Impact of probiotic fermentation of food on their prebiotic activity and digestibility

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Fermented foods are traditionally used for human nutrition from centuries, all over the world.

Different types of raw materials are used as substrate for food fermentation processes.

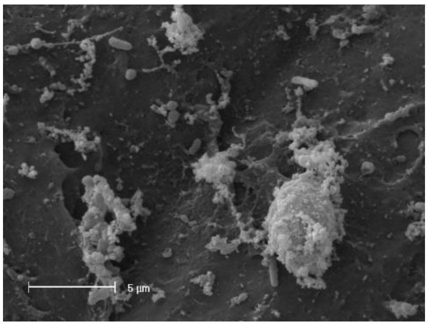
Fermentation is known to improve the nutritional value of the original substrate as well as to extend the so-called shelf-life.

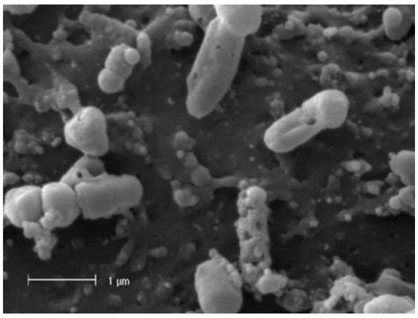
In addition to the effects of food, beneficial health effects are also traditionally attributed to fermented foods through the interaction of food with human gut microbiota and the positive impact that the improvement of the intestinal functions can have on the wellbeing of consumers.

However, the mechanisms underlying these beneficial effects have not been clarified yet.

What is clear is that bacteria used for fermenting foods are not *per se* beneficial to consumers, i.e. they are NOT *a priori* probiotics.

Carefully selected probiotics can be associated to different food substrates to maximize the properties of the substrate in terms of beneficial effect for special categories of population.



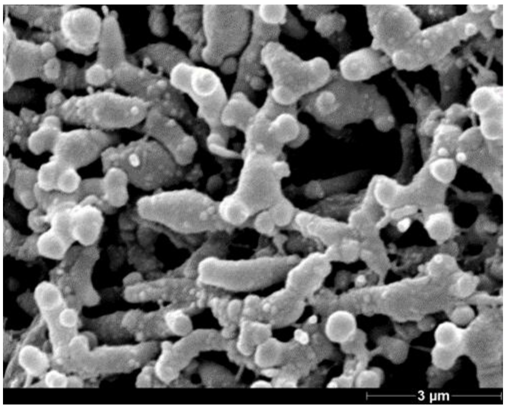


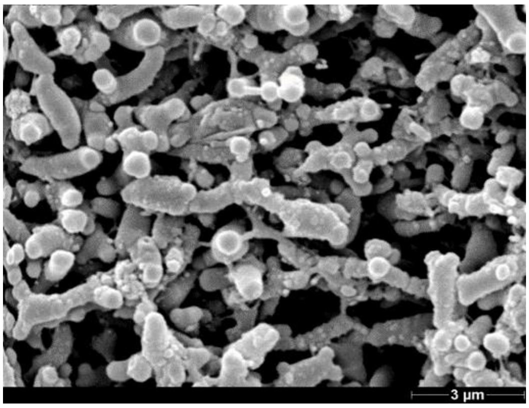
Here we introduce the area of POSTBIOTICS:

Postbiotics comprise metabolites and/or cell-wall components released by probiotics.

Postbiotics may, together with probiotics, improve host health.

Women, lactating mothers, baby during the weaning period usually suffer from an endogenous lack of bifidobacteria, a key group of bacteria whose beneficial functions mainly rely on the production of short chain fatty acids essential for the colonic functions. In order to promote the selective development of bifidobacteria, some prebiotics, such as fructo- and galacto-oligosaccharides are exogenously added to weaning foods





CASE STUDY:

Improvement of nutritional and prebiotic properties of oat by fermentation with probiotics

Development and patenting of a production process of oat fermented by selected probiotic strains in order to improve its prebiotic potential and nutritional value

Dealing with Oat

Oat (Avena sativa)

- Many health benefits that have been attributed to oat and oat products including lowering blood pressure and

  preventing radical damage to DNA, RNA, proteins, and

  cellular organelle.

- Oat contain soluble dietary fiber, unsaturated fatty acids,

  several vitamins and minerals, abundant antioxidant

  compounds, and have a well-balanced protein composition.

- The primary protein in oat is avenalin and this makes oats suitable for inclusion in a gluten free diet

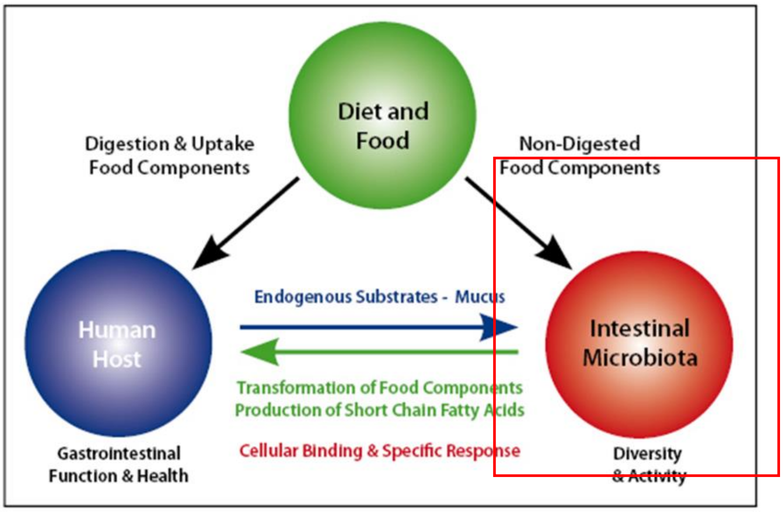
Beneficial effects of β-glucans from oat have been recognized by EFSA

The Panel concludes that a cause and effect relationship has been established between the consumption of oat beta-glucan and lowering of blood LDL-cholesterol concentrations. The following wording reflects the scientific evidence: "Oat beta-glucan has been shown to lower/reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of (coronary) heart disease".

The Panel concludes that a cause and effect relationship has been established between the consumption of oat and barley grain fibre and an increase in faecal bulk.

The Panel considers that in order to obtain the claimed effect a food should be at least "high in fibre" from oats or barley as per Annex to Regulation (EC) No 1924/2006. The target population is the general population.

First objective of the research



First goal was to ferment oat in order to obtain an ingredient able to act as prebiotic when added to formula milk

Exploiting the prebiotic potential of oat fermented by probiotic bacteria:

comparison of different selected probiotic blends

- Oat was fermented by three different blend of probiotics
- Then the fermented oat was dried
- The dried powder was then used as «prebiotic ingredient» to commercial formula milks.

Exploiting the prebiotic potential of oat fermented by probiotic bacteria:

▶ **comparison of different selected probiotic blends**

Reference bifidobacteria	Formula							
	brand A				brand B			
	Reference formula	1% fermented ingredient			Reference formula	1% fermented ingredient		
		BLEND A	BLEND B	BLEND C		BLEND A	BLEND B	BLEND C
Bif. breve LMG S-29966	7,64E+07	2,12E+08	2,61E+08	1,96E+08	4,73E+07	1,18E+08	9,64E+07	2,66E+08
Bif. breve LMG P-30999	4,09E+07	3,64E+08	1,29E+08	3,45E+08	2,55E+07	1,47E+08	1,39E+08	1,06E+08

B. breve LMG S-29966 and *B. breve* LMG P-30999 were used as reference strains to measure the prebiotic effect.

▶ **comparison of different selected probiotic blends during *in vitro* stool fermentation**

Carbon source	Count per gr	log	Growth gain (log10)
Glucose	4,74E+08	8,7	1,1
NF oat powder	1,57E+08	8,2	0,6
Blend A	2,21E+08	8,3	0,7
Blend B	3,17E+08	8,5	0,9
Blend C	4,45E+08	8,6	1,0
T0	3,88E+07	7,6	₩

Quantification of bifidobacteria in stools by comparing glucose as conventional carbon source or non fermented oat versus the fermented oat based ingredients.

Time zero concentration was also measured as well as value obtained in the presence of non-fermented natural ingredient.

Counts obtained by Q-PCR

An *in vitro* dose-response study

Quantification of bifidobacteria in FM

Data in table compare different final concentrations of the fermented ingredient.

Glucose was used as positive control as conventional carbon source.

Time zero level of bifidobacteria was also measured as well as the value obtained in the presence of non-fermented ingredient (used as negative control).

	CFU/ml	Log10	Growth gain (log10)
T0	4,20E+07	7,6	/
T48 NF powder	5,90E+07	7,8	/
T48 glucose	1,30E+09	9,1	1,3
T48 0.5% ingr	8,70E+08	8,9	1,2
T48 1% ingr	1,00E+09	9	1,2
T48 1.5% ingr	1,30E+09	9,1	1,3
T48 2% ingr	1,50E+09	9,2	1,4
T48 3% ingr	2,10E+09	9,3	1,5
T48 5% ingr	1,60E+09	9,2	1,4

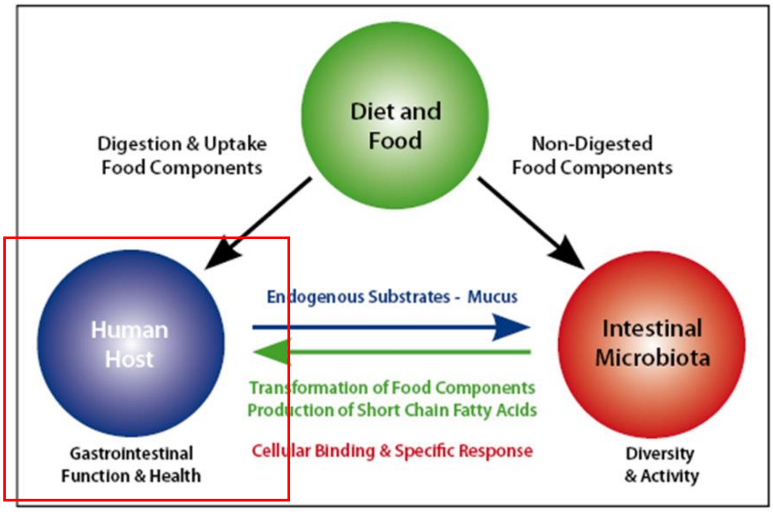
A positive side-effect: reduction in anti-nutrients (phytates) by fermentation

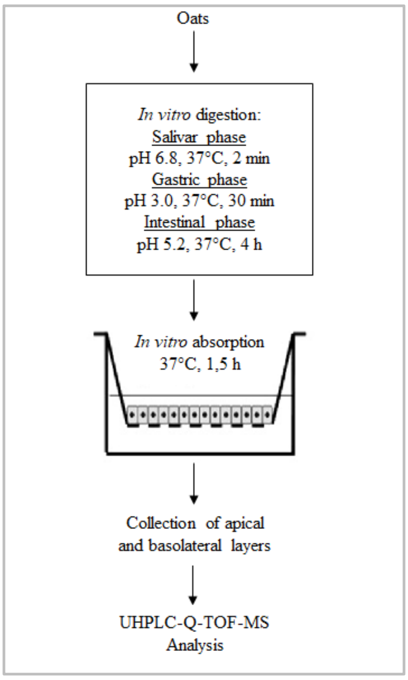
Decrease in the final concentration of phytates in fermented ingredient

Substrate	Phytate (g/100g)
Non-fermented oat	2,98
Fermented oat blend A	1,34
Fermented oat blend B	1,17
Fermented oat blend C	1,44

Microbe-to-Human relationships mediated by food:

focus on human host



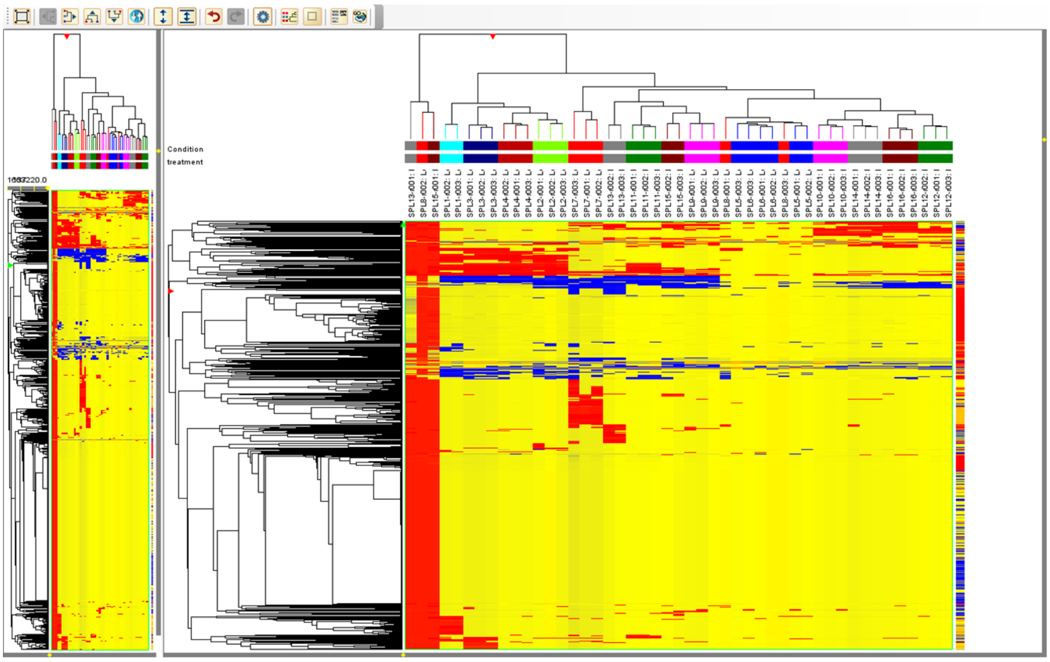


(Modified from Netzel et al. 2011, Food Res Int vol 44(4):868-874)

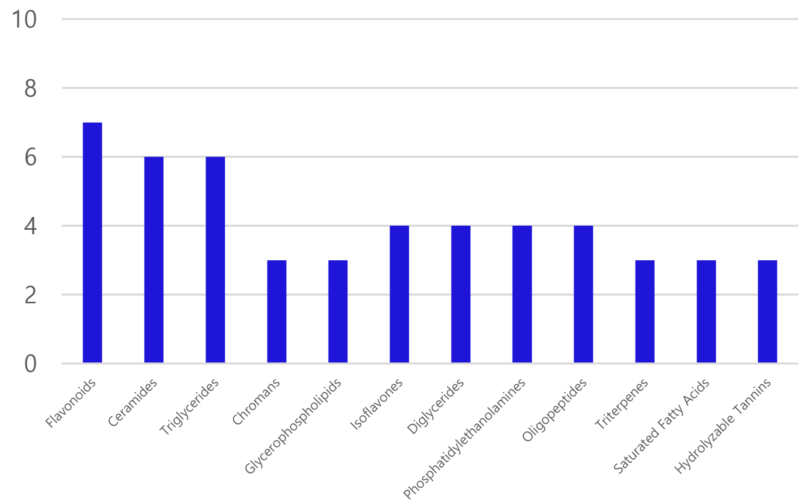
Metabolomic analysis: a hierarchical analysis of *in vitro* simulation of digestion and absorption.

The profile of absorbed compounds is similar among different samples both raw or processed ones.

Similitude of absorbed metabolites by enterocytes-type cell lines.



Flavonoids
Ceramides
Triglycerides
Chromans
Glycerophospholipids
Isoflavones
Diglycerides
Phosphatidylethanolamines
Oligopeptides
Triterpenes
Saturated Fatty Acids
Hydrolyzable Tannins

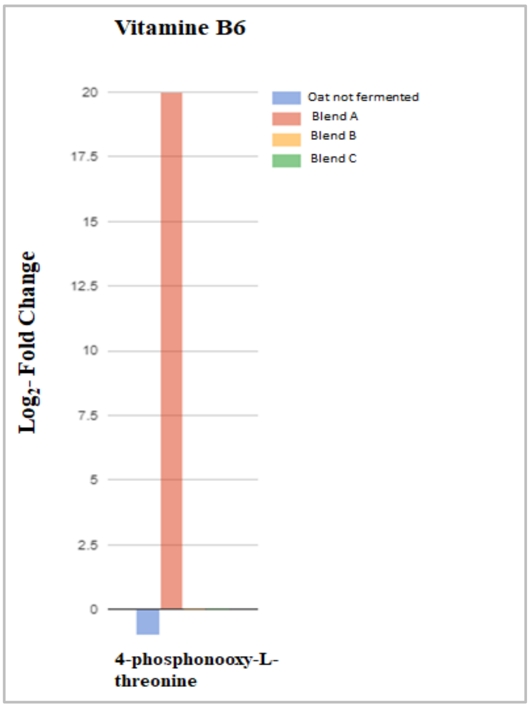


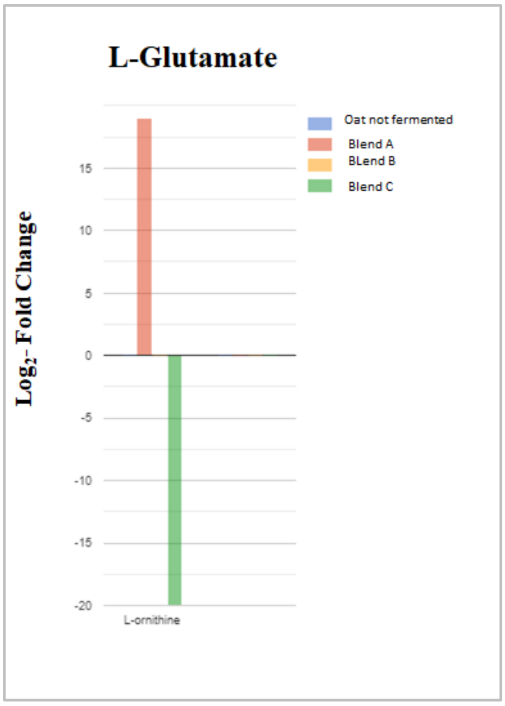
Metabolomic analysis: focusing on single metabolite = a blend related effect

Different absorption by enterocyte cells

of compounds involved in the biosynthesis

of important co-factors, like vitamins and amino acids.





To conclude

- ❑ **PROBIOTIC BACTERIA CAN INTERACT WITH FOOD MATRIX IN A POSITIVE WAY**
- ❑ **THIS INTERACTOIN CAN RESULTS IN A PREBIOTIC EFFECT BUT ALSO IN A BETTER ABSORPTION PROFILE**
- ❑ **FOOD MATRIX FERMENTATION CAN THEN RESULT IN TO A *SYNBIOTIC* FOOD**