INFLUENCE OF CHITOOLIGOSACCHARIDE DERIVATIVES OBTAINED BY MAILLARD REACTION ON THE GROWTH OF PROBIOTIC BACTERIA

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Introduction

Recent advances in the study of prebiotics are addressed to the design of new oligosaccharides able to reach unaltered the colon distal regions and to promote the growth of specific probiotic bacteria. Chitooligosaccharides (COS) obtained from chitosan posses antimicrobial properties due to the presence of amino groups in its structure. Chemical modification of COS by substitution of their amino groups could eliminate this antimicrobial effect and convert chitosan in a new interesting prebiotic ingredient. Although these kind of modifications have already been carried out [1,2] the evaluation of these COS derivatives as potential prebiotic ingredients has not been yet investigated.

Objective

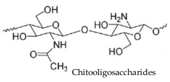
The aim of this work has been the study of the effect of COS modification by Maillard reaction on the growth of several strains of *Bifidobacterium* and *Lactobacillus* spp.

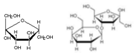
Material and Methods

DERIVATIVE SYNTHESIS

(Chung et al. (2005)) (2)

Chitooligosaccharides (COS) + Carbohydrate





Dissolution in water at 2% (w/v)

Incubation (121ºC, 20 min)
↓
Dialysis (96 h)
↓
Lyophilization
↓
Chitooligosaccharide derivatives

CHITOOLIGOSACCHARIDE FERMENTATION

A. Culture preparation: strains and broth

Aerobic incubation:
- *L. casei LC-01*
- *L. casei LC-431*
- *L. paracasei L-26*
- *L. acidophilus LA-10*
- *L. acidophilus LA-5*
- *L. acidophilus Ki*
- *L. brevis*

Anaerobic incubation:
- *B. longum*
- *B. pseudolongum*
- *B. animalis BB-12*
- *B. animalis Bo*

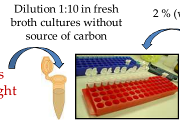
Broth culture: MRS without glucose + 0.05% cysteine

B. Inoculation

Cell cultures grown overnight at 37ºC

Dilution 1:10 in fresh broth cultures without source of carbon

2 % (w/v)



Chitooligosaccharide derivatives. Stock at 10% (w/v) in MRS broth

300 µL/well

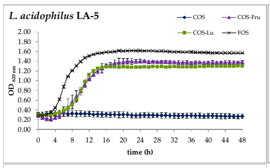
C. Measurement of optical densities at 620 nm (OD_{620})

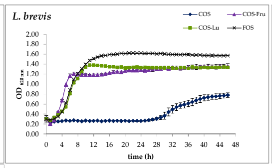
Aerobic cultures: values of OD_{620} recorded at 60 min intervals.
Anaerobic cultures: values of OD_{620} recorded at 0, 14, 24 and 48 h of fermentation. OD for *B. animalis* BB-12 *and B. animalis* Bo were recorded at 60 min intervals using paraffin to maintain anaerobic conditions. Treatment of data was carried out using the software DMfit v.2.1.

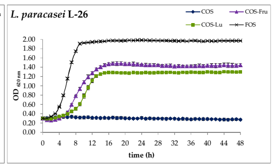
Results

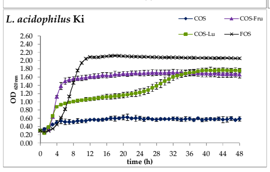
AEROBIC CULTURES: GROWTH RATES.

Figures show growth curves for all the aerobic strains tested with unmodified COS (—◆—) and COS derivatives , COS-Fru (—▲—) and COS-Lu (—■—). FOS (—✕—) were used as controls.

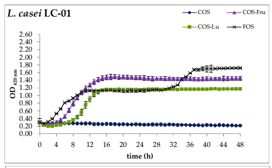


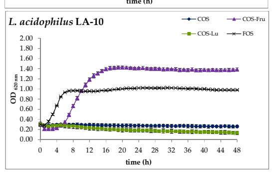






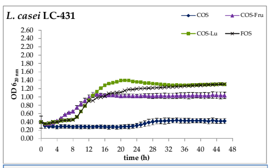
- ***L. acidophilus* LA-5, *L. paracasei* L-26, *L. brevis and L. acidophilus* Ki** showed a similar behaviour for the substrates under study. These strains were not able to use COS as a fermentable substrate whereas they were able to ferment both COS derivatives, COS-Fru and COS-Lu as source of carbon. Values of OD_{620} reached (at different times) were similar for COS derivatives.
- ***L. acidophilus* Ki** showed a slower growth rate than the other strains, reaching the maximum values of OD_{620} at 40 h of fermentation. COS were not used as a fermentable substrate.

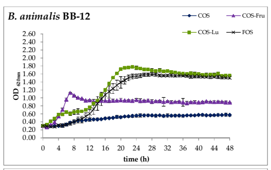




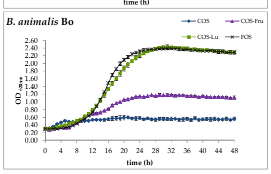
- ***L. casei* LC-01** *showed higher growth values for the derivative* COS-Fru.
- ***L. acidophilus* LA-10** was, also, able to ferment the COS-Fru derivative, however, it did not use COS-Lu as a source of carbon.

Values reached for OD using FOS as source of carbon were significantly lower than those reached using the COS-Fru derivative.





- ***L. casei* LC-431*, B. animalis* BB12 *and B. animalis* Bo** showed a similar behaviour for the derivatives under study. They preferibely grew using COS-Lu as source of carbon, specially *B. animalis* BB12 and *B. animalis* Bo.Regarding to COS, no growth was observed.
- ***B. animalis* Bo** showed the maximum OD for COS-Lu and FOS among all the tested bacteria.



ANAEROBIC CULTURES: VALUES OF OD_{620}

Table 1.- OD_{620} values after 14, 24 and 48 h of anaerobic growth with COS and COS derivatives

	B. pseudolongum				*B. longum*			
	0 h	14h	24h	48 h	0 h	14h	24h	48 h
COS	0.300	0.300	0.378	0.394	0.300	0.289	0.371	0.417
COS-Fru	0.300	0.374	0.343	0.320	0.300	0.387	0.354	0.364
COS-Lu	0.300	0.610	1.111	1.205	0.300	1.473	1.441	1.425

- ***B. pseudolongum*** reached maximum values of OD_{620} after 48 h of fermentation when COS-Lu was used as source of carbon. This strain was not able to grow on COS-Fru and COS.
- ***B. longum*** reached maximum values of OD_{620} after 14 h of fermentation. This strain, like *B. pseudolongum,* was not able to grow in COS-Fru and COS.

Conclusions

- ✓ **This study about the fermentation of COS derivatives by Maillard reaction by selected strains of *Bifidobacterium* and *Lactobacillus* spp. has been carried out for the first time.**
- ✓ **All the probiotic strains tested in this study were not able to use COS as carbon source. COS derivatives, however, were used by most of the tested strains. Modification by Maillard reaction converts COS into fermentable substrates by *Bifidobacterium* and *Lactobacillus* strains.**
- ✓ **Although more studies are necessary to evaluate the possible prebiotic effect of COS derivatives obtained by Maillard reaction, these preliminary results indicate that they are fermented by probiotic bacteria and that they could be good candidates to be used as prebiotics.**

References
[1]Yang, T. C., Chou, C. C., & Li, C. F. (2002). *Food Research International*, 35, 707–713
[2] Chung, Y.C., Wang, H.L., Chen, Y.M., Li, S.L., 2003. *Bioresource Technology*. 88 (3), 179–184.

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Assessment of the Bifidogenic Potential of Arabinoxylooligosaccharides derived from Wheat Bran

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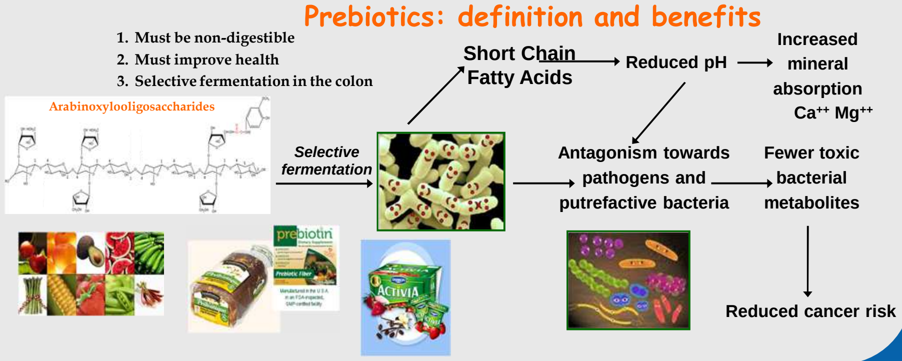
Background

The importance of human intestinal microbiota in maintaining host health is well known. Probiotics, prebiotics and the combination of these two components (synbiotics) can contribute to support an adequate balance of the bacterial population in the human large intestine.

From a nutritional point of view, Arabinoxylooligosaccharides (AraXOS) are considered as Non Digestible Oligosaccharides and one of its most important features as food ingredients is the ability to stimulate the growth of intestinal bifidobacteria.



Experimental

RAW MATERIAL

Wheat Bran is a byproduct of wheat flour milling, of a lignocellulosic nature, rich in arabinoxylan

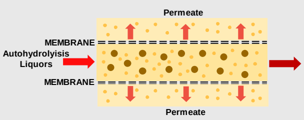
AUTOHYDROLYSIS

Reaction with hot, compressed water and Wheat Bran under optimized conditions

✓Hydrolytic degradation of arabinoxylan into AraXOS

✓Side processes leading undesired compounds: monosaccharides and non- saccharides

REFINING PROCESS



Membrane Technology

Ion Exchange

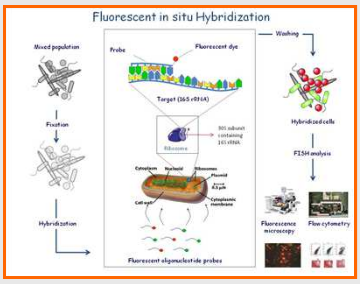
BIOACTIVE COMPOUNDS

Arabinoxylooligosaccharides (AraXOS)

(with similar purity to that of commercially available prebiotics)

In vitro FERMENTABILITY ASSESSMENT

MICROBIOTA ANALYSIS



Results

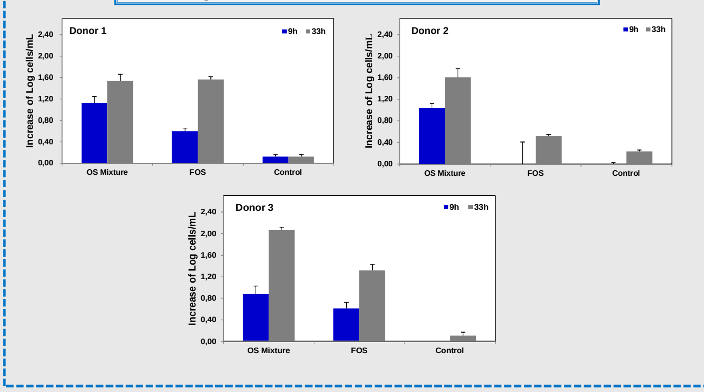
Composition of the purified mixture of oligosaccharides (OS) from Wheat Bran

Component	Mass fraction (g component/g [1]NVC)
[2]GlucOS	0.304
[3]XOS	0.362
[4]AOS	0.169
[5]AcOS	0.009
[6]OGaU	0.041
[7]ONVC	0.049

Total Oligosaccharides (TOS): 88.5% of NVC

[1]NVC: Non Volatile Compounds
[2]GlucOS: Glucooligosaccharides (as glucose)
[3]XOS: Xylooligosaccharides (as xylose)
[4]AOS: Arabinooligosaccharides (as arabinose)
[5]AcOS: Acetyl groups linked to oligosaccharides (as acetic acid)
[6]OGaU: Uronic acids linked to oligosaccharides (as uronic acids)
[7]ONVC: Other Non Volatile Compounds

Population Dynamics: *Bifidobacterium* in faecal cultures grown in mixture OS from Wheat Bran



Conclusions

The experimental results confirmed the ability of wheat bran (AraXOS concentrate) to promote the growth of *Bifidobacterium* population acting as carbon sources, leading mainly to the generation of acetic and lactic acid.

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COMPETE POPH QREN FCT Fundação para a Ciência e a Tecnologia

Prebiotic Potential of Oligosaccharides derived from Wheat Bran

Universidade de Vigo

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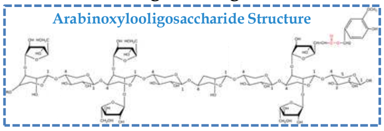
Introduction

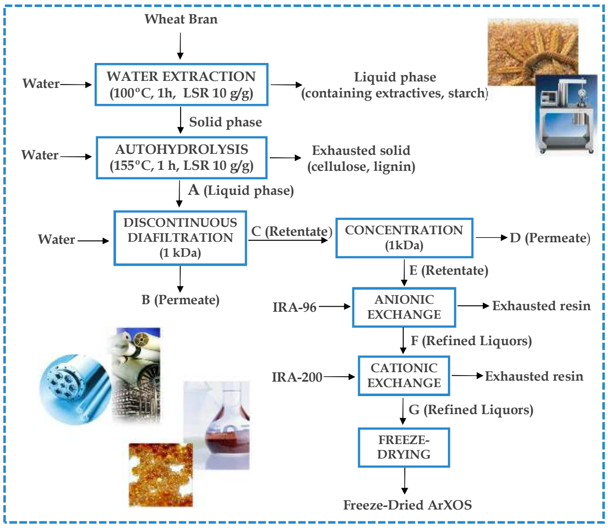
As it is well known, the human intestinal microbiota has significant effects on the host health, so that the interest in the maintenance of its balance and activity has fostered the research and development of new prebiotics and probiotics.

Non-Digestible Oligosaccharides (NDO) are the most known prebiotics. Oligosaccharides (OS) with prebiotic properties (including inulin, fructooligosaccharides (FOS), galactooligosaccharides (GaOS) and lactulose) are commercially available, and many others are under study. From a nutritional point of view, arabinoxylooligosaccharides (AraXOS) behave as NDO and are classified as "emerging prebiotics" owing to their potential in this field, although strong additional scientific evidence is necessary.

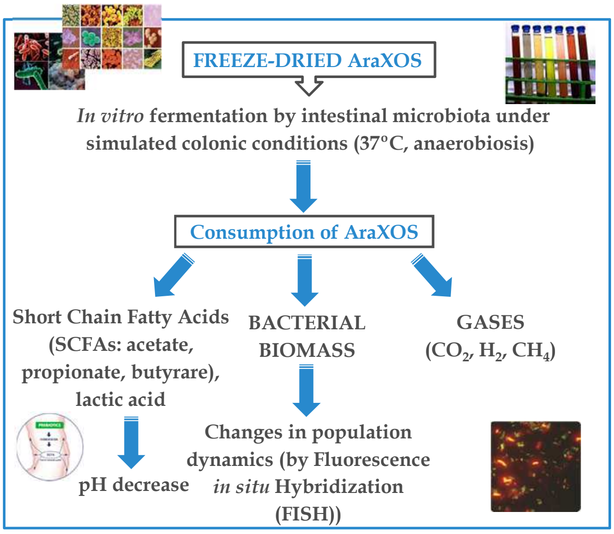


Methods

PROCESSING SCHEME FOR MANUFACTURING AraXOS



In vitro ASSESSMENT OF THE FERMENTABILITY BY HUMAN FAECAL INOCULA



Results

EFFECTS ACHIEVED WITH THE PURIFICATION SCHEME

Oligosaccharides in autohydrolysis liquors: xylooligosaccharides, Arabinosyl groups, Acetyl groups, Uronyl groups, glucooligosaccharides

0.64 g/g Non Volatile Compounds

Other Non Volatile Compounds

0.09 g/ g NVC

↓

Oligosaccharides in refined liquors: xylooligosaccharides, Arabinosyl groups, Acetyl groups, Uronyl groups, glucooligosaccharides

0.89 g/g Non Volatile Compounds

Other Non Volatile Compounds

0.00 g/ g NVC

STUDY OF *in vitro* FERMENTABILITY ON AraXOS MIXTURE

% OS Consumption	Donor 1	Donor 2	Donor 3
9 h	36	66	41.3
12 h	68.5	79.2	64.8
33 h	86.8	89.5	90.7

Total SCFA (mM)	Donor 1	Donor 2	Donor 3
9 h	36.1	70.4	45.0
12 h	64.7	86.7	70.8
33 h	86.3	99.7	98.9

pH	Donor 1	Donor 2	Donor 3
0 h	7.2	7.3	7.3
9 h	6.7	6.2	7.1
12 h	5.4	5.5	6.6
33 h	5.2	5.4	5.4

Conclusions

Based on the obtained results, it can be concluded that the AraXOS concentrate generated by using an environment friendly technology can be considered as a potential prebiotic; however, more scientific evidence is needed to confirm that these oligosaccharides are prebiotics.

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