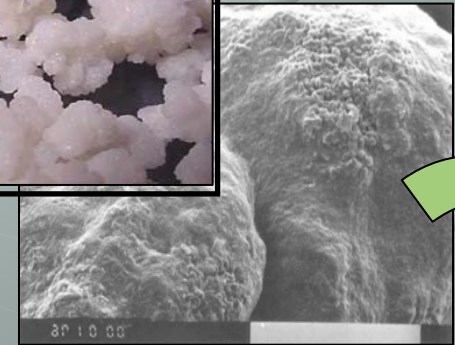


Kooiman 1968, Micheli et al 1999

Kefir grains

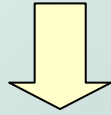
Kefir grains are natural immobilized starter consisting of a complex association of microorganisms

Microorganisms are embedded in a proteins and polysaccharide matrix.



- Lactic acid bacteria (lactococcus, homo y hetero fermentatives lactobacillus)
- Yeast
- Acetic acid bacteria

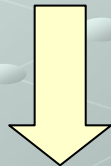
Kefir grains



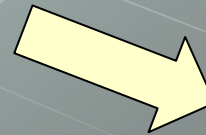
Milk or whey



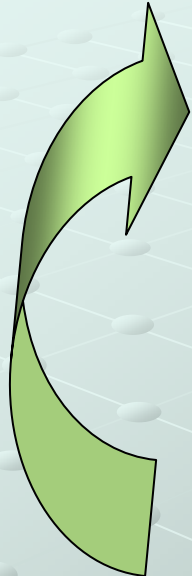
Incubation at 20 °C



**Filtration
plastic sieve**



**Fermented
product**

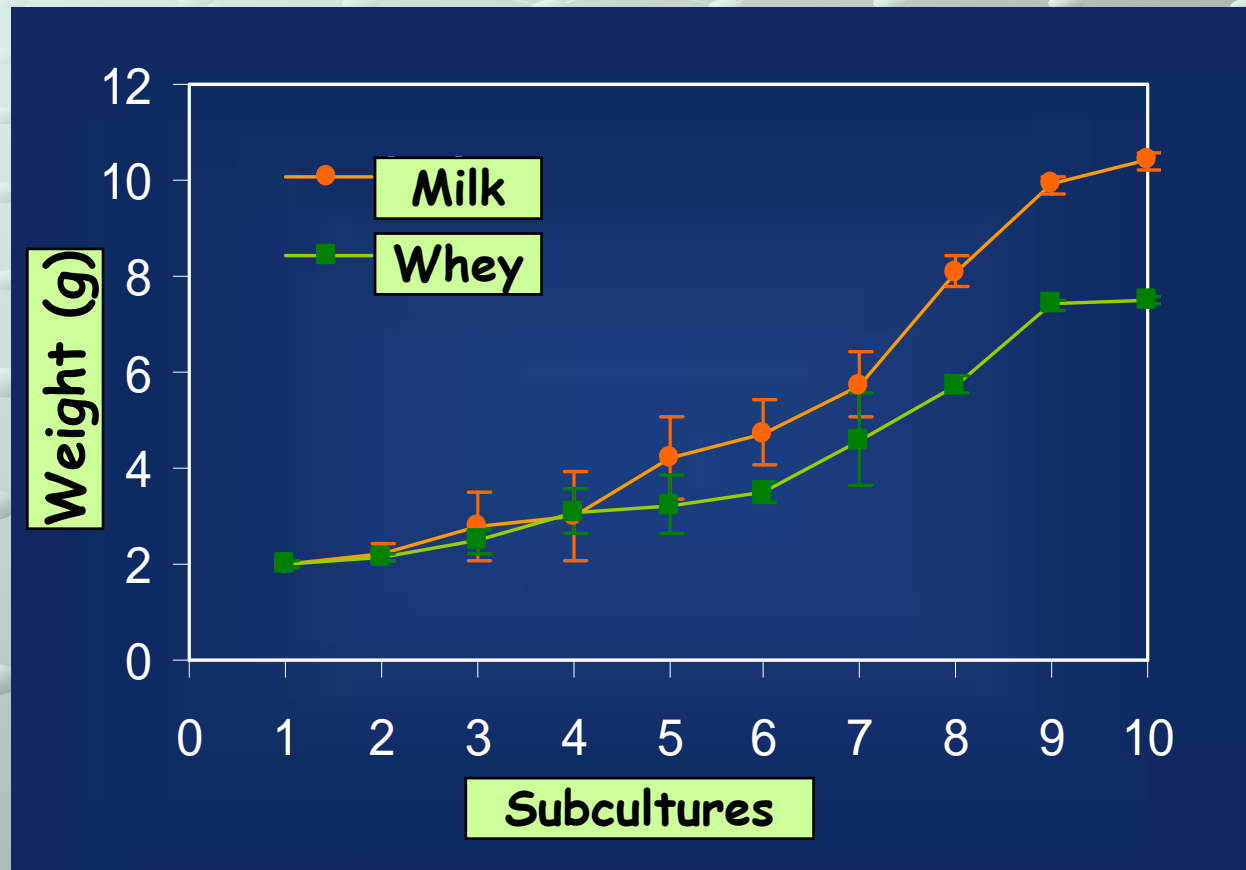


grains



Kefir grains

Grain weight increases a function of subcultures



Kefir grain composition



	Milk	Whey
➤ Protein	5-6%	6-8%
➤ Polysaccharide	8-9%	8-9%
➤ water	86-87%	83-84%
➤ LAB	$1.8 \cdot 10^8$ cfu/g	
➤ Yeast	$2.1 \cdot 10^7$ cfu/g	
➤ AAB	$2.5 \cdot 10^5$ cfu/g	

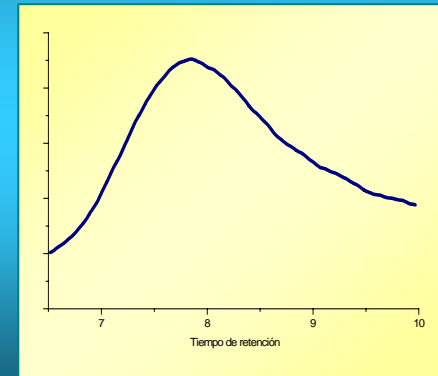
Kefiran: Physicochemical properties

Intrinsic Viscosity

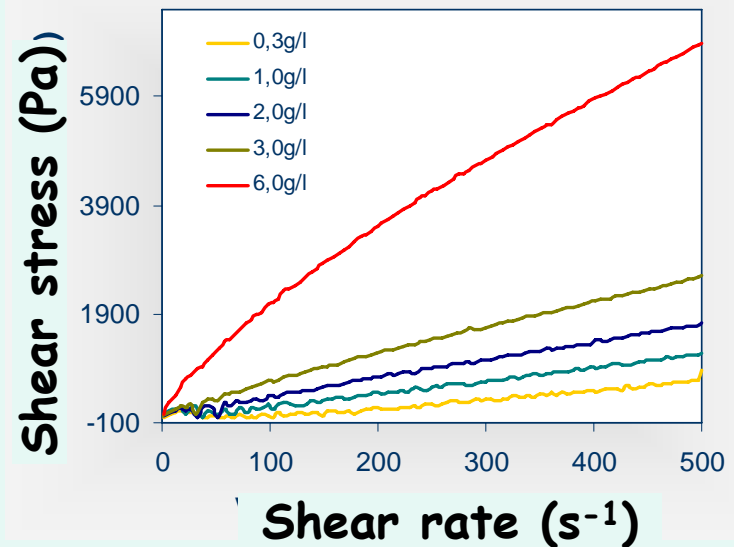
$6.0 \pm 0.05 \text{ dL/g}$



Molecular mass by gel permeation chromatography

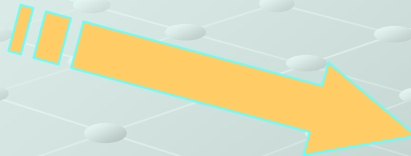


$MW > 4 \cdot 10^6$



Polysaccharide and biological function

Polysaccharides and oligosaccharides



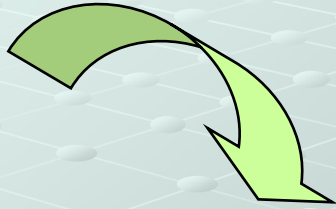
Dietary fibers
FOSs
GOSs
inuline
lactulose

Functional Aspects

- Prebiotic
- Non-digerible fibers
- Immune system stimulation
- Antiulcer
- Antitumoral

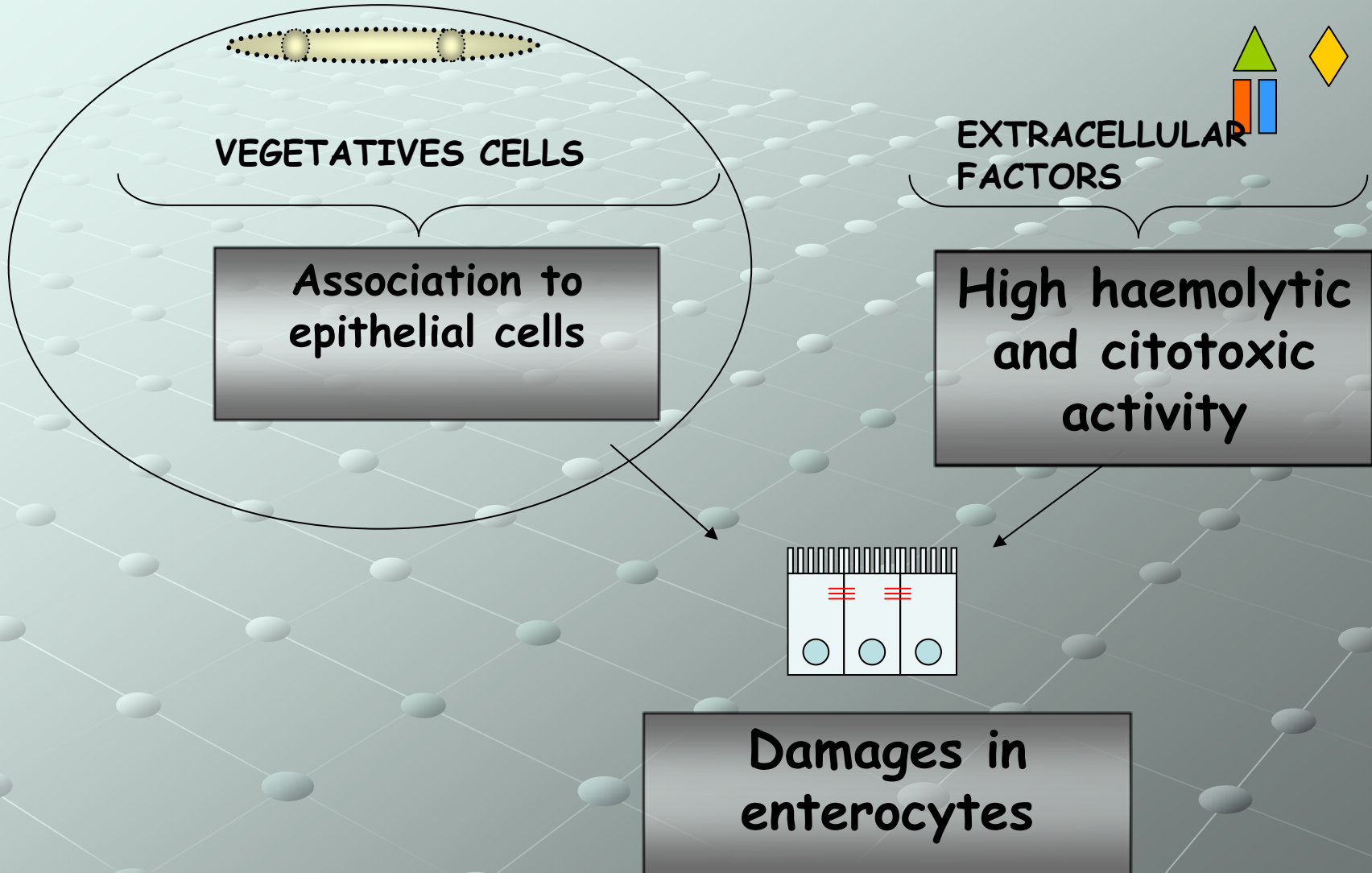
Polysaccharide and biological function

Kefiran

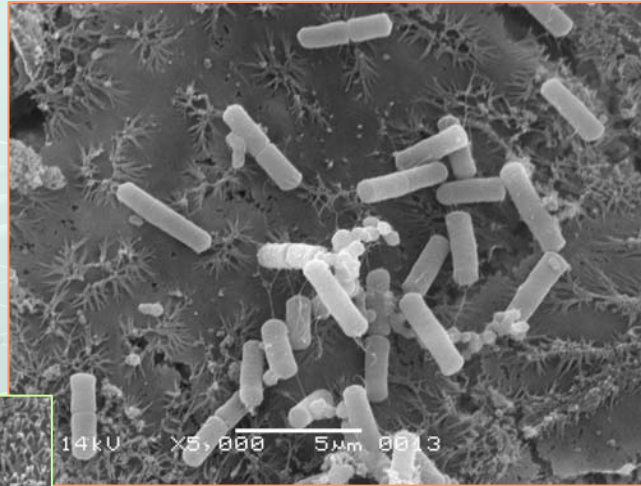


Effect of kefiran on cytopathic events triggered by *Bacillus cereus* on Caco 2 cells (In vitro study)

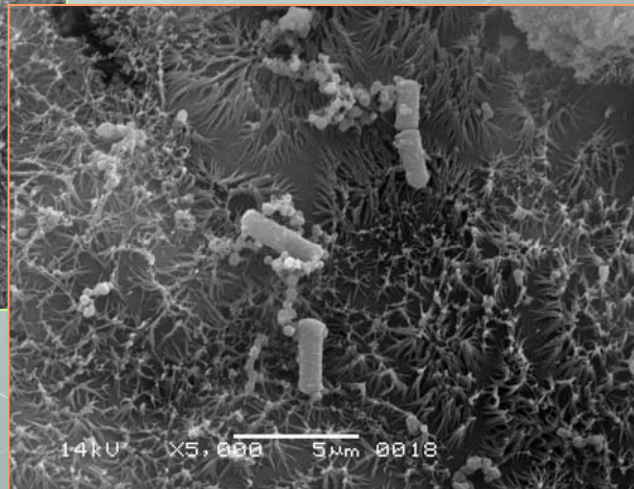
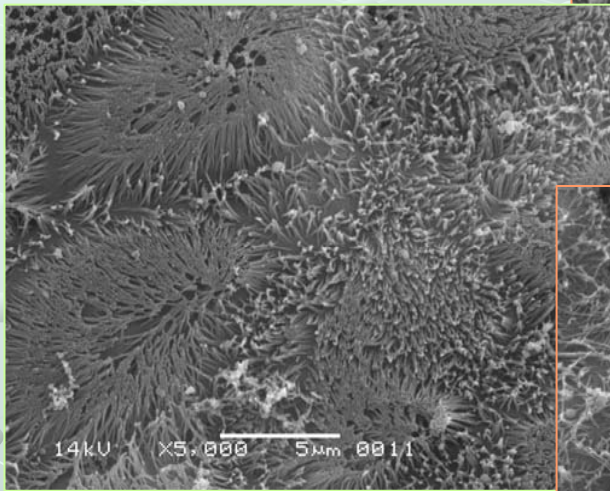
B. cereus: virulence factors



Bacillus cereus on Caco 2 cells



Bacillus cereus on Caco 2:



Caco 2 preincubated with kefir

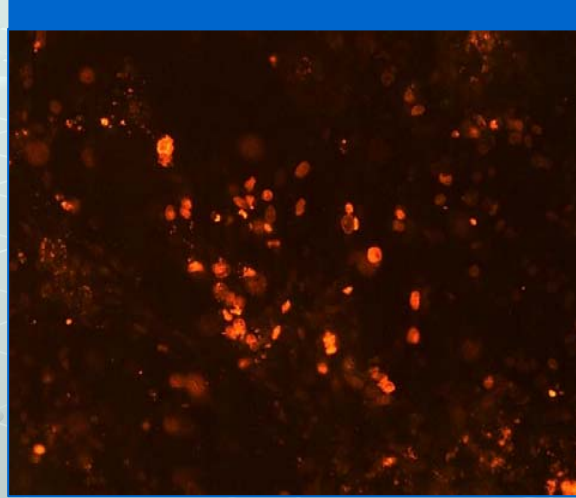
Scanning electron microscopy

Labelling of necrotic cells with Propidium iodide

Control

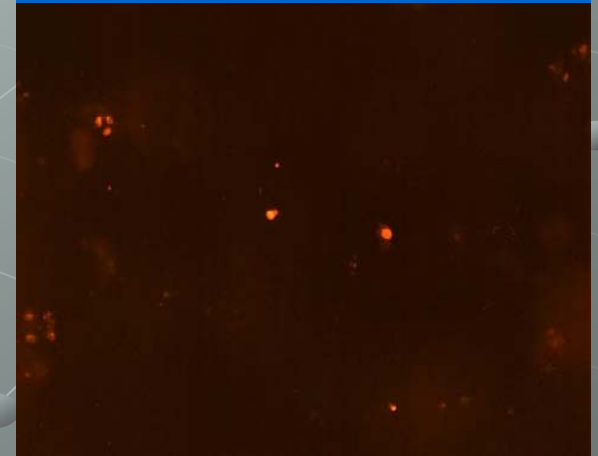


B. cereus



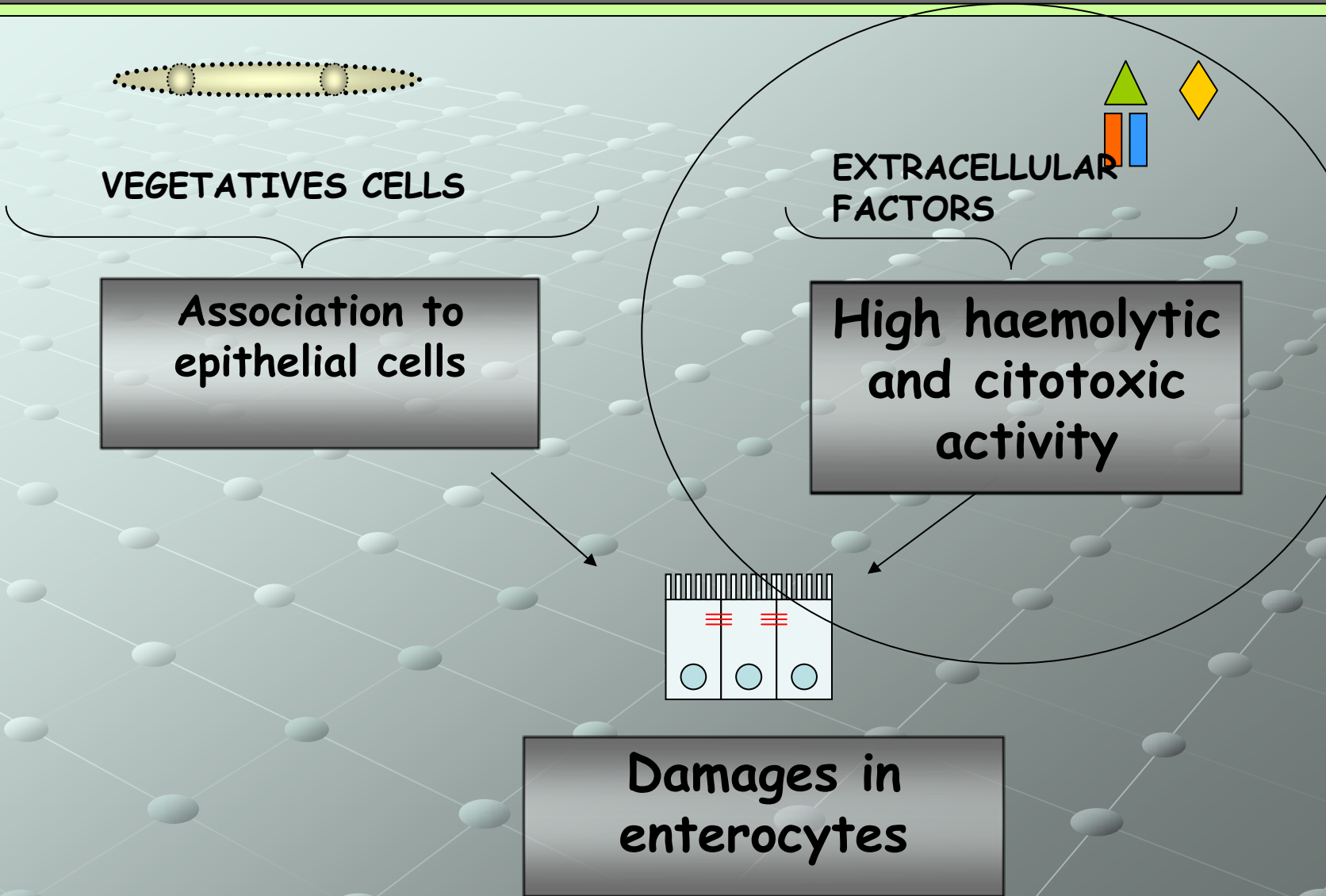
B. cereus + kefiran

preincubation

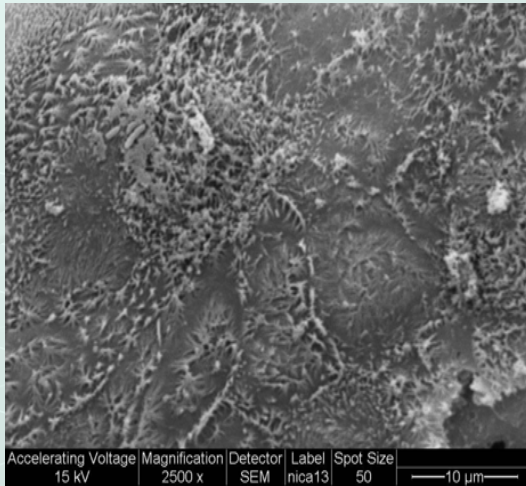


Kefiran antagonize cell death events
triggered by *B. cereus*

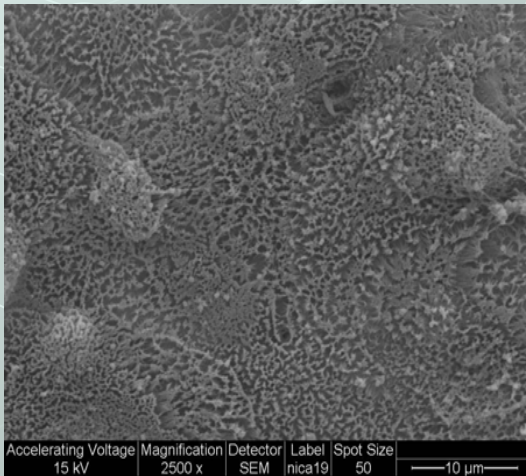
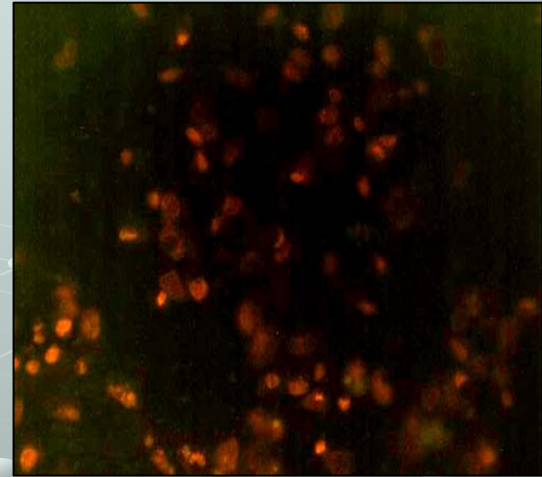
B. cereus: virulence factors



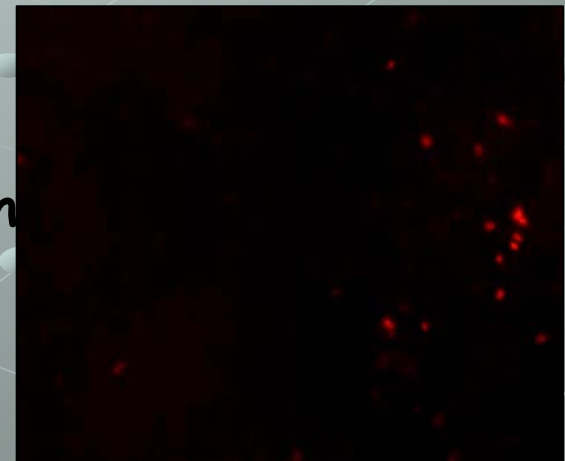
Effect of Supernatant of *Bacillus cereus* on Caco 2 cells



Without
kefiran



with kefiran



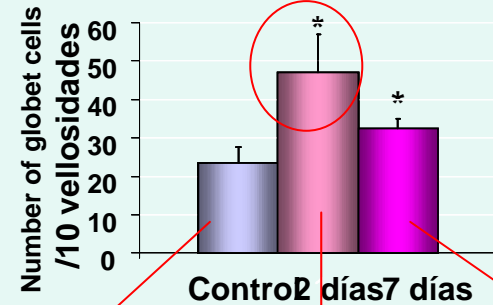
Scanning electron
microscopy

Propidium iodide
labelling

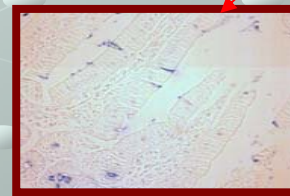
In vivo studies: Balb C mice

Increment in mucus producing cells in small gut

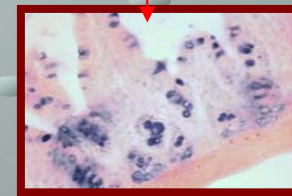
oral
administration
of kefir



Increment in number of IgA
producing cells in *lamina propria*



Control



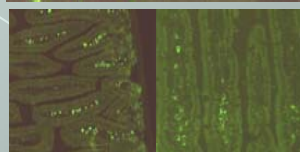
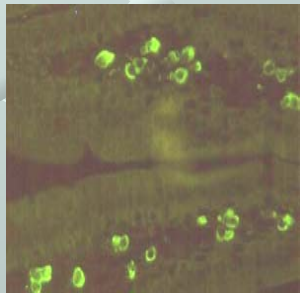
2 days



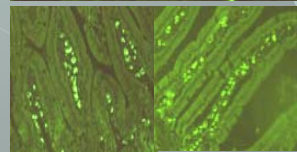
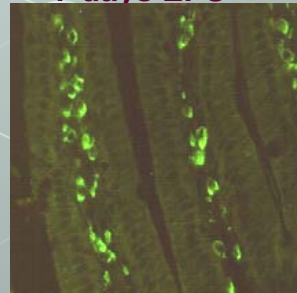
7 days

*

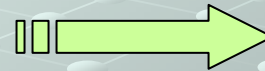
Control



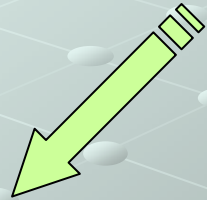
7 days EPS



Kefiran



Fermented



Grain



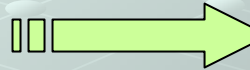
8g%



250mg/L

Technological application of kefiran

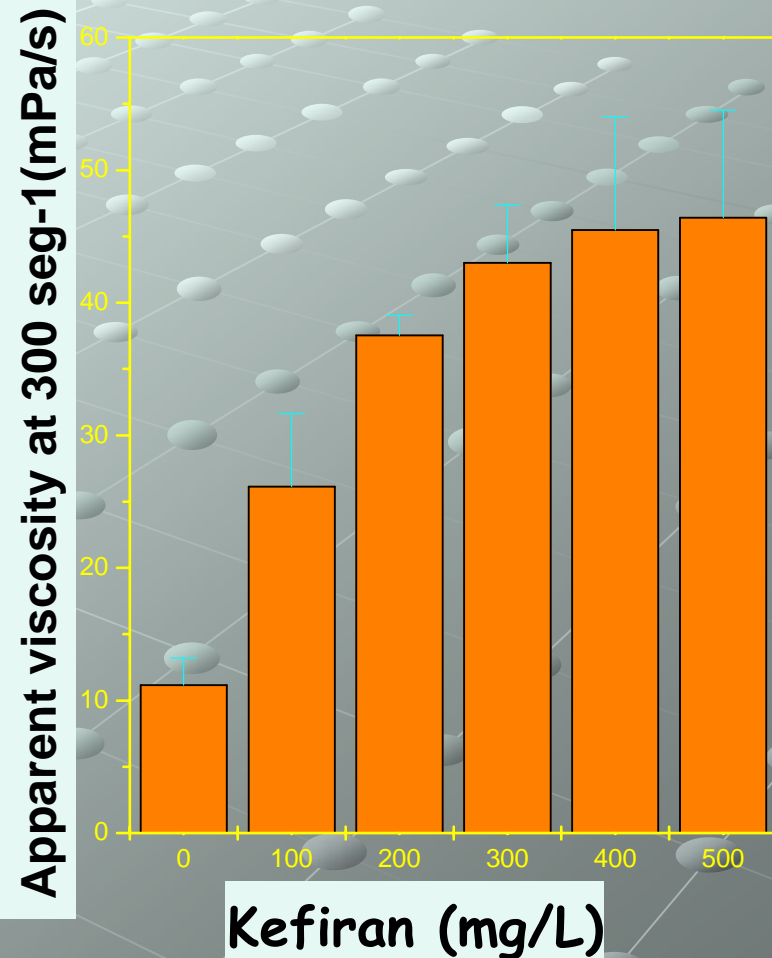
**Kefiran as
food
additive**



**Does Kefiran improve
texture
of fermented food?**

Rheological properties of glucono- δ -lactone induced skim milk gels

Kefiran improves texture of acid milk gels

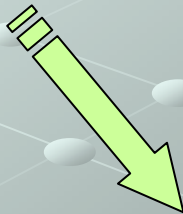
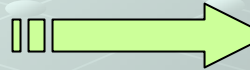


Technological application of kefiran : functional properties

**Kefiran as
food
additive**

**Kefiran improves
texture
of fermented food**

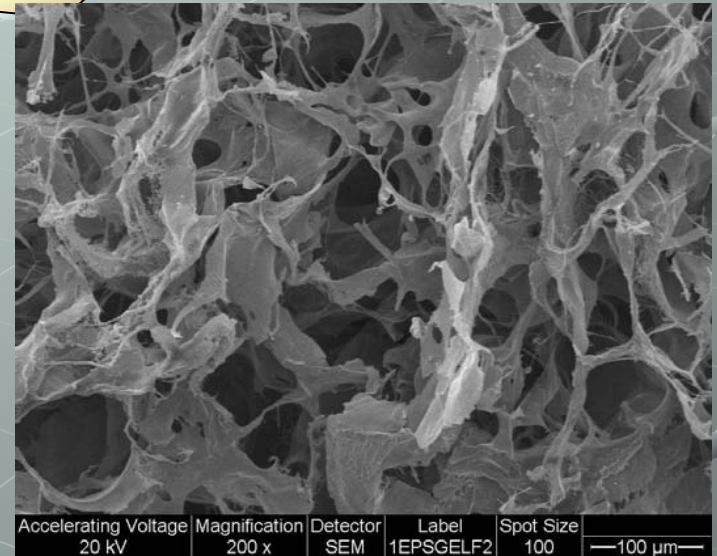
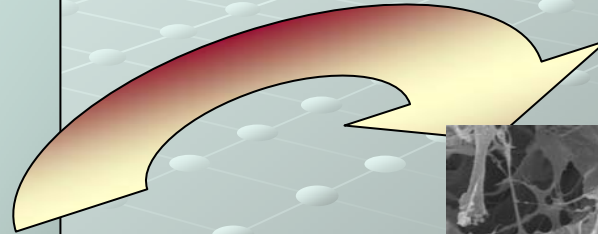
Gel forming ability



Kefiran cryogels :



**Water holding capacity
(WHC)
 $90.43 \pm 1.51\%$**



**Microscopic aspect of cryogels
(magnification 200X)**

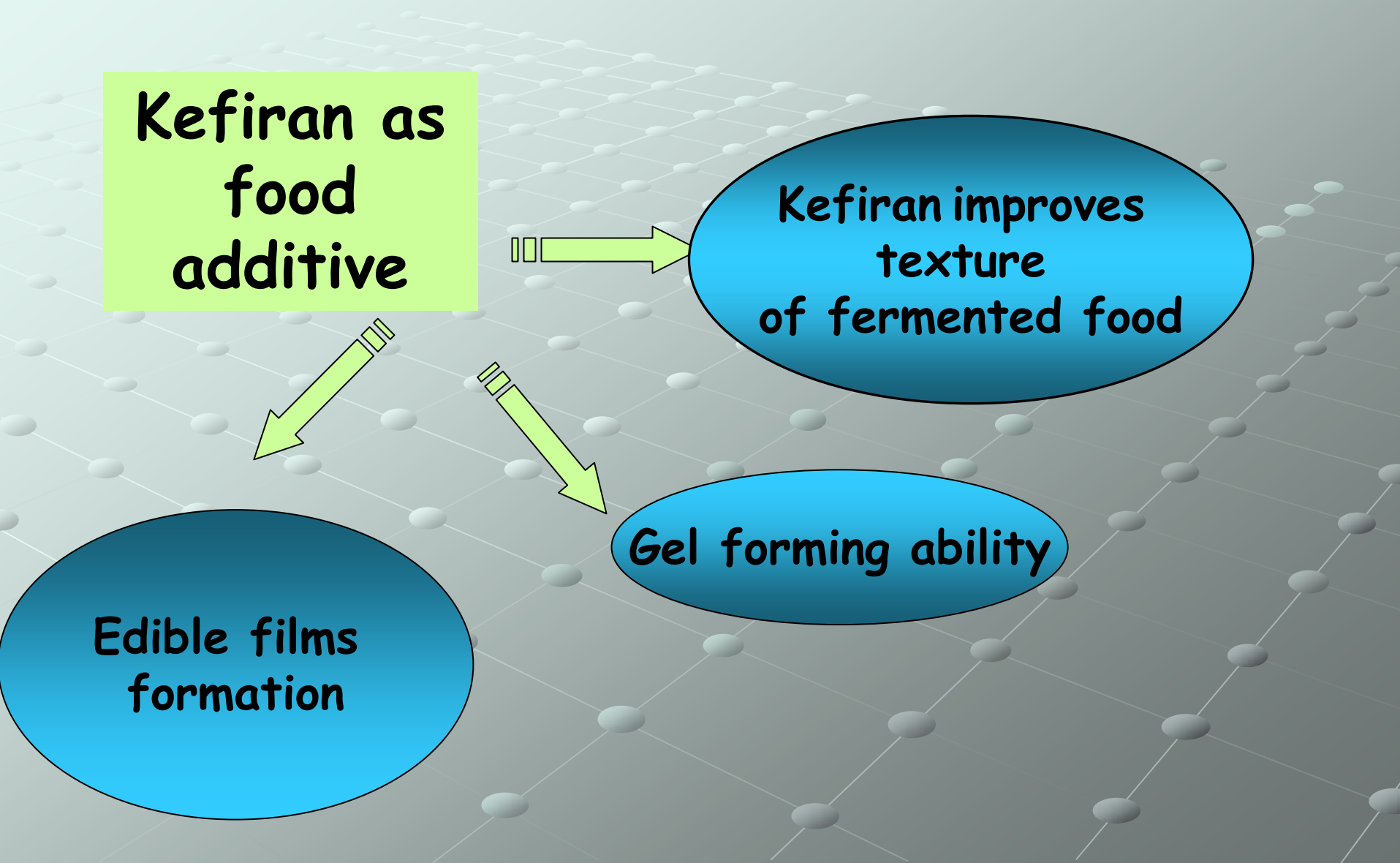
Technological application of kefiran : functional properties

**Kefiran as
food
additive**

**Kefiran improves
texture
of fermented food**

Gel forming ability

**Edible films
formation**



Edible films formation

kefiran

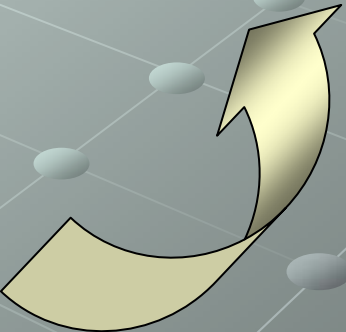
- 5g/l
- 7,5g/l
- 10g/l



57 cm²



Dry in oven at 50°C



Films: Water vapour barrier properties

Films	Water vapour permeability (WVP)
Kefiran (10 g/L)	5.43×10^{-11}
Chitosan (10g/L)	9.03×10^{-11}
Cellophane	8.4×10^{-11}
LDPE	9.14×10^{-13}

LDPE: low density polyethylene.

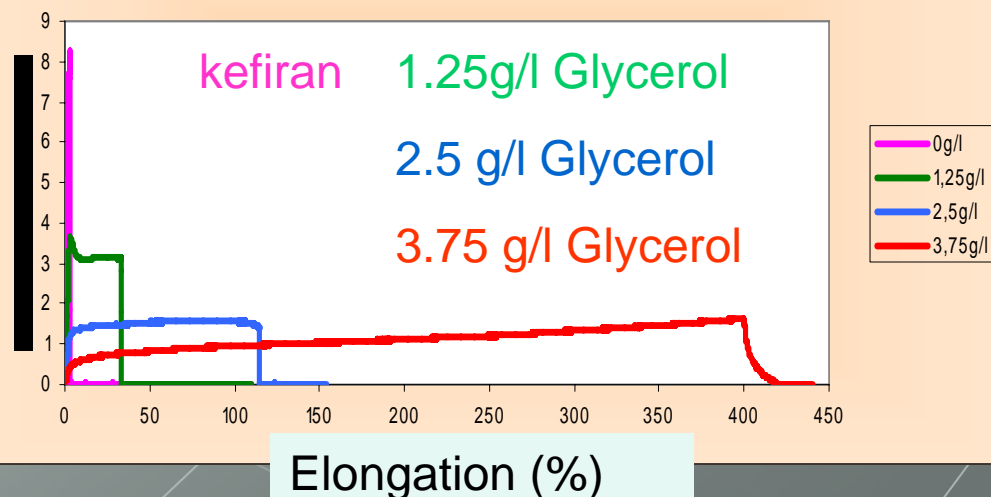
Films: mechanical properties

Tensile tests of films plasticized with glycerol

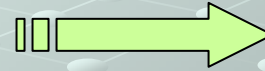


Texturometer Ta xT2i (TA.XT2i-Stable Micro Systems).

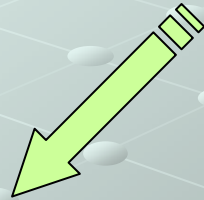
Traction profiles



Kefiran



Fermented product



Grains



8g%



250mg/L

Fermented milk



- ✓ Microorganisms with probiotic properties

- ✓ High inhibitory capacity of pathogens and contaminant microorganisms.

- ✓ Low lactose concentration (3.2%)

- ✓ Functional components **Kefiran**

Frozen Kefir

	Stability	Crystal size
Frozen kefir	30.3±1.8	99±41
Frozen kefir with cream	36.8±0.6	98±40
Frozen yogurt	18.8±1.1	87±28
Commercial ice cream	27.7±1.6	81±32

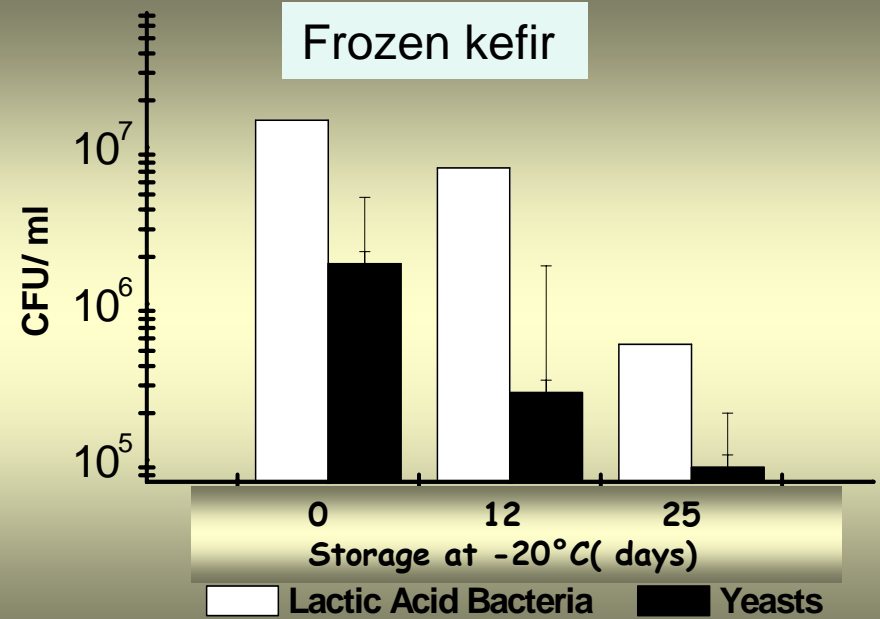
Frozen kefir without cream has higher stability than frozen yogurt and similar to commercial formulation



Kefiran

Frozen Kefir

Kefir microflora maintains viable at least 12 days at -20°C



Non-trained sensorial panel

High acceptability

Kefir grains

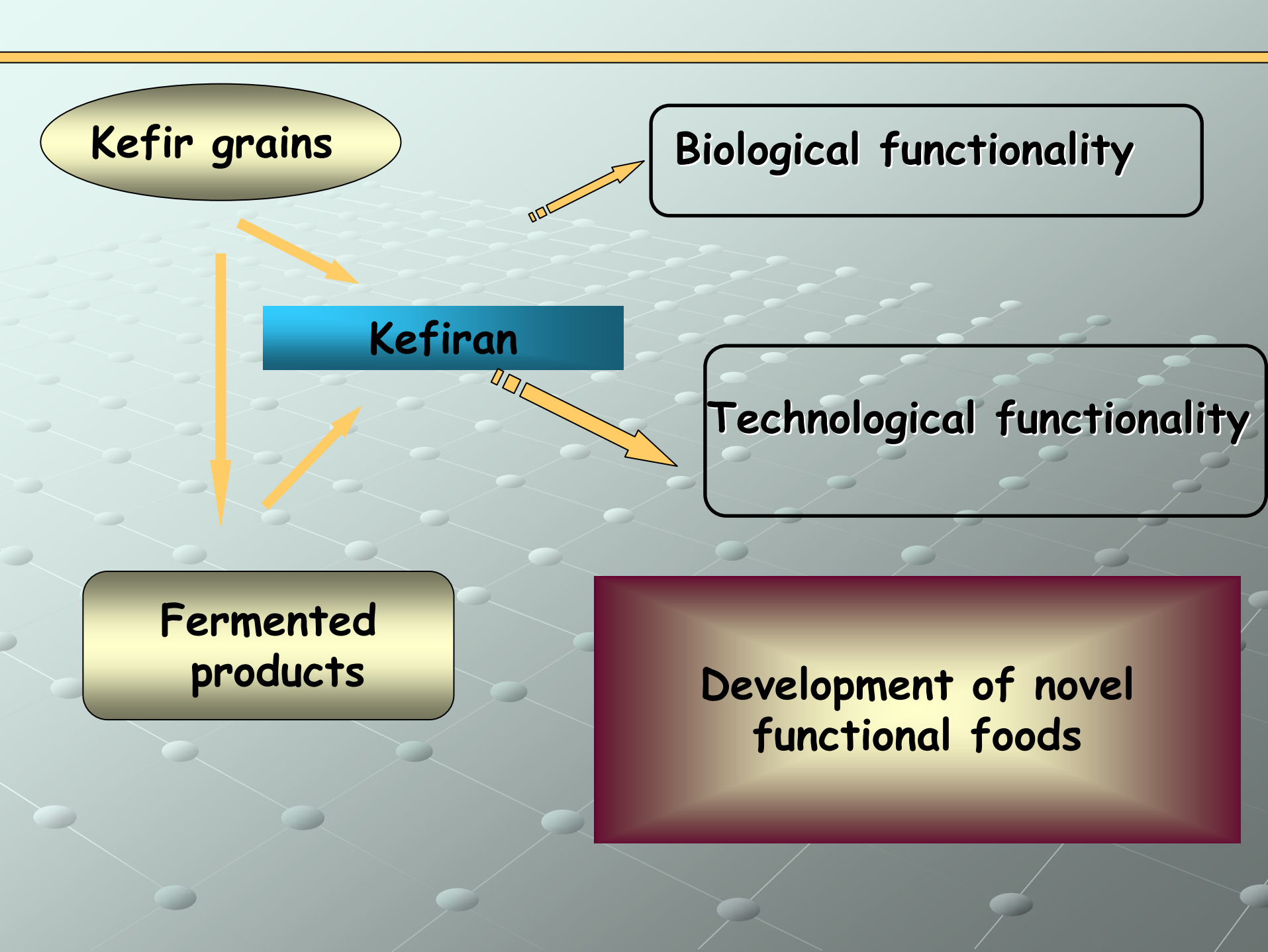
Biological functionality

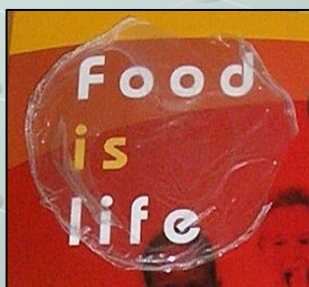
Kefiran

Technological functionality

Fermented products

Development of novel functional foods





Judith Piermaria
Micaela Medrano
Fernanda Hamet
Pablo Rimada
Jessica Minnaard
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Ivanna Rolny
Pablo Pérez
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