Plant prebiotics in BLSS for human nutrition in space

Simona Proietti[1], simona.proietti@cnr.it
Stefano Moscatello[1] stefano.moscatello@cnr.it
Filomena Nazzaro[2] filomena.nazzaro@cnr.it
Walter Stefanoni,[3] walter.stefanoni@crea.gov.it
Alberto Battistelli[1] alberto.battistelli@cnr.it

1 Istituto di ricerca sugli ecosistemi terrestri. Consiglio Nazionale delle Ricerche Viale Marconi 2, 05010 Porano (TR) Italy
2 Istituto di scienze dall'alimentazione. Consiglio Nazionale delle Ricerche Via Roma, 64, 83100 Avellino (Italy).
3 Centro di ricerca Ingegneria e trasformazioni agroalimentari. Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria CREA Via della Pascolare 16, 00015 Monterotondo (RM) Italy

WHAT ARE WE TALKING ABOUT?
DEFINITION OF PREBIOTICS

https://isappscience.org/

nature reviews gastroenterology & hepatology

Open Access | Published: 14 June 2017

Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics

Glenn R. Gibson, Robert Hutkins, Mary Ellen Sanders, Susan L. Prescott, Raylene A. Reimer, Seppo J. Salminen, Karen Scott, Catherine Stanton, Kelly S. Swanson, Patrice D. Cani, Kristin Verbeke & Gregor Reid

Nature Reviews Gastroenterology & Hepatology **14**, 491–502(2017) | Cite this article

37k Accesses | **906** Citations | **349** Altmetric | Metrics

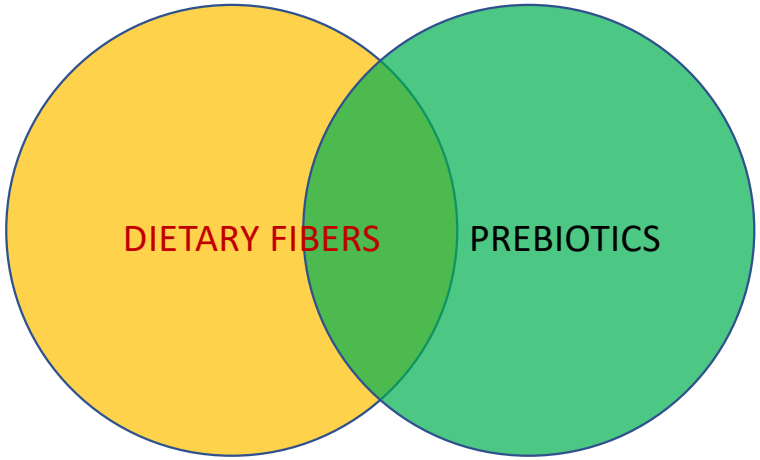
Current accepted definition 2016

“a substrate that is selectively utilized by host microorganisms conferring a health benefit”

A WIDE OPEN DEFINITION

- Many microbe species
- Many host organisms
- Many target organs
- Many target functions
- Many candidate molecules
- …….

GUT MICROBIOTA



Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

Developments in understanding and applying prebiotics in research and practice—an ISAPP conference paper

K.P. Scott[1], R. Grimaldi[2], M. Cunningham[3], S.R. Sarbini[4], A. Wijeyesekera[2], M.L.K. Tang[5], J.C.-Y. Lee[6], Y.F. Yau[6], J. Ansell[7], S. Theis[8], K. Yang[9], R. Menon[10], J. Arfsten[11], S. Manurung[12], V. Gourineni[13] and G.R. Gibson[2]

Table 1 Adapted from Joshi *et al.* (2018)

Confirmed prebiotics	Food Source Content of specific prebiotic fibre (%)
Galacto-oligosaccharides (GOS)	β-GOS produced enzymatically from lactose
Fructo-oligosaccharides (FOS)	Asparagus (5%), leeks (11·7%), garlic (17·5%),
Inulin	Chicory (64·4%), onion (8·6%), Jerusalem artichoke (31·5%), wheat (2%), banana (1%)
Lactulose	Synthetic disaccharide

But the list is growing: polyphenols…

Polyphenols, the new frontiers of prebiotics.

Nazzaro F[1]. Fratianni F[1]. De Feo V[2]. Battistelli A[3]. Da Cruz AG[4]. Coppola R[5]

Advances in Food and Nutrition Research, 07 Jul 2020, 94:35-89

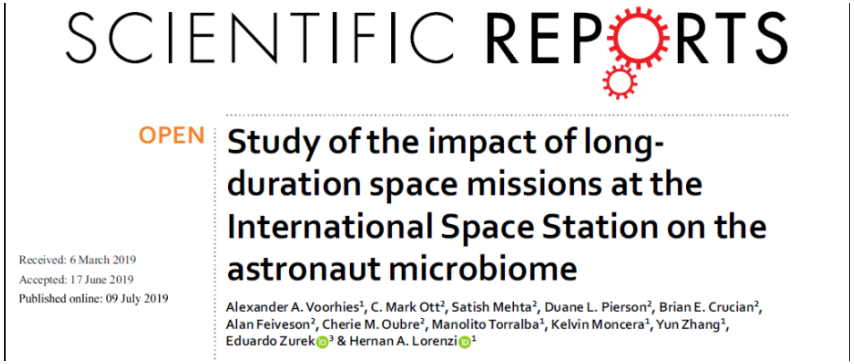
DOI: 10.1016/bs.afnr.2020.06.002 PMID: 32892838

INTERNATIONAL SCIENTIFIC ASSOCIATION FOR PROBIOTICS AND PREBIOTICS

https://isappscience.org/

Candidate prebiotics	
Soy, Soybean oligosaccharides	Soybeans (2·3 stachyose, 7 raffinose)
Pectin	Cell wall component of many fruits
Cellulose	General component of plant cell walls
Resistant starch	Multiple food sources (corn, potato, tapioca, etc.)
Xylan, Xylo-oligosaccharides, Arabinoxylo-oligosaccharides	Wheat bran
Mannose	Many fruits and vegetables
Maltose, Malto-oligosaccharides	Breakdown products from starch
Isomaltulose	Honey, sugarcane juice, sucrose
Palatinose	Patented form of isomaltulose, made from beet sugar
Polydextrose	Synthetic fibre
Raffinose oligosaccharides	Lentils (0·16%), peas (0·5%), beans (0·33%), chickpeas (0·4%)
β-glucans	Soluble fibre found in oats and barley cereals (3–6%)

ASTRONAUT MICROBIOME IS AFFECTED DURING SPACE MISSIONS



SCIENTIFIC REPORTS

OPEN

Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome

Received: 6 March 2019
Accepted: 17 June 2019
Published online: 09 July 2019

Alexander A. Voorhies[1], C. Mark Ott[2], Satish Mehta[2], Duane L. Pierson[2], Brian E. Crucian[2], Alan Feiveson[2], Cherie M. Oubre[2], Manolito Torralba[1], Kelvin Moncera[1], Yun Zhang[1], Eduardo Zurek[3] & Hernan A. Lorenzi[1]

…. then ***treatments known to block inflammation in the GI, such as the administration of prebiotics or treatment with the probiotic bacteria A. muciniphila, could be implemented in space to reduce the risk of diseases associated with chronic inflammatory responses****. …..*

EXPERIMENTS WITH PREBIOTICS ARE CONDUCTED ON BOARD THE ISS

npj | Microgravity

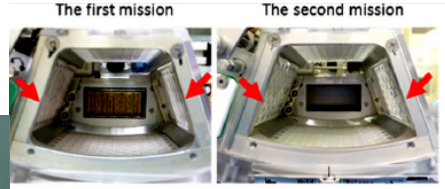
www.nature.com/npjmgrav

BRIEF COMMUNICATION OPEN

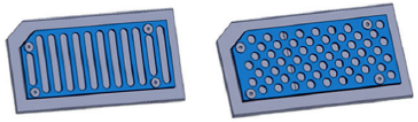
Dietary intervention of mice using an improved Multiple Artificial-gravity Research System (MARS) under artificial 1 *g*

Chie Matsuda[1], Tamotsu Kato[2,3], Sayo Inoue-Suzuki[4,2], Jun Kikuchi[5,3], Toshiko Ohta[1,2,4], Masaharu Kagawa[4], Masahira Hattori[2,6], Hiroe Kobayashi[1], Dai Shiba[7], Masaki Shirakawa[7], Hiroyasu Mizuno[7], Satoshi Furukawa[1], Chiaki Mukai[1] and Hiroshi Ohno[2,3]

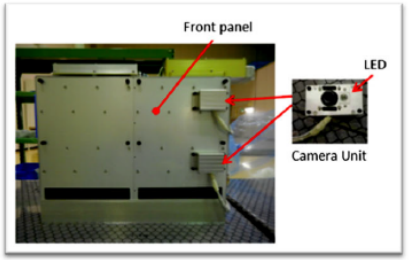
(http://www.understandinganimalresearch.org.uk)



Habitat Cage Unit (HCU)



Cover of paper sheet



Improved Transportation Cage Unit (TCU)

EXPERIMENTS WITH PREBIOTICS ARE TARGETED TO BLSS

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

Relationship between the Gut Microbiome and Energy/Nutrient Intake in a Confined Bioregenerative Life Support System

Juanjuan Chen,[a,b] Qi Wang,[b,c] Zikai Hao,[d] Zhongxia Li,[b] Sunil Kumar Sahu,[b] Hong Liu,[d] Liang Xiao[b]

[a]School of Biology and Biological Engineering, South China University of Technology, Guangzhou, China
[b]BGI-Shenzhen, Shenzhen, China
[c]BGI Education Center, University of Chinese Academy of Sciences, Shenzhen, China
[d]Institute of Environmental Biology and Life Support Technology, School of Biological Science and Medical Engineering, Beihang University, Beijing, China

….In addition, alterations in …. such as the synthesis of propionate, butyrate, and glutamate, in our study also indicated ***that the gut microbiome may play a role in mental disorders such as anxiety … and depression … in those confined in the BLSS****….*

EDEN ISS

HORIZON 2020

European Commission

ID: 636501 https://eden-iss.net/

Preliminary results in previous projects

Raffinose, stachyose and total NSC content on produce obtained in the FEG. (mg 100 g FW^{-1})

	Raffinose + Stachyose		Total Carbohydrate	
	Mean	s.e.	Mean	s.e.
Lettuce *cv* Batavia Normal light	6,6	0,9	652,7	73,0
Lettuce *cv* Batavia Increased light			1027,5	97,2
Lettuce *cv* Outredgeous Normal light	18,1	6,8	614,0	38,8
Lettuce *cv* Outredgeous Increased light	27,4	2,9	988,1	54,4
Rocket *cv* coltivata	7,24	0,5	767,8	233,8
Radish leaves	5,34		640,9	291,3
Swiss chard	3,7	1,1	157,6	69,1
Mustard *cv* Red giant	3,1	0,4	234,3	49,1
Mustard *cv* Frizzy Lizzy	3,2	0,2	102,8	16,9

INT. GEN. X ENV.

MIN. 0,8 % of NSC

MAX. 3,1 % of NSC

EDEN ISS

Preliminary results in previous projects

HORIZON 2020

European Commission

ID: 636501

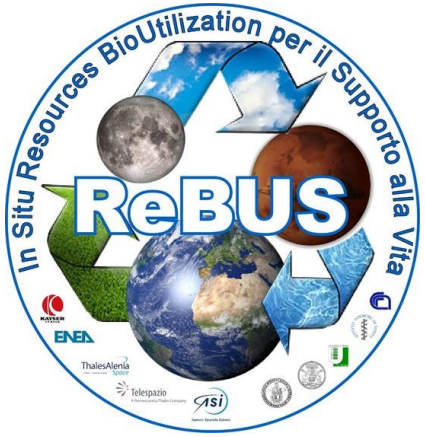
FOS in different lettuce tissues obtained in the FEG facility during the mission in Antarctica. (% of the DW).

Lettuce variety and tissue	% of Fructo-Oligosaccharides	
	Mean	s.e.
Batavia Leaf	0,84	0,01
Batavia Root	1,97	0,27
Outredgeous Leaf	1,55	0,19
Outredgeous Root	1,23	0,63

INT. GEN. X TISSUE

CONCLUSIONS

Limited amounts on vegetables not selected for prebiotic content



The new project

In-situ REsource Bio-Utilization for life Support system (ReBUS)

Agenzia Spaziale Italiana

Agenzia Spaziale Italiana

WP 5000 CNR

WP 5200 Prebiotics in plants and waste materials

Production and analysis of materials → Prebiotics

Prebiotics ↓

WP 6000 ISS

WP 6100 Prebiotics and well being: animal model and study proposal on board the ISS

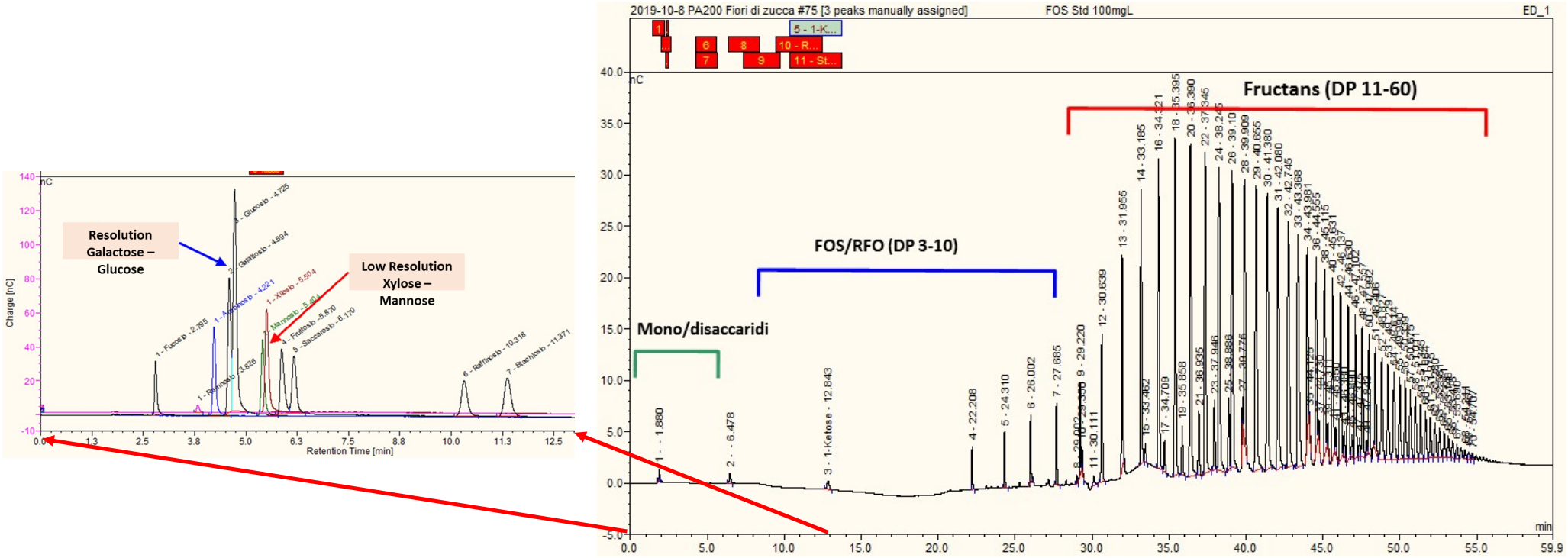
Simone Macrì & Francesca Zoratto
Centre for Behavioral Sciences and Mental Health
Istituto Superiore di Sanità (ISS), Roma

Rationale

→Support the hypothesis that the prebiotic properties of fructans, extractable from vegetables grown in BLSS, have a positive effect on individual psychophysical well-being

METHODS UPGRADE

CarboPac PA 200 in HPAEC-PAD - (ICS-5000 ThermoScientific)



Chromatographic profile of polysaccharides analysed by IC:

- degree of polymerization (DP)
- quantification of the FOS

(through mild acid hydrolysis to obtain the cleavage of glucosidic bonds of fructo-oligosaccharides in glucose and fructose monomers)

First step:
Selection of species that:

- Are small in size
- Fast growing
- Known to be edible (the full plant!)
- Contain a lot of prebiotics
- Can grow nicely in fully controlled environment
- Respond to modulation of growth conditions

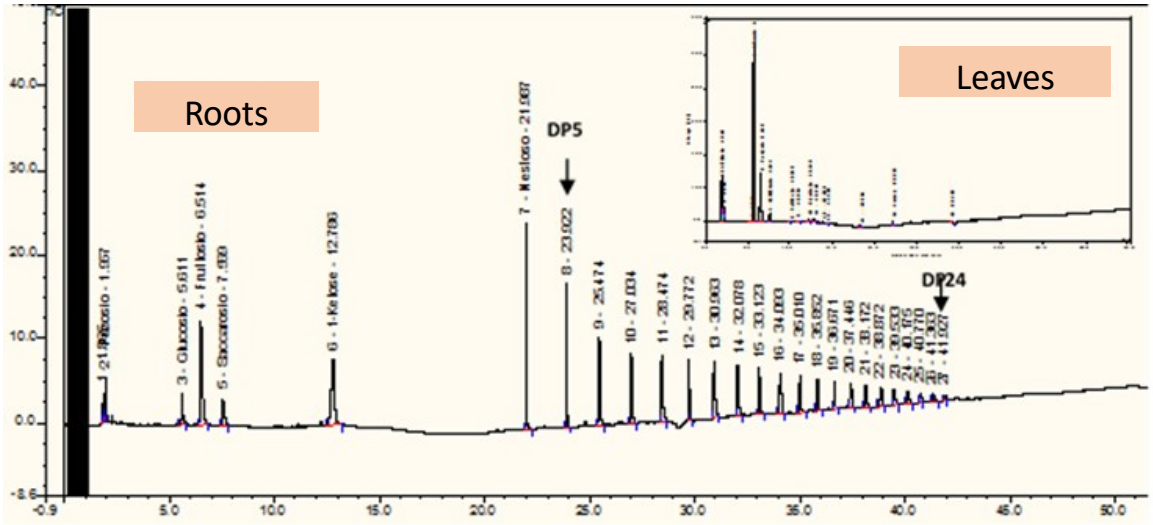
Fructo-oligosaccharides (FOS) in different plant species, tissues and development stages

Plant species	Tissue and development stage
Dandelion (*Taraxacum officinale*)	Leaves and roots of plant
Raponzolo (*Campanula rapunculus*)	Leaves and roots of plant

Dandelion (*Taraxacum officinalis*)

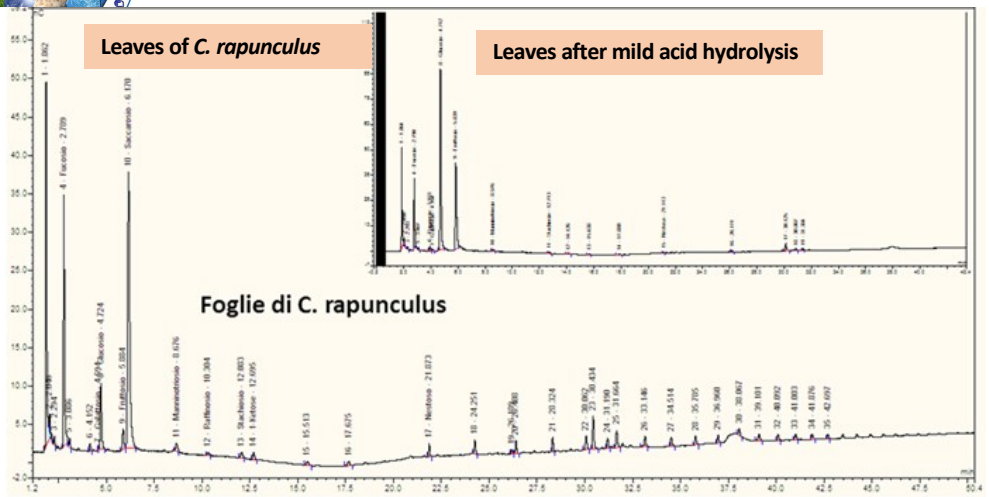
Raponzolo (*Campanula rapunculus*)

Dandelion (*Taraxacum officinale*): leaves and roots

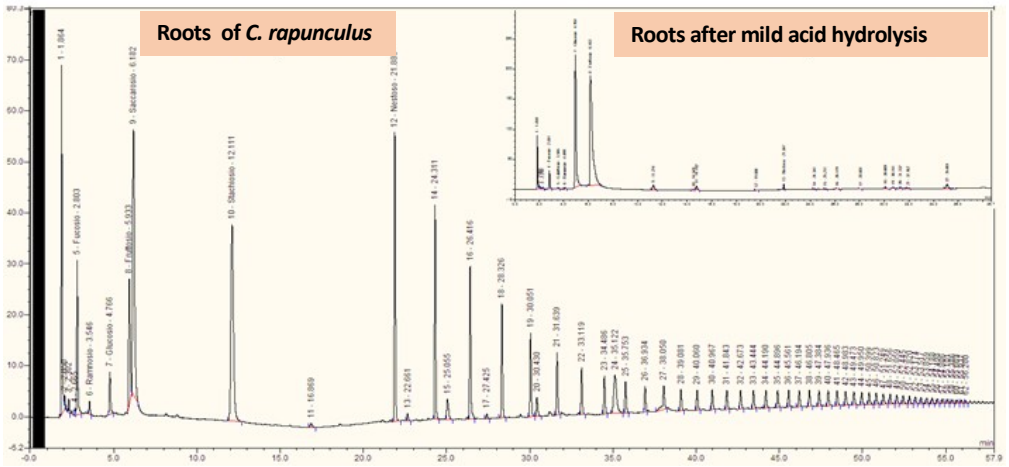


Carbohydrate content on leaves and roots on dandelion (Total content of soluble carbohydrate in roots and leaves = 4,0 % of the DM)						
	Glucose	Fructose	Sucrose	Raffinose	1-Kestose	Nestose
Root	2,7	36,8	10,0	0,4	22,1	28,0
Leaf	43,2	44,3	7,2	0,5	3,3	1,5

Campanula rapunculus: leaves and roots



THE WHOLE PLANT IS EATEN



Fructans in *Campanula rapunculus*
DP an content as % of the DW

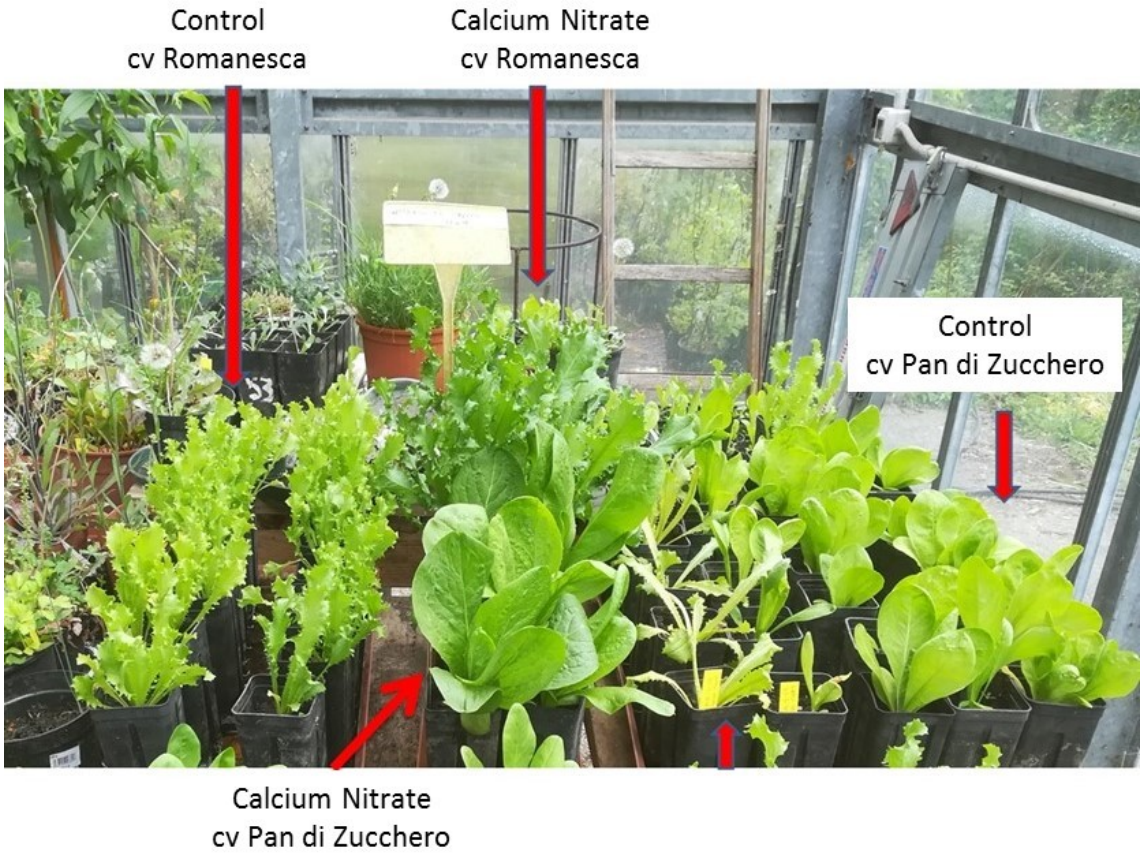
	DP		Fructans % (mainly FOS)	
	mean	s.e	mean	s.e
Leaf	8,10	2,15	1,11	0,17
Root	16,91	0,63	14,80	0,18

Second step:

Control of fructans accumulation:

- Nitrogen
- Light

Cultivated species (*Cichorium*) with high fructans content
Effects of growth conditions (N availability) on prebiotics content



In greenhouse: leafy vegetables accumulating fructans:

Cichorium intybus cv Pan di zucchero
Cichorium endivia cv Romanesca
Treatments: Calcium Nitrate (12 mM)

LIMITED ENVIRONMENTAL CONTROL

Fructo-oligosaccharides contents of *Cichorium* varieties

LIMITED ENV. CONTROL

EFFECT OF N TREATMENT

Cichorium intybus cv Pan zucchero

	Control		N treatment	
TAP ROOTS	**mean**	**se**	**mean**	**se**
Fructans % (g 100 g F.W.$^{-1}$)	7,9	0,5	6,4	1,1
Fructans (mg plant^{-1})	82,16	2,1	64,64	3,7
LATERAL ROOTS	**mean**	**se**	**mean**	**se**
Fructans % (g 100 g F.W.$^{-1}$)	2,4	0,3	1,4	0,3
Fructans (mg plant^{-1})	158,4	3,4	81,62	2,2
Fructans (mg plant^{-1})	240,56		146,26	

Cichorium endivia cv Romanesca

	Control		N treatment	
TAP ROOTS	mean	se	mean	se
Fructans % (g 100g F.W.$^{-1}$)	5,9	0,8	10,8	0,5
Fructans (mg plant^{-1})	48,97	2,1	258,12	10,3
LATERAL ROOTS	**mean**	**se**	**mean**	**se**
Fructans % (g 100 g F.W.$^{-1}$)	1,8	0,4	3	0,3
Fructans (mg plant^{-1})	217,26	9,1	448,5	4,0
Fructans (mg plant^{-1})	266,23		706,6	

NITROGEN EFFECT MIGHT DEPEND ON SPECIES AND ORGANS

Fructo-oligosaccharides contents of *Cichorium* varieties

EFFECT OF LIGHT INTENSITY

HIGH ENV. CONTROL

Cichorium intybus cv Cicoria di Chiavari				
Fructans in the TAP ROOT				
	% (DW basis)		g plant^{-1}	
	mean	se	mean	se
HIGH LIGHT	57,5	3,7	4,5	0,9
LOW LIGHT	55,6	3,2	2,6	0,8

GROTH LIGHT INTENSITY MIGHT FAVOUR FRUCTANS PRODUCTIVITY VIA PROMOTION OF GROWTH

Conclusions

- Prebiotic producing vegetables can be included in BLSS as leafy-root vegetables
- Species selection is still an open question although promising candidate are available
- Physiology (and molecular biology) of prebiotics accumulation is a largely open question
- Growth variables in BLSS can help controlling prebiotics accumulation and productivity
- The role of prebiotic rich vegetables on human (and animal) psychophysical well-being is a relevant research topic for both the Earth and Space


THANK YOU FOR YOUR ATTENTION