



CATÓLICA PORTO
BIOTECNOLOGIA

Bioacessibilidade de fitoquímicos e impacto na microbiota

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**Current
consumer**

**Better health status
Reduce risk of disease**

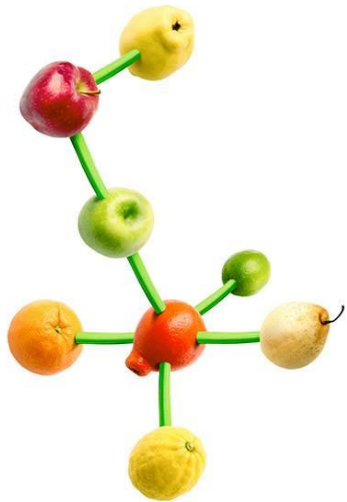
**Functional
Foods**



Bioactive Ingredients/additives

Limitations

Advantages



Functionality
Interaction with Food or GIT

Bioaccessibility

Bioavailability

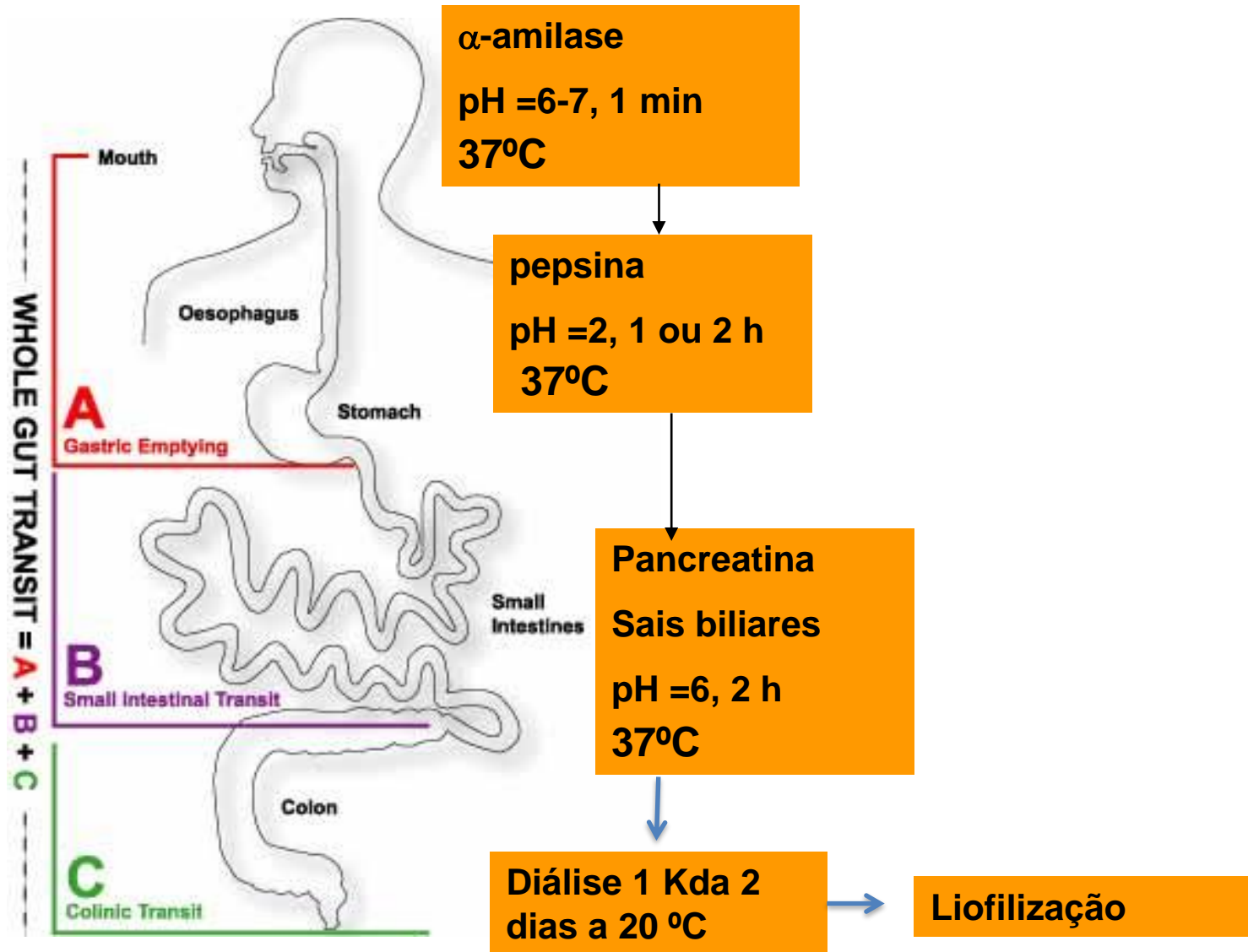
Foods with new features

Increase food safety and shelf-life

Promote human health

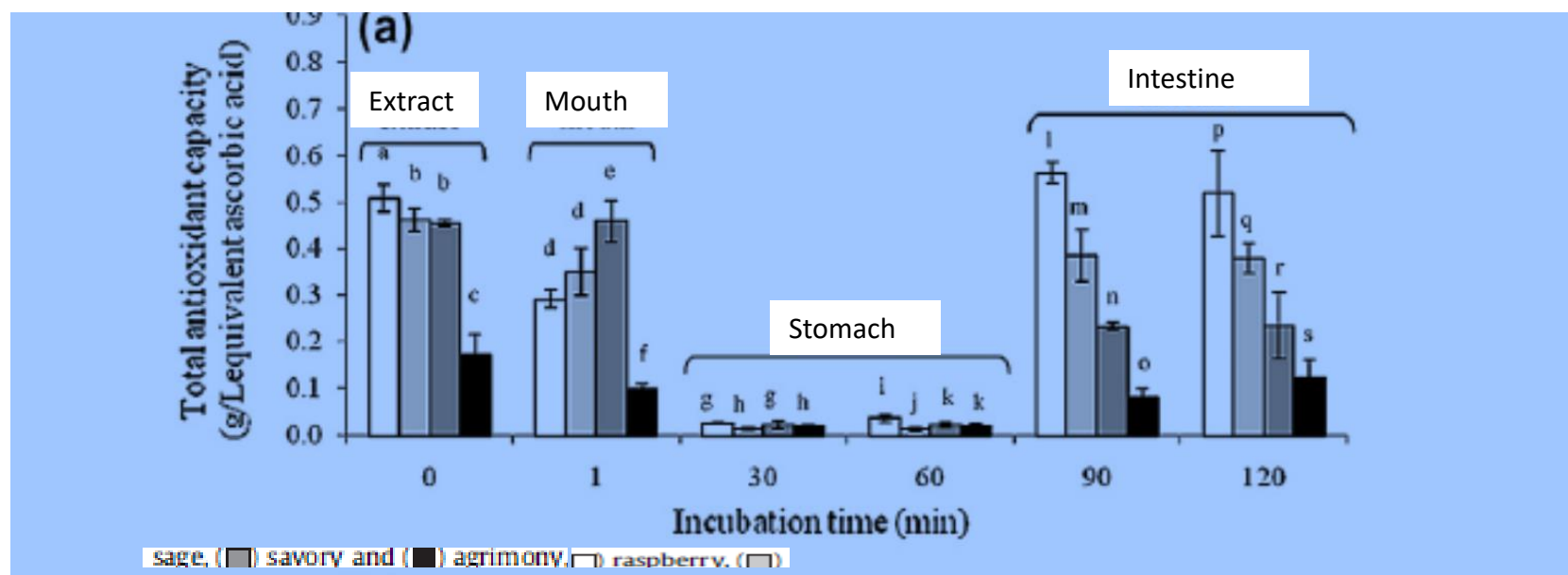
Reduce risk of disease

Biodisponibilidade no tracto gastrointestinal

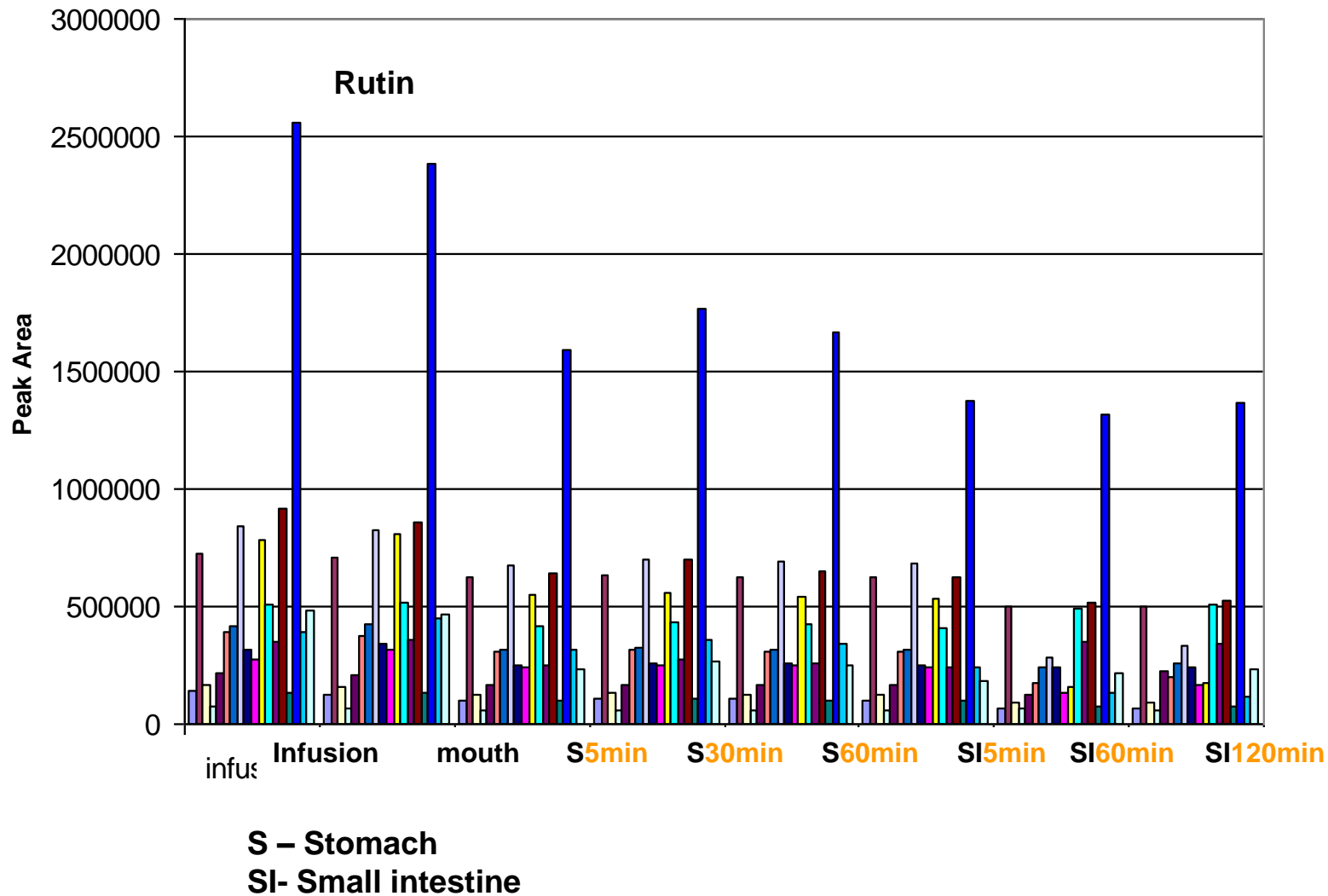


Effect of *in vitro* digestion upon the antioxidant capacity of aqueous extracts of *Agrimonia eupatoria*, *Rubus idaeus*, *Salvia sp.* and *Satureja montana*

Maria S. Gião^a, Susana Gomes^a, Ana R. Madureira^a, Ana Faria^{b,c}, Diogo Pestana^b, Conceição Calhau^b, Manuela E. Pintado^a, Isabel Azevedo^b, F. Xavier Malcata^{d,e,f,*}



Raspberry



Effect of processing and storage on the nutritive and functional properties of fruits — strawberry and peach

Fruit Yoghurt



28 d

4° C

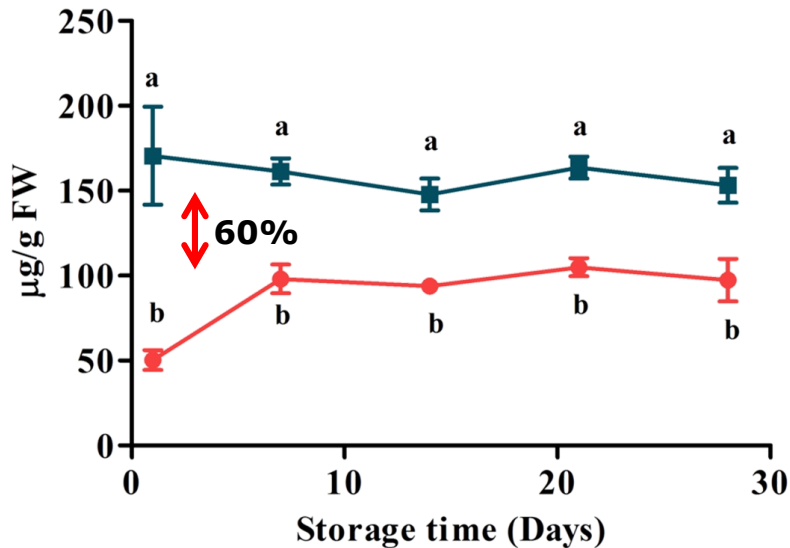
Extractions

Effect of processing and storage on the nutritive and functional properties of fruits — strawberry and peach

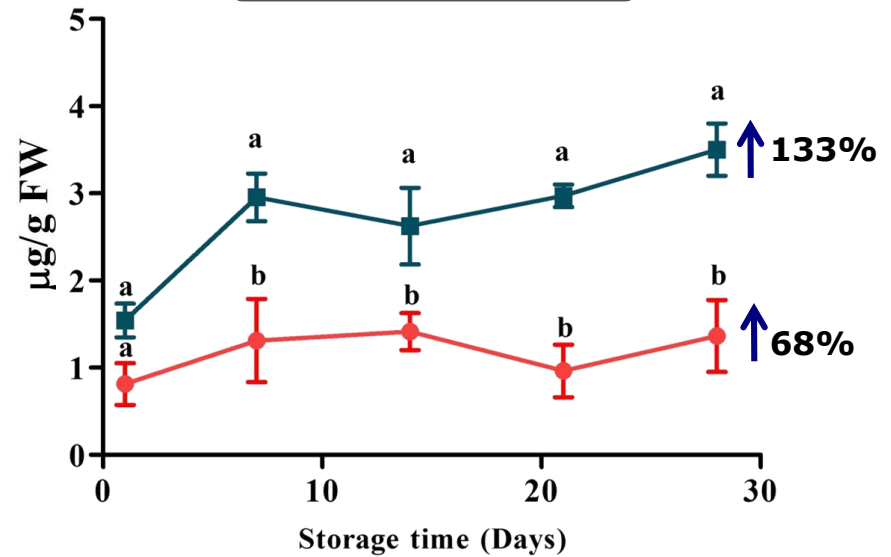
Fruit Yoghurt		Antioxidant activity	Total phenolics	Anthocyanins/ Carotenoids
Strawberry		↓	↓	↓
Peach		↓	↓	↑

—●— Peach yogurt
—■— Peach prepare

(+)-Catechin



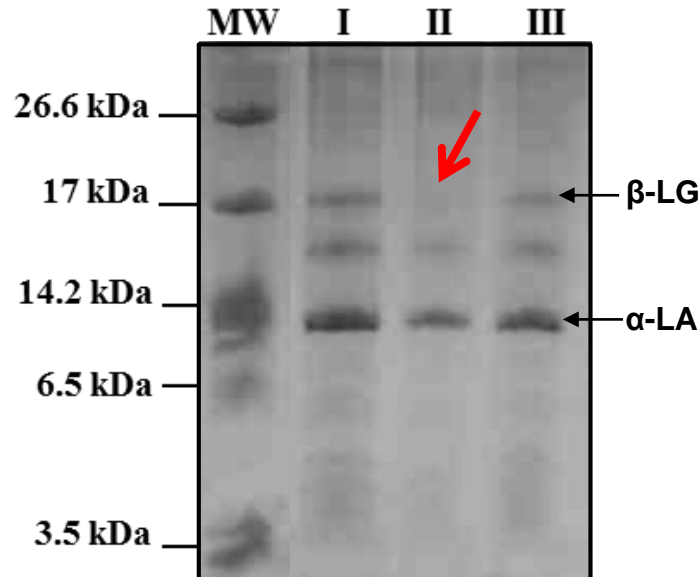
Lutein+zeaxanthin



Effect of processing and storage on the nutritive and functional properties of fruits — strawberry and peach

Fruit Yoghurt

Strawberry yoghurt



I- Yoghurt

II- Yoghurt with strawberry prepare with hydrocolloid

III- Yoghurt with strawberry prepare without hydrocolloid

Effect of processing and storage on the nutritive and functional properties of fruits — strawberry and peach

Fruit Yoghurt

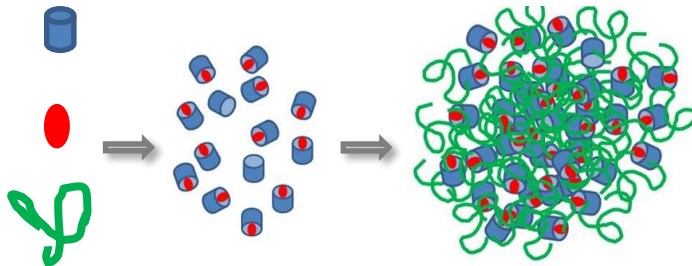


Model system of phenolics with key food components

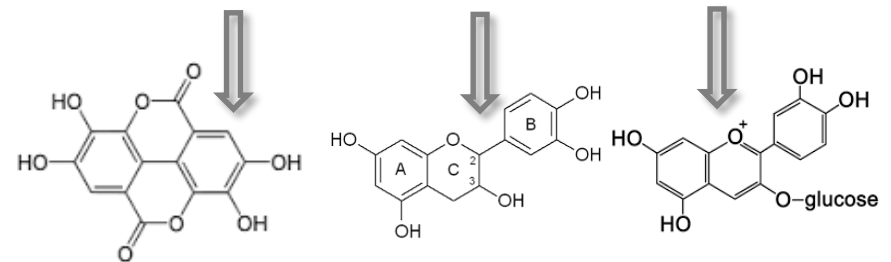
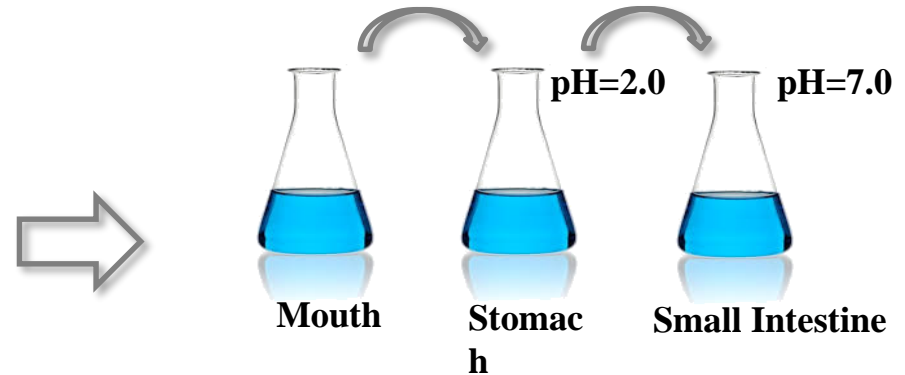
β -Lactoglobulin

Polyphenol

Polysaccharide



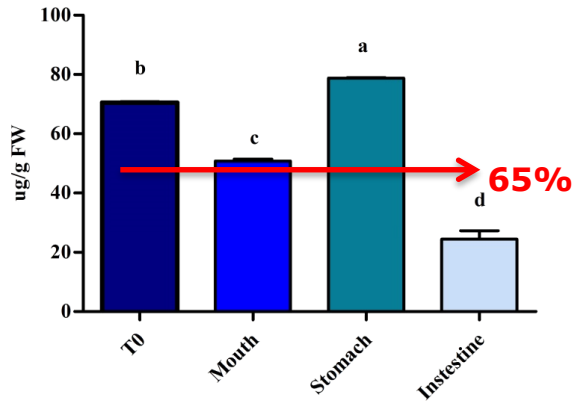
GASTROINTESTINAL SYSTEM *IN VITRO*



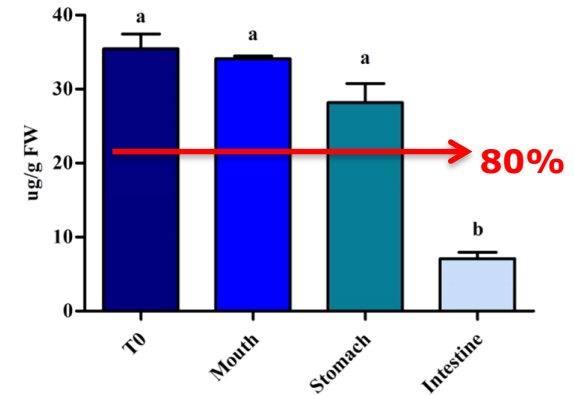
Effect of processing and storage on the nutritive and functional properties of fruits — strawberry and peach

Fruit Yoghurt

Pelargonidin-3-glucoside



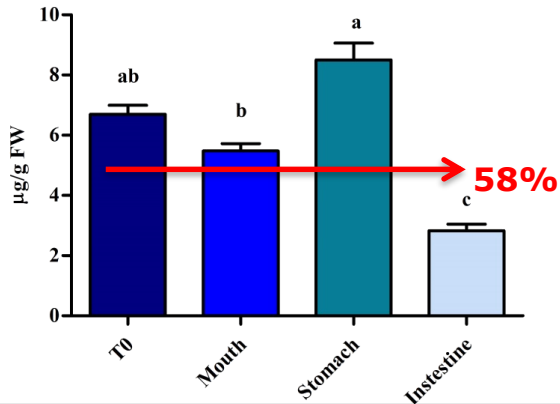
(+)-Catechin



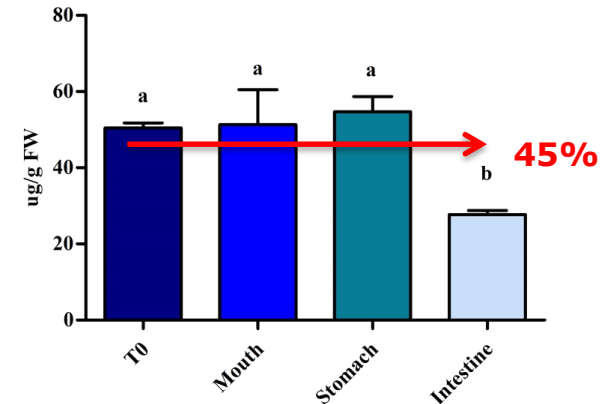
Strawberry

Peach

Pelargonidin-3-rutinoside



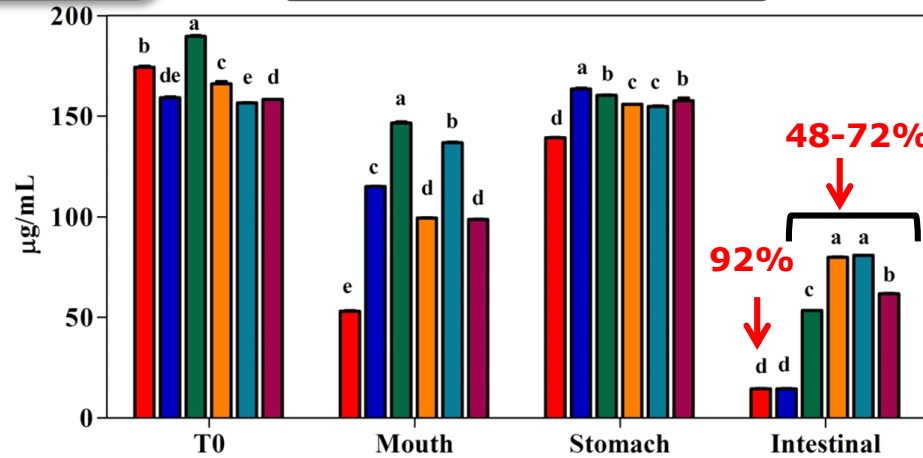
Neochlorogenic acid



Effect of processing and storage on the nutritive and functional properties of fruits — strawberry and peach

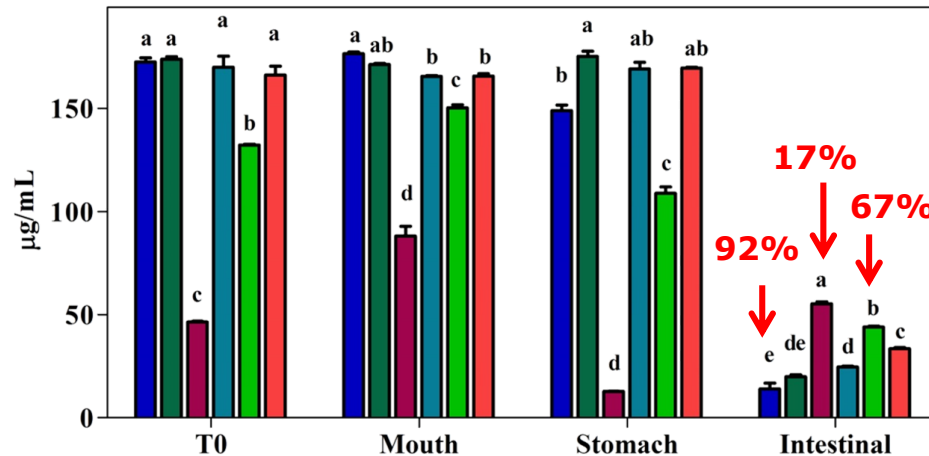
Model system of phenolics with key food components

(+)-Catechin



- (+)-Catechin
- (+)-Catechin+B-LG
- (+)-Catechin+Pectin
- (+)-Catechin+Chitosan
- (+)-Catechin+P+B-LG
- (+)-Catechin+Chi+B-LG

Cyanidin-3-glucoside

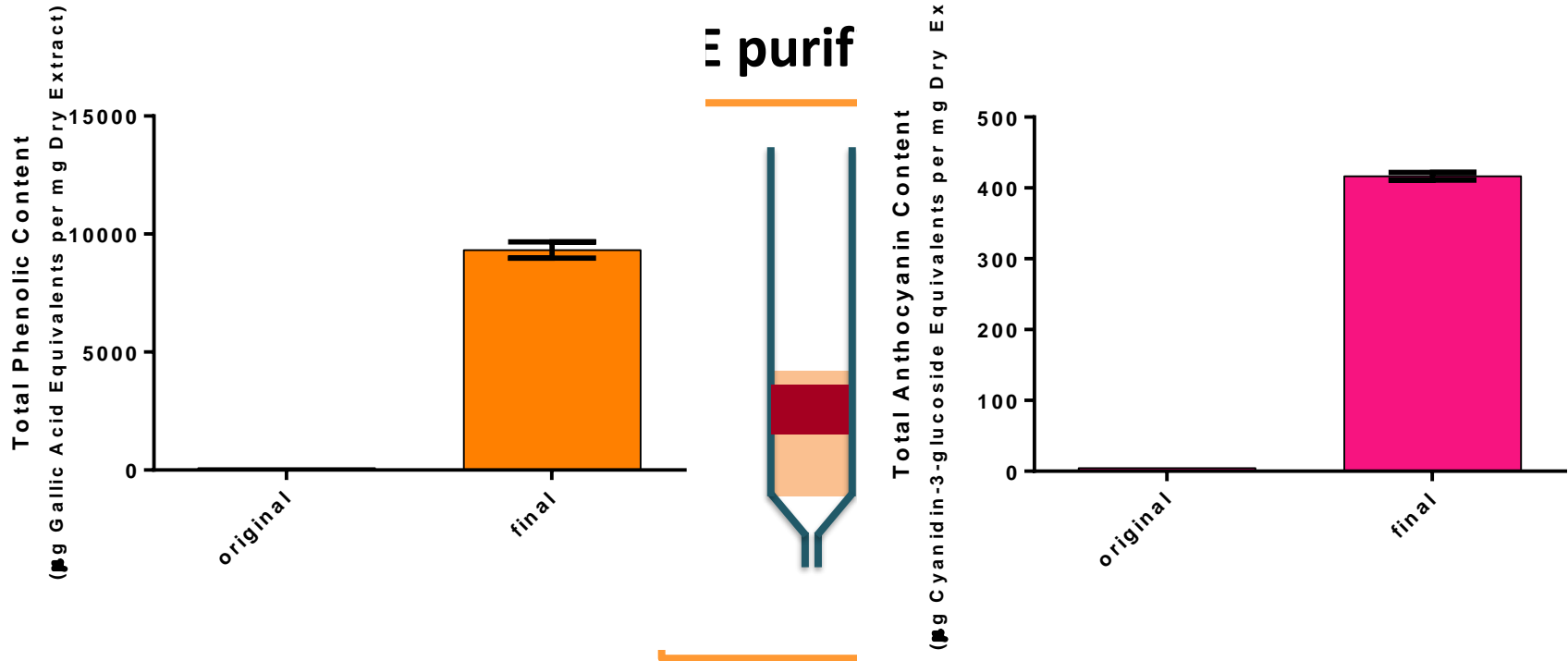


- Cy-3-gluc
- Cy-3-gluc +β-LG
- Cy-3-gluc +Pectin
- Cy-3-gluc +Chitosan
- Cy-3-gluc +β-LG+Pectin
- Cy-3-gluc +β-LG+Chitosan

Study of *Vaccinium corymbosum* blueberries: Characterization of extracts

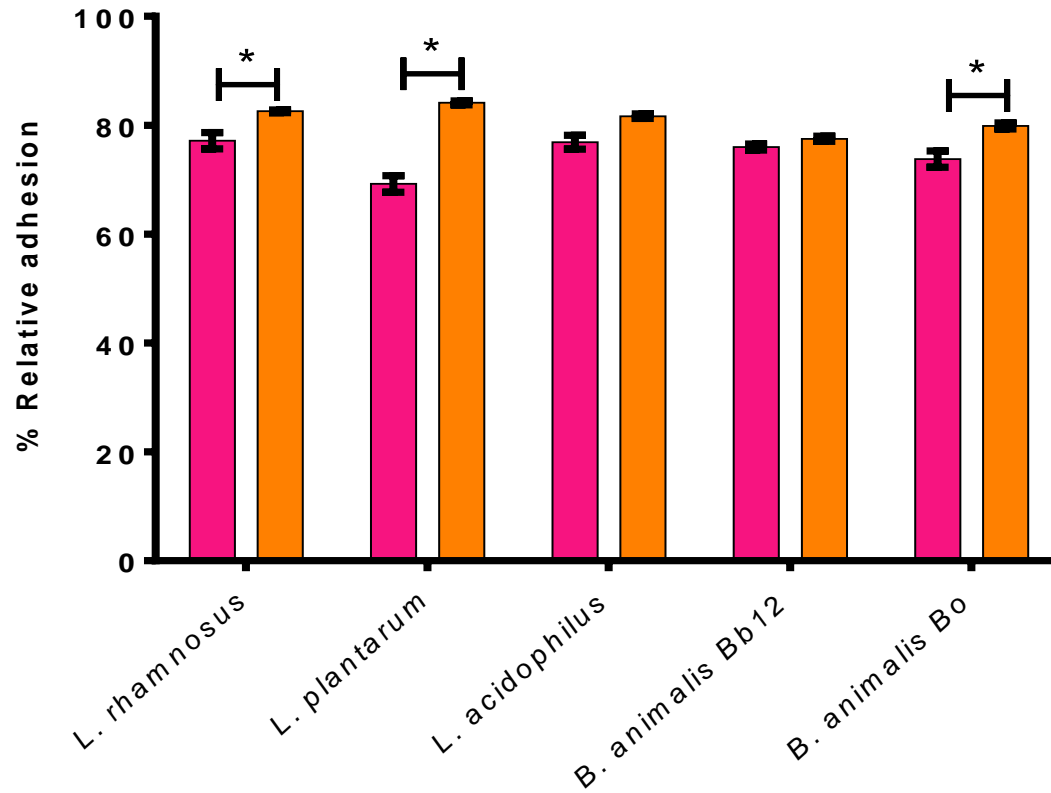
Definition of an extraction protocol

Selected Protocol: Ethanol with 0.01% HCl, 1 h 40 °C + 15 min , 35 KHz



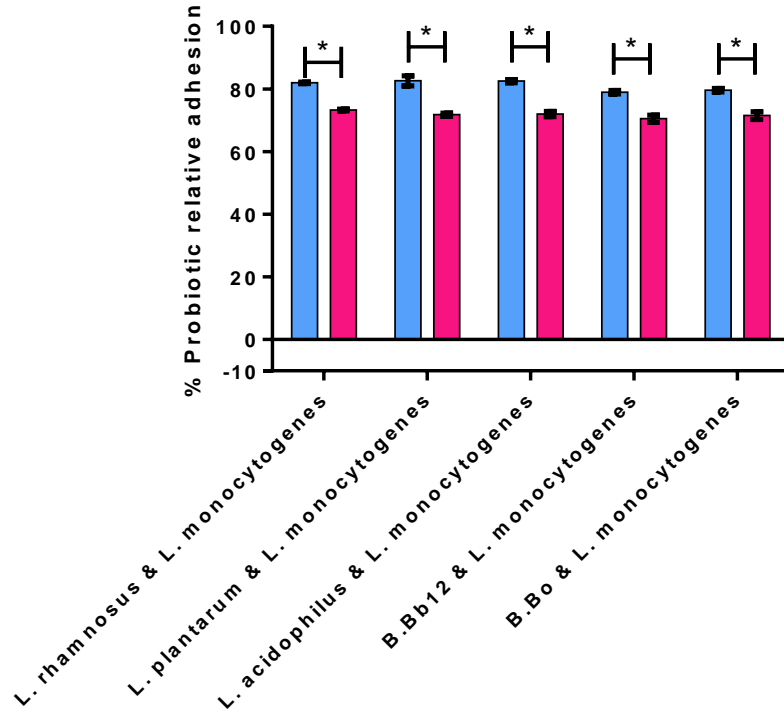
Study of *Vaccinium corymbosum* blueberries: Characterization of extracts

Impact on probiotic & probiotic/pathogen systems: Effect on probiotic adhesion

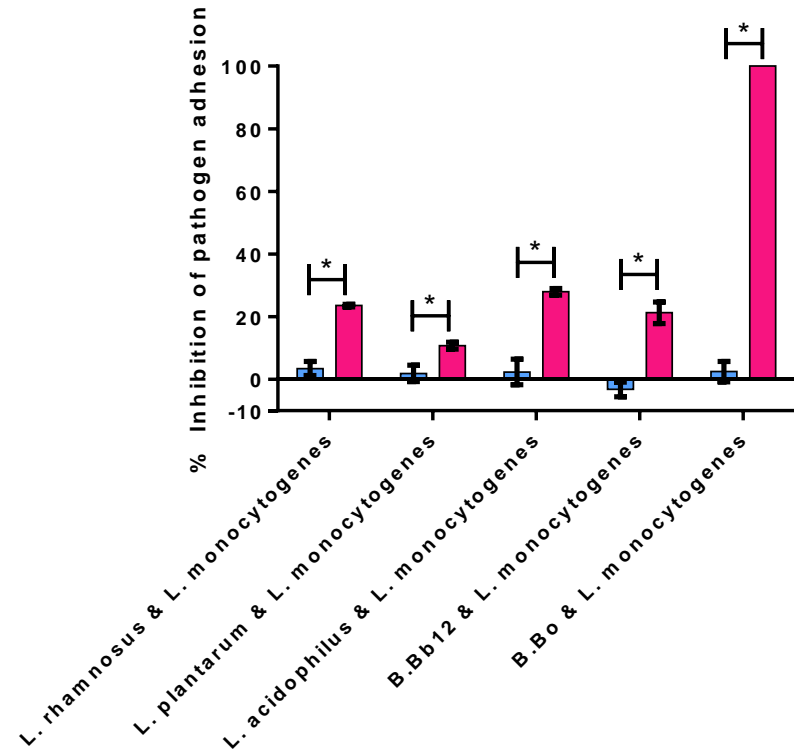


Study of *Vaccinium corymbosum* blueberries: Characterization of extracts

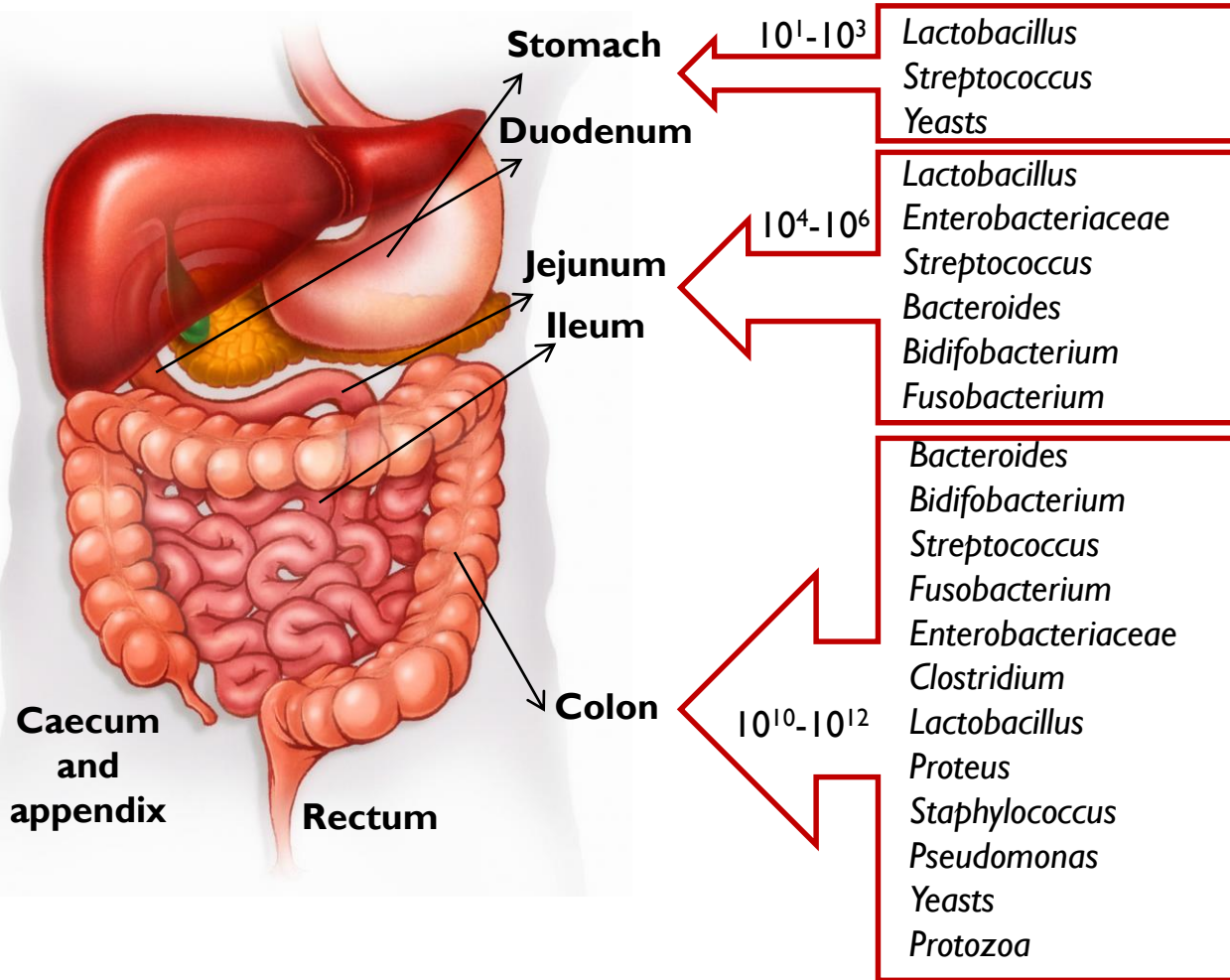
Probiotic adhesion



L. monocytogenes adhesion inhibition



GUT MICROBIOTA DIVERSITY AND FUNCTIONS



METABOLIC

Production of vitamins
 Digestion of dietary carcinogenics
 Production of short chain fatty acids

PROTECTIVE

Colonization resistance
 Innate and adaptative immunity

STRUCTURAL

Intestinal villi
 Mucus layer

DIET



Dietary pattern:

Western diet
Vegetarian diet

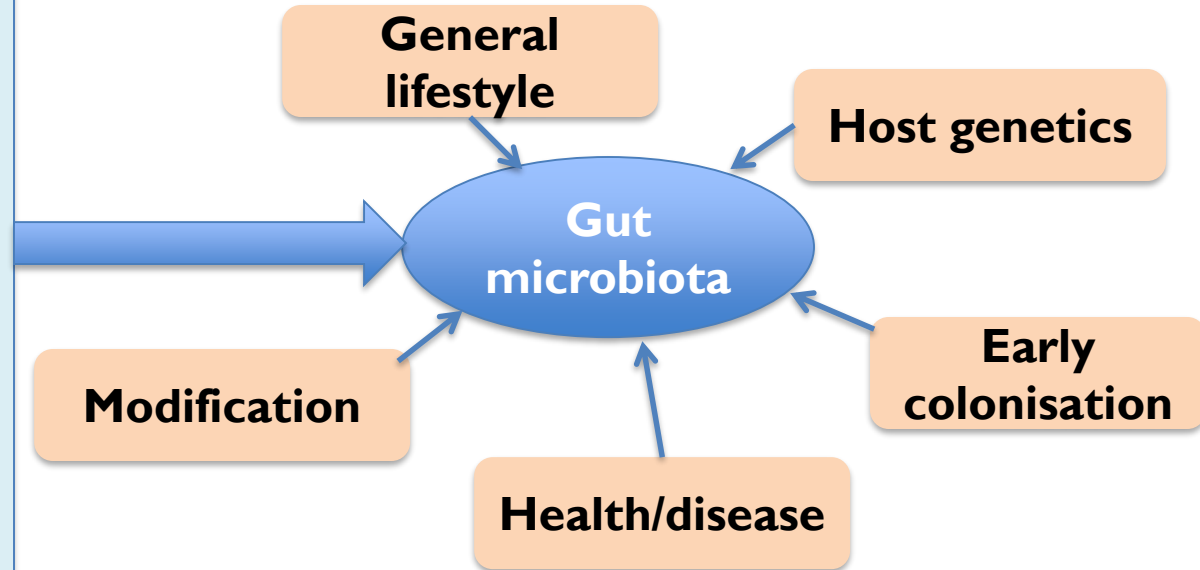
Specific foods:

Whole grain
Fruits and nuts
Vegetables and legumes

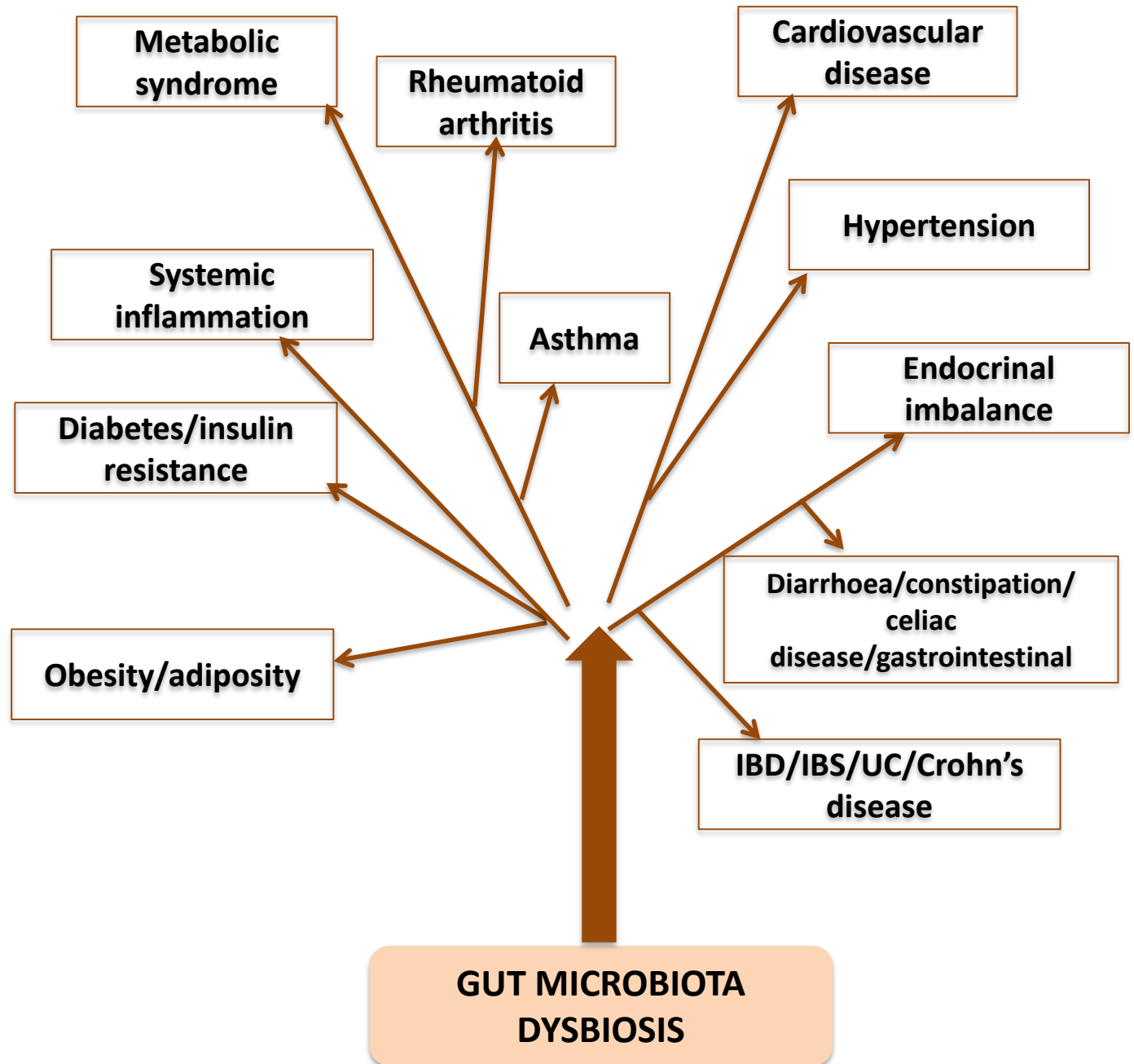
Foods composition:

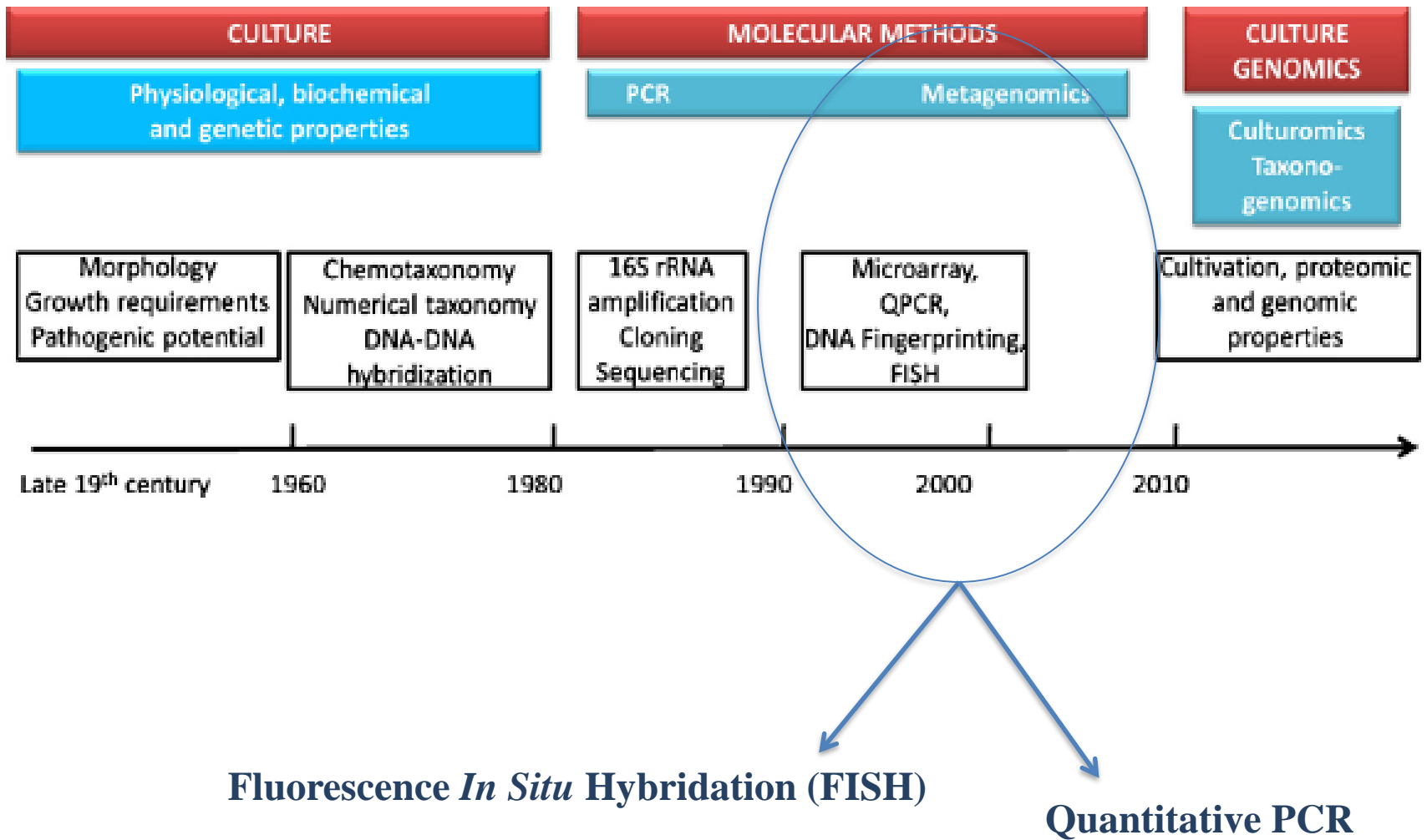
Fiber/carbohydrates
Fat
Protein
Phytochemicals

Foods-associated commensal microbes



Health states associated with gut microbiota dysbiosis (microbial imbalance)

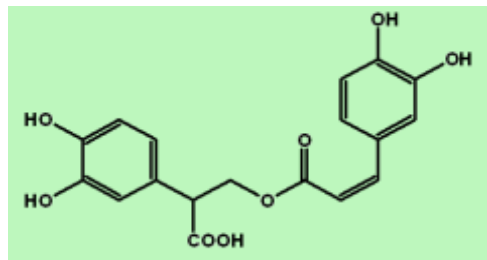




ROSMARINIC ACID AND HERBAL EXTRACTS RICH IN ROSMARINIC ACID



Sage and savoury
(~28mgRA/g)



ANTIMICROBIAL

Cancer risk reduction

Colon, breast and oral cancer

Treatment (adjuvant) of rheumatoid arthritis

Treatment (adjuvant) of cataracts

Treatment (adjuvant) of allergic asthma

Neurologic diseases

Prevention of cell damage/cytotoxic effects

ANTI-INFLAMMATORY

ANTIOXIDANT

ANTIMUTAGENIC



Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: <http://www.tandfonline.com/loi/bfsn20>

Therapeutic and Nutraceutical Potential of Rosmarinic Acid - Cytoprotective Properties and Pharmacokinetic Profile

Sara Nunes, Raquel Madureira, Débora Campos, Bruno Sarmento, Ana Maria Gomes, Manuela Pintado & Flávio Reis

RA ORAL INGESTION -----WEAKNESSES

Absorption
compromised by other
phenolic compounds

Metabolized by gut
microbes to less
bioavailable forms

Insufficient gastric
residence time

Low permeation
at intestinal cells-
low absorption

Unstable during
digestion
(stomach)



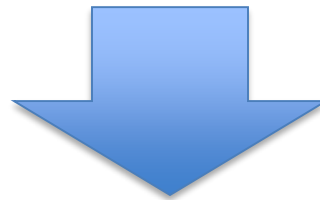
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Therapeutic and Nutraceutical Potential of
Rosmarinic Acid - Cytoprotective Properties and
Pharmacokinetic Profile

Sara Nunes, Raquel Madureira, Débora Campos, Bruno Sarmento, Ana
Maria Gomes, Manuela Pintado & Flávio Reis



BIOACTIVITY COMPROMISED

SOLID LIPID NANOPARTICLES

Drug/bioactive compounds delivery systems

Nanometric spheres (10-1000 nm)

Produced with lipids

↓
Waxes
Mono, di or triglycerides
Fatty acids
Steroids
+
Emulsifiers
(prevention of agglomeration)

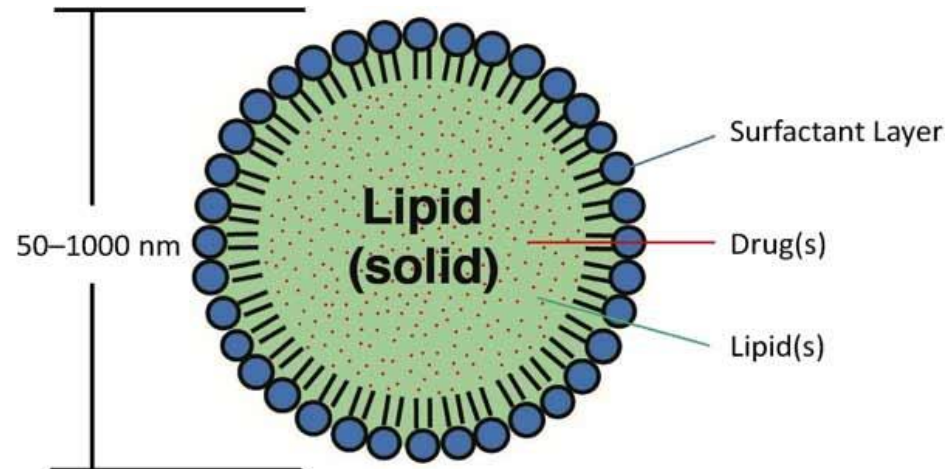
Resistant to digestion

Intact phenolic compounds release at intestine

Stable during storage

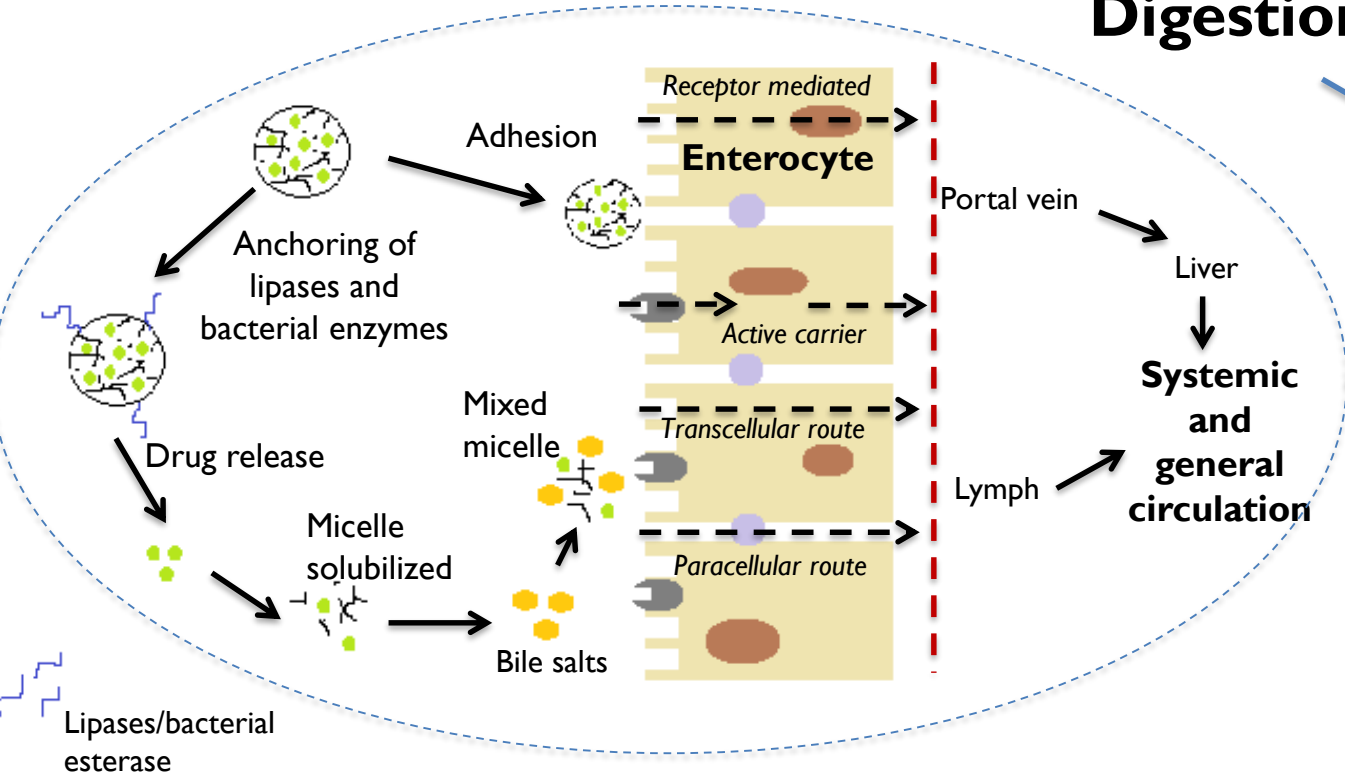
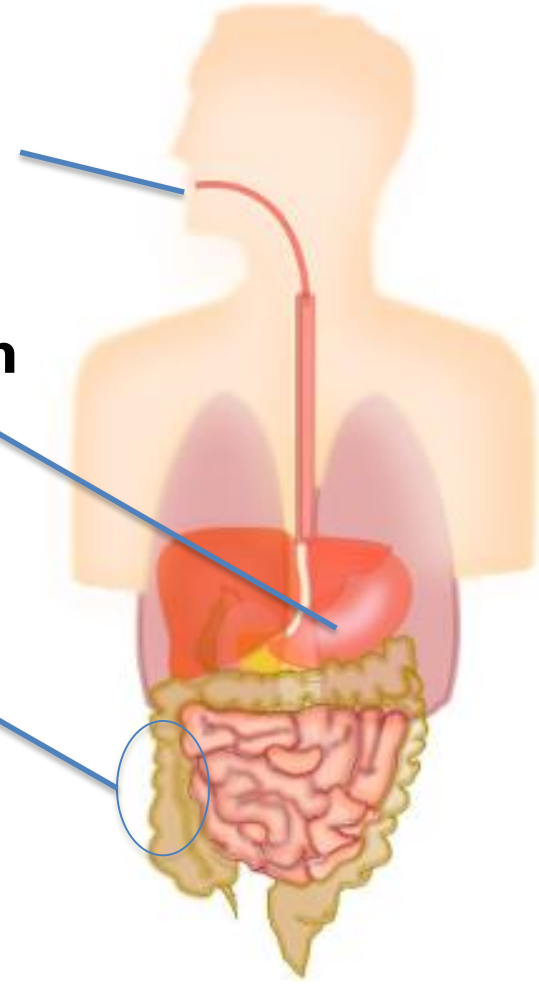
Not toxic

↓
Development of a functional food ingredient



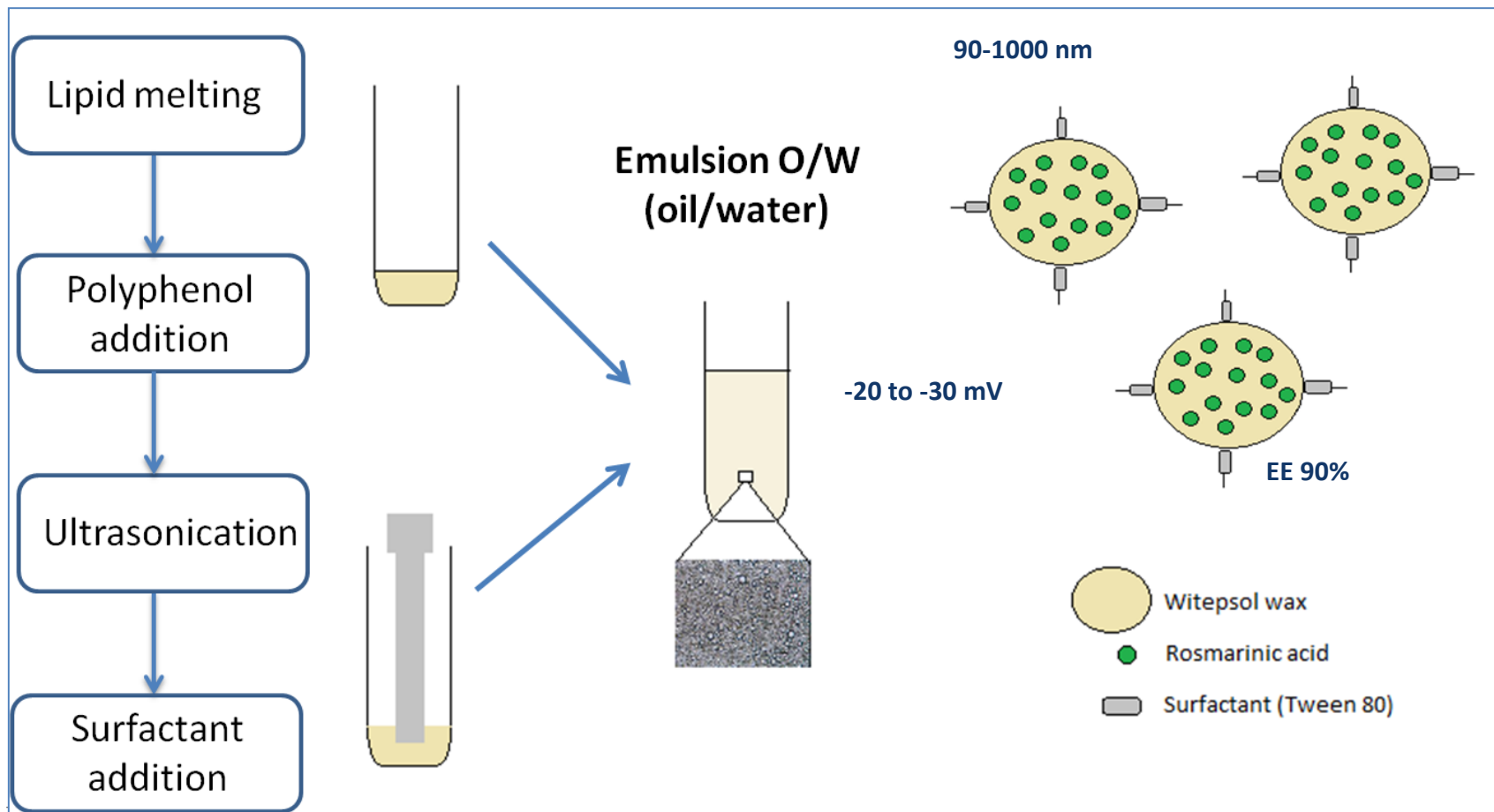
Ingestion

Digestion



Solid lipid nanoparticles and loaded compounds fate

SOLID LIPID NANOPARTICLES PRODUCTION AND CHARACTERIZATION



Optimization of the production of solid Witepsol nanoparticles loaded with rosmarinic acid



Débora A. Campos^a, Ana Raquel Madureira^{a,*,}, Ana Maria Gomes^a, Bruno Sarmento^{b,c}, Maria Manuela Pintado^{a,*,}

RSC Advances

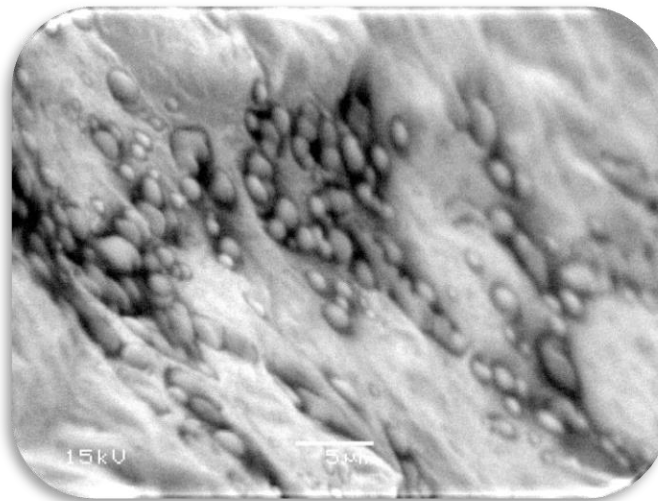
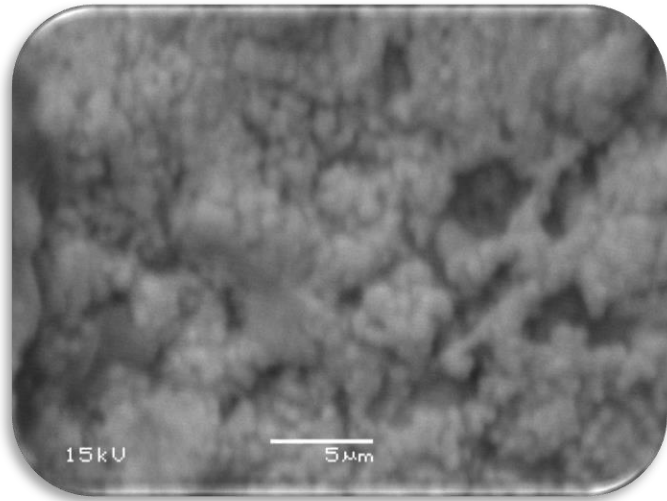
PAPER



Cite this: RSC Adv., 2015, 5, 22665

Characterization of solid lipid nanoparticles produced with carnauba wax for rosmarinic acid oral delivery

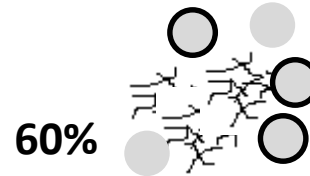
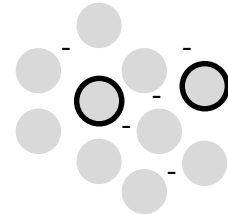
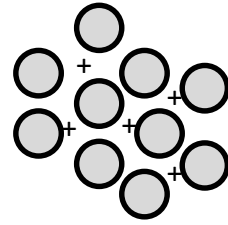
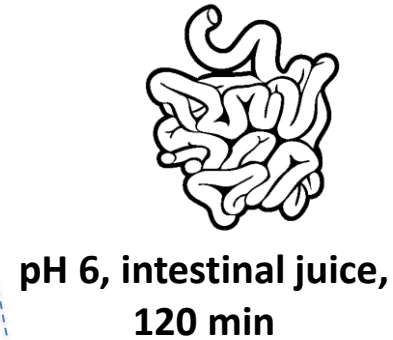
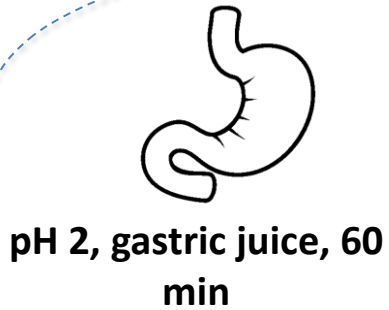
Ana Raquel Madureira,^{a*} Débora A. Campos,^a Pedro Fonte,^b Sara Nunes,^c Flávio Reis,^c Ana Maria Gomes,^a Bruno Sarmento^{b,c} and Maria Manuela Pintado^a



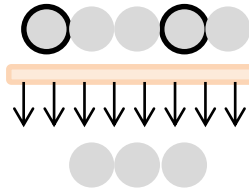
SEM MICROGRAPHS



FOOD INGREDIENT POWDER Optimized final lyophilized SLN



Micelles solubilisation



Intestinal epithelium (dialysis tube/ cell lines)

CONCENTRATIONS?

IN VITRO TOXICITY STUDYS

Non encapsulated RA at 1.5 mg/mL decreases lymphocytes proliferation by necrosis (cell integrity damage, no DNA damage)

SLN were produced with concentrations of RA (free or provided by extracts) that release a max. 1.5 mg/mL of RA at intestine

COLON ENVIRONMENT SIMULATION

ATMOSPHERE

Anaerobic mixture
(H₂, CO₂, N₂)



GUT BACTERIA RICHNESS

Human faeces collection

BODY TEMPERATURE

37°C Incubation chamber

NUTRIENTS AND CHEMICAL CONTENT

Basal medium

BATCH FERMENTATION

SAMPLES TESTED:

- Free extracts (sage and savoury) and RA
- Witepsol and Carnauba SLN empty (WSLN and CSLN)
- WSLN and CSLN loaded with extracts and RA

1% (w/v) (0.1 g) de prebiótico

+

8 mL Meio nutriente de base:

5,0 g / L de caldo de tripticase de soja (TSB) sem dextrose,

5,0 g / l de bactopectona,

0,5 g / L-cisteína-HCl a

1,0% (v / v) de solução de sal de A e solução de sal B (200,0 g / L de $K_2HPO_4 \cdot 3H_2O$ e 0,2% (v / v) de

0,5 g / solução G resazurina, em água destilada.

O pH final ajustado a 6,8.

+

2 % (v/v) (0.2 mL) - Amostras de fezes de três voluntários humanos saudáveis:

(dieta normal, sem doenças do aparelho digestivo e sem antibióticos há 3 meses).

Diluição 1: 10 em solução salina fisiológica (cisteína-HCl, 0,5 g / L e NaCl 8,5 g / L)

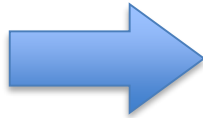


0-24 h, T: 37 °C, anaerobiose

BATCH FERMENTATION PROCEDURES



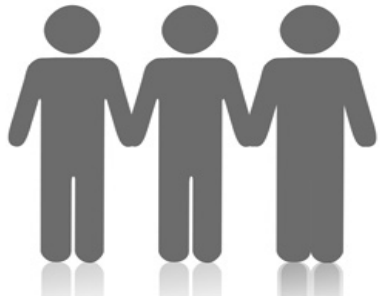
Fermentation nutrient medium without oxygen



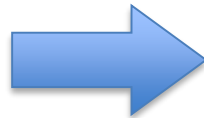
Medium at anaerobic conditions



Distribution in anaerobic sealed serum bottles



Collection of human faeces



Faecal slurry preparation

Distribution in bottles

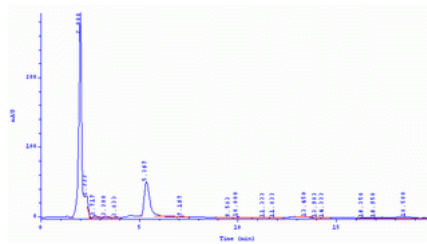
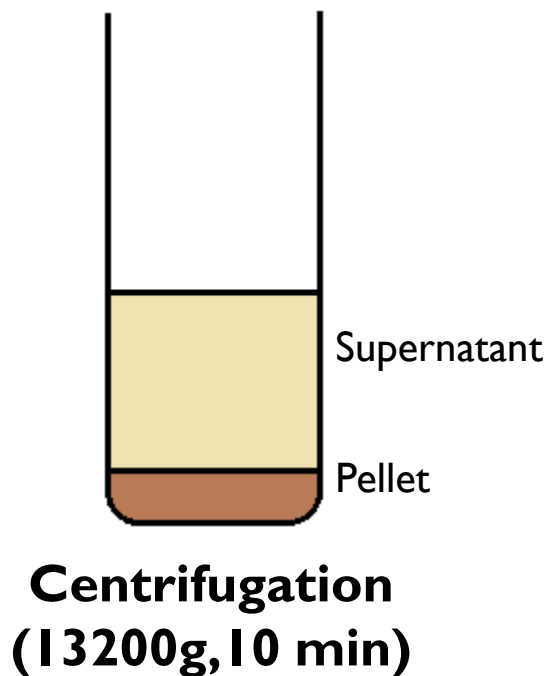


RA, extracts and SLN addition



Incubation of serum bottles at 37°C for 24h

SAMPLING AND ANALYSIS PROCEDURES (0, 8 AND 24 H)

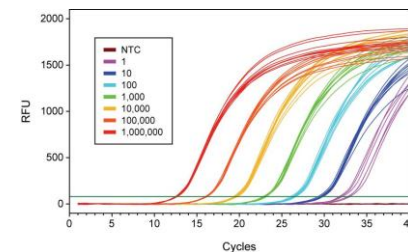


HPLC

**Quantification of
phenolic compounds
and
short chain fatty acids**

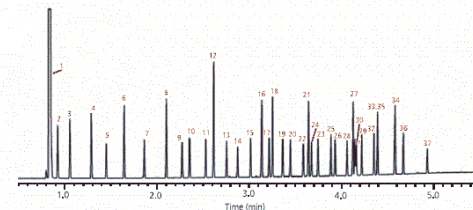


**Bacterial DNA
extraction**



PCR real time

**Fats SLN lipids
quantification by GC
(FAME)**

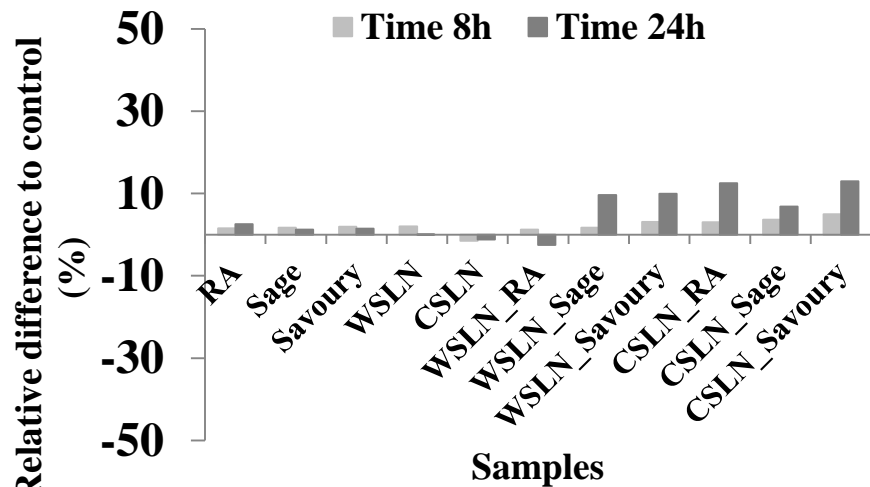


GENERAL FAECAL MICROBIOTA COMPOSITION OF VOLUNTEER PARTICIPANTS (N=3)

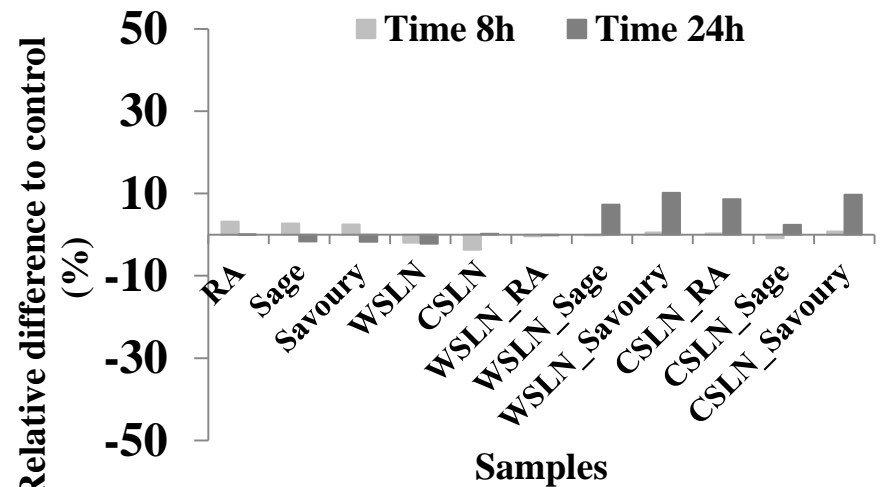
Division (genus)	Number of copies (n=3)*
Universal	5.90±0.416
Firmicutes	6.05±0.487
<i>Clostridium leptum</i>	5.12±1.06
<i>Lactobacillus</i> spp.	2.63±0.526
<i>Roseburia hominis</i>	4.92±0.470
Bacteroidetes	5.34±0.247
<i>Bacteroides</i>	5.06±0.957
Actinobacteria	
<i>Bifidobacterium</i> spp.	6.17±0.277

*Values are presented as mean \pm SD and expressed as \log_{10} 16S rRNA gene copies/20 ng of DNA

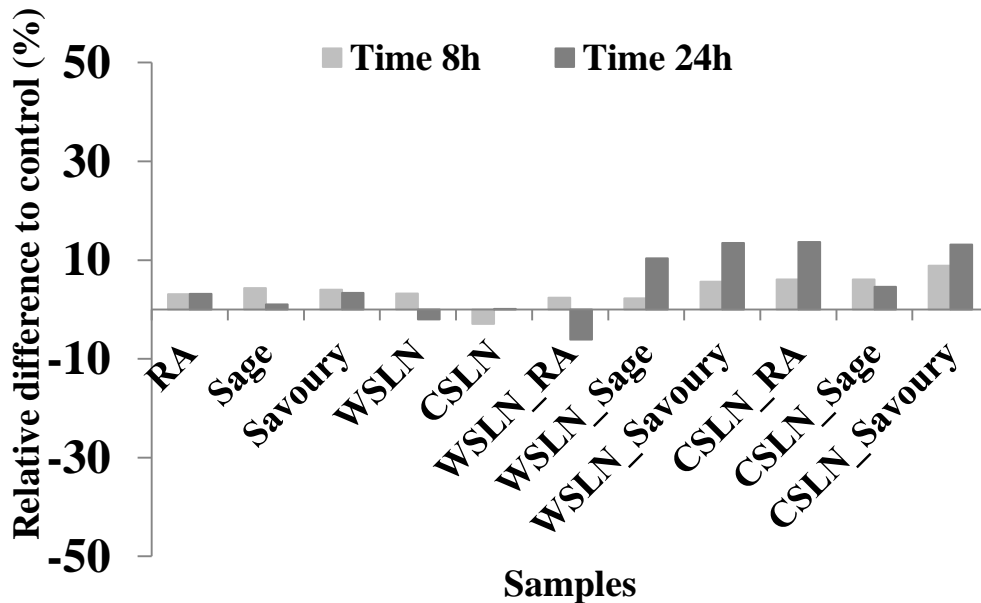
Universal



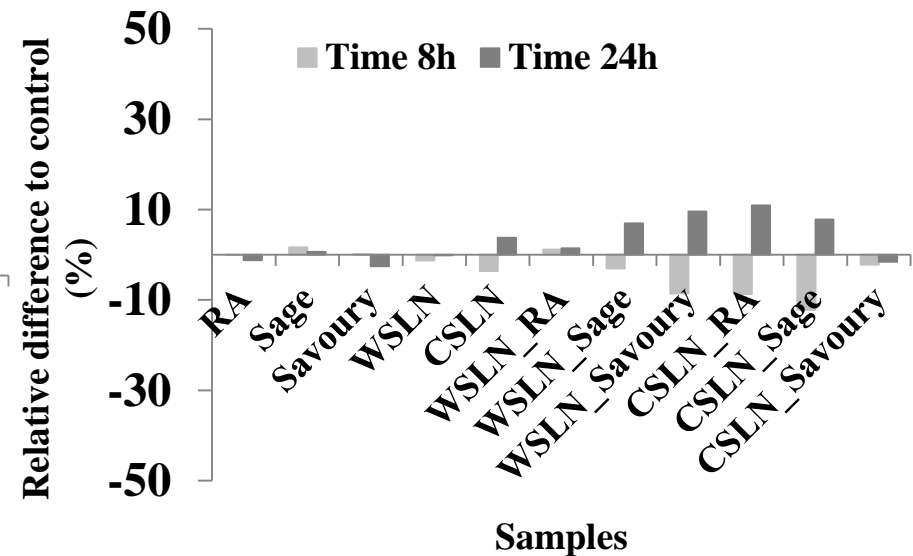
Firmicutes



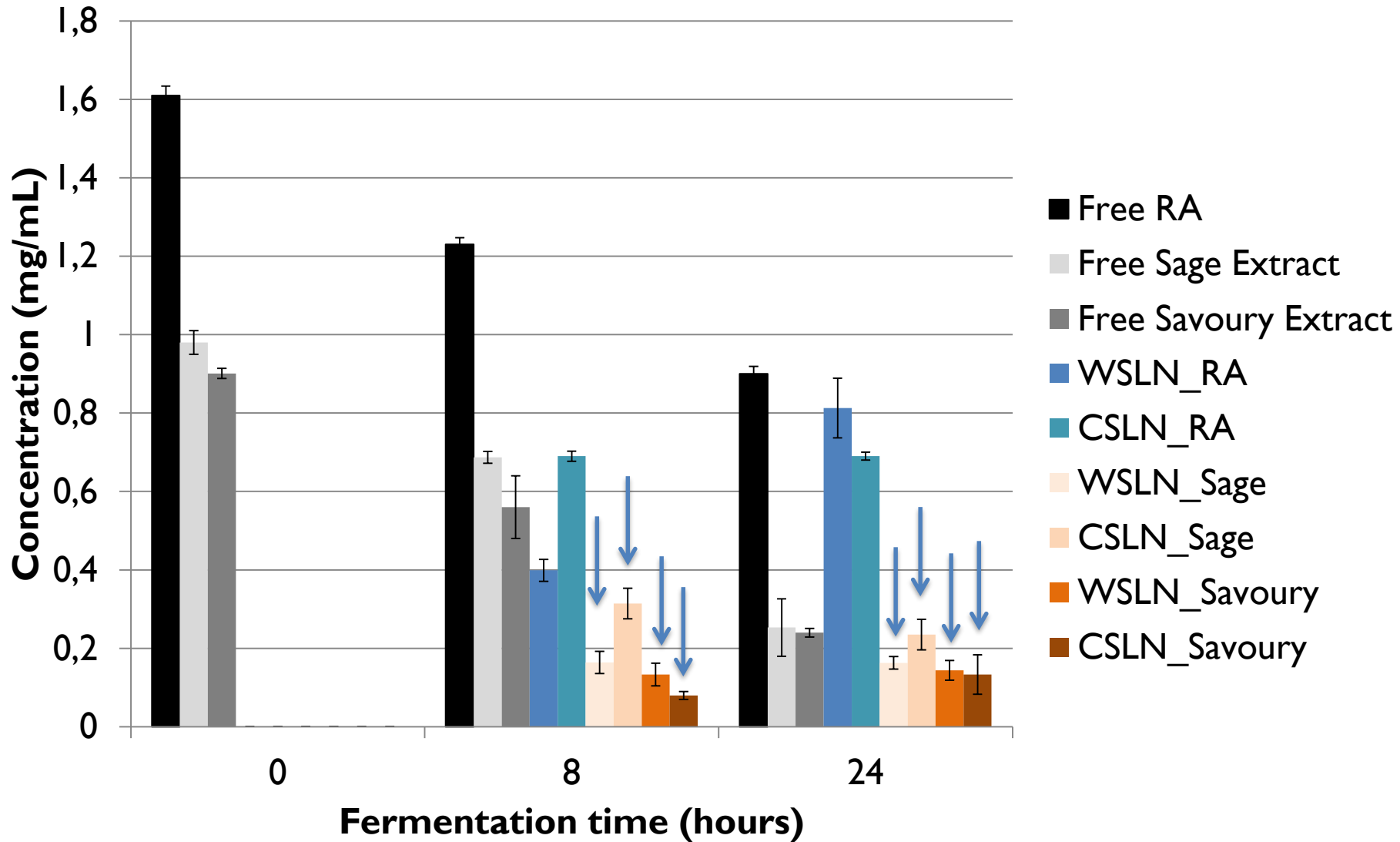
Bacteroidetes

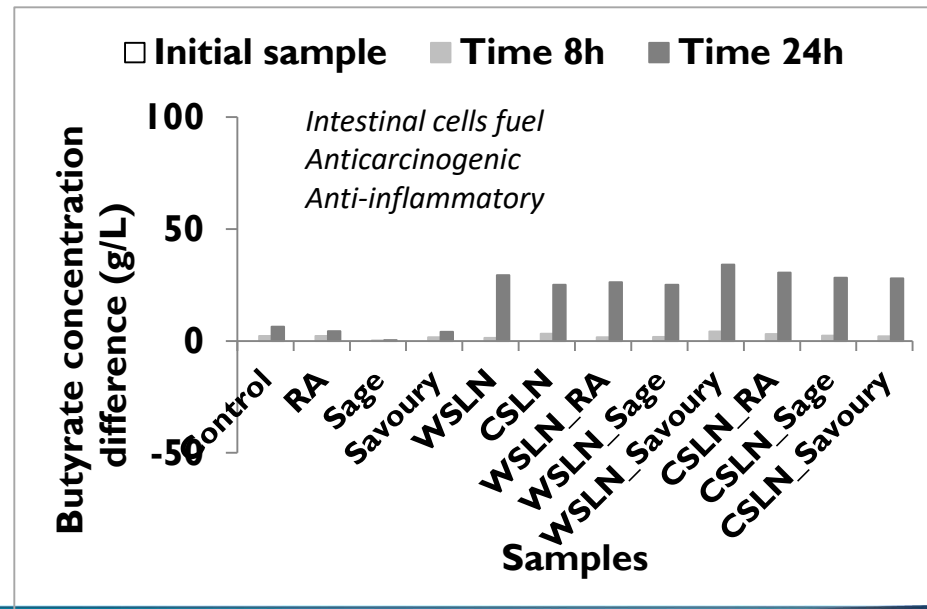
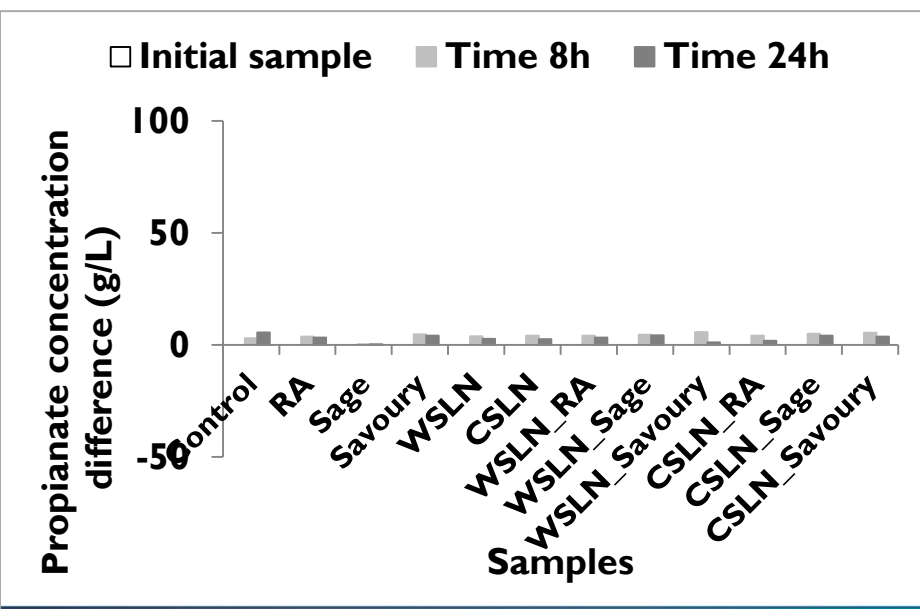
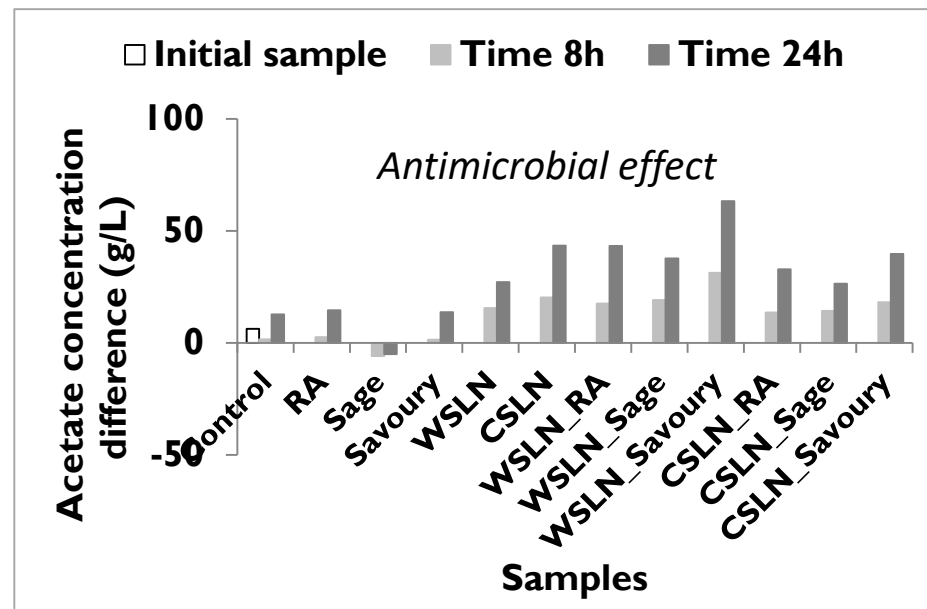
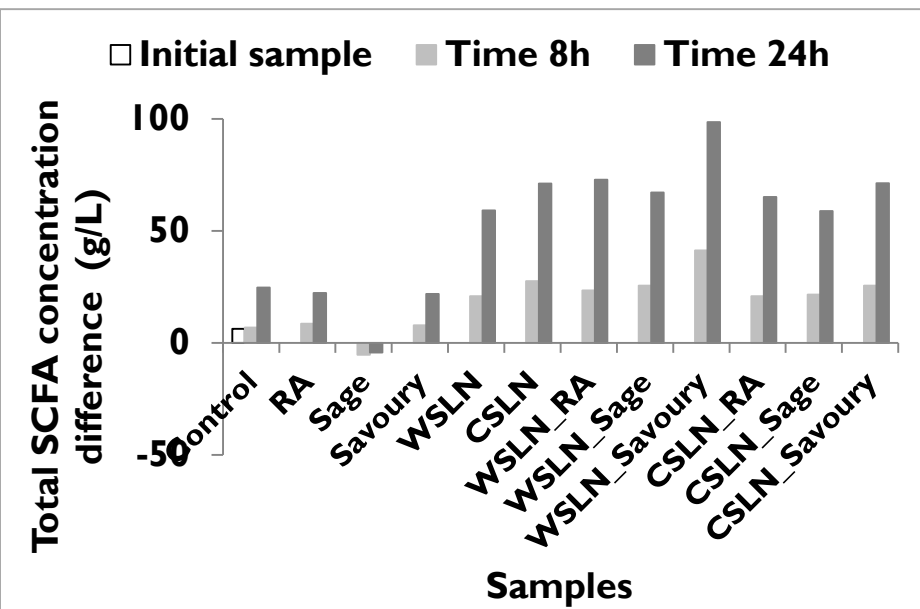


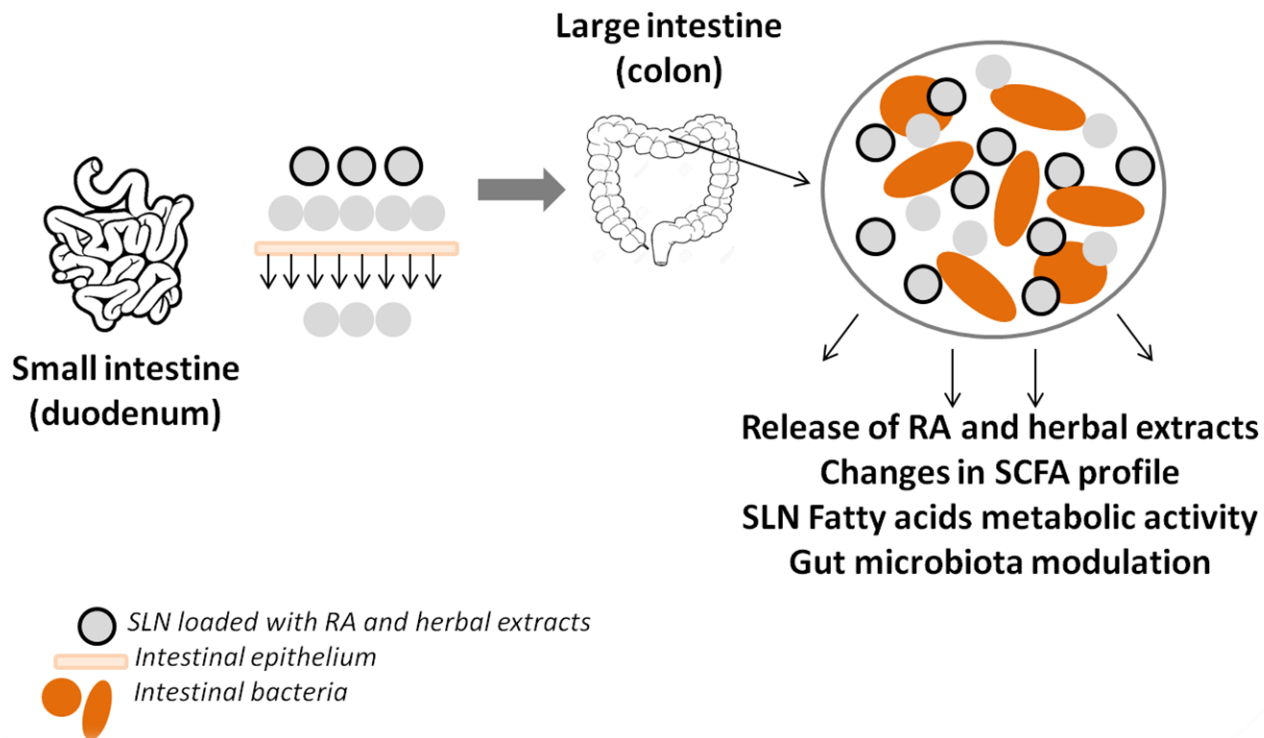
Bifidobacterium



ROSMARINIC ACID CONCENTRATION ALONG FERMENTATION TIME







RA and herbal extracts should be used at non-inhibitory concentrations to gut bacteria
Encapsulation of RA and herbal extracts can control the delivered concentrations in order to not have an antimicrobial effect on gut bacteria
Studies in gut microbiota should be done before approving a bioactive compound to consumption

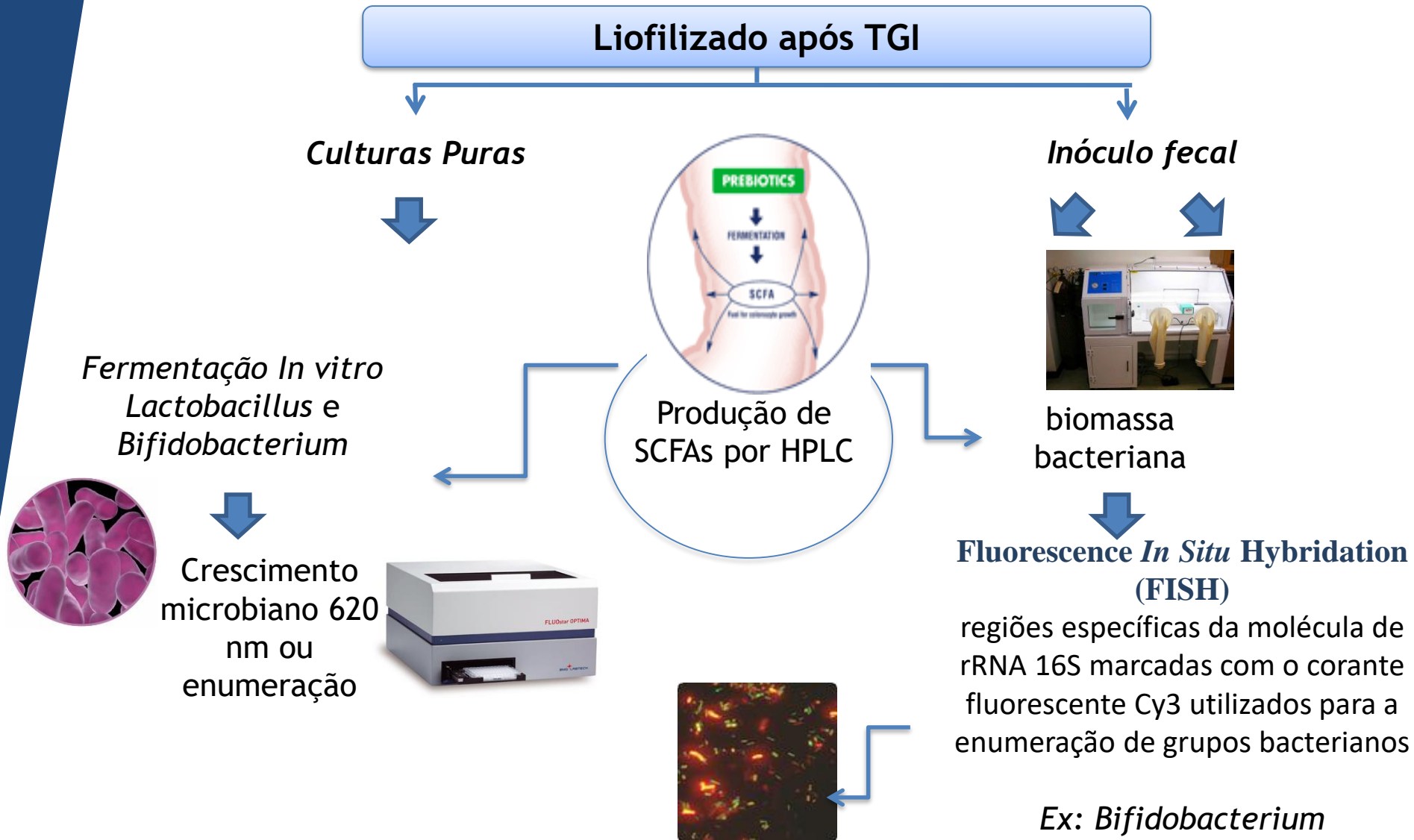


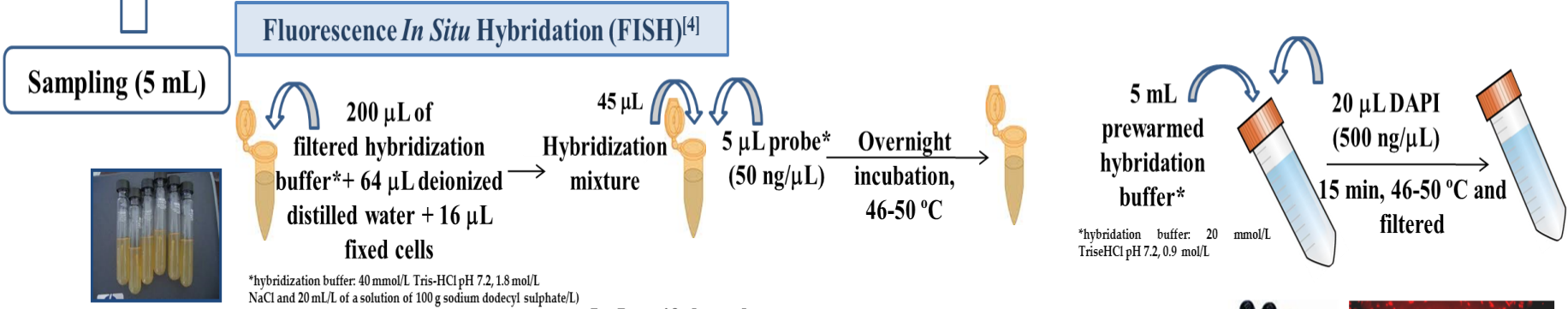
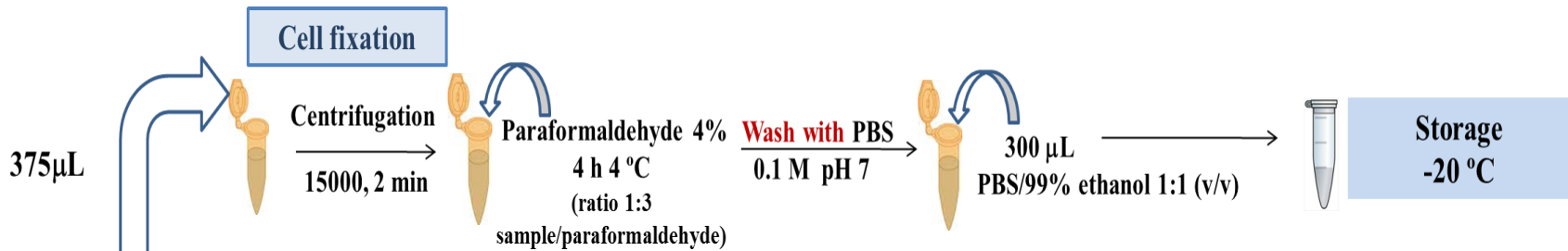
Cite this: DOI: 10.1039/c4fo00857j

In vitro assessment of the prebiotic potential of *Aloe vera* mucilage and its impact on the human microbiota

Beatriz Gullón,^a Patricia Gullón,^a Freni Tavaría,^a José Luis Alonso^b and Manuela Pintado^{*a}

Estudo da atividade prebiótica de aloe vera

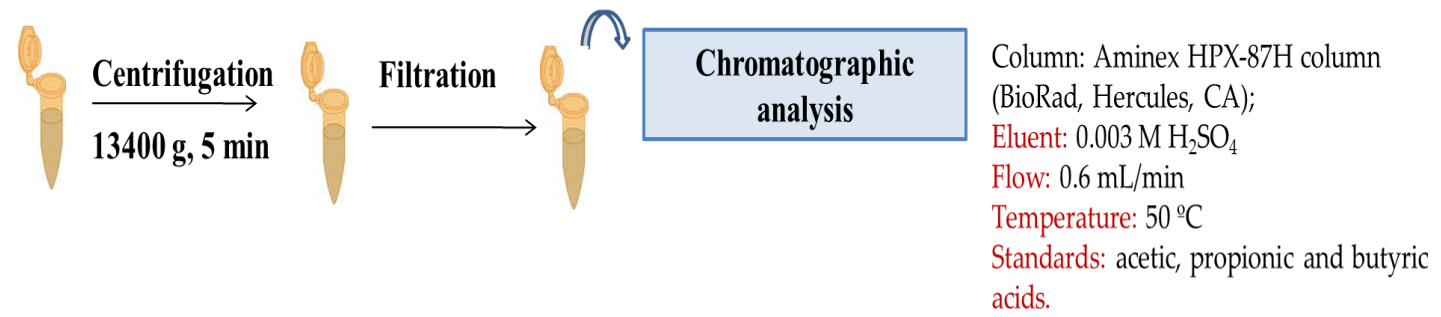




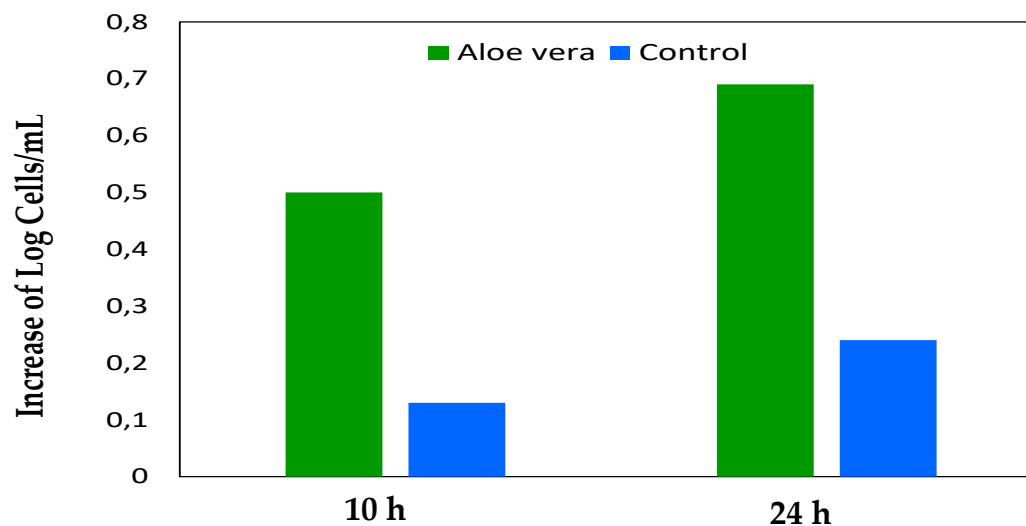
Bacterial count

*Synthetic oligonucleotide probes
Bifidobacterium spp.
Lactobacillus/Enterococcus spp.
Bacteroides/Prevotella
Clostridium coccoides/Eubacterium
rectale spp.
Atopobium (*Ato291*)
Total bacteria (DAPI ; 4,6-diamidino-2-
fenilindol)

Determination of short chain fatty acids (HPLC-RI)



Carbon source	Time (h)	pH	Acetate	Propionate	Butyrate	Total SCFA
<i>Aloe vera</i>	5	6.4	13.60 ^b ± 2.73	5.71 ^c ± 0.63	2.49 ^b ± 0.90	21.79 ^b ± 4.10
	10	5.8	28.35 ^c ± 3.60	9.39 ^c ± 0.62	5.95 ^b ± 1.37	43.69 ^c ± 5.18
	24	5.2	39.23 ^c ± 2.03	11.03 ^b ± 1.18	7.18 ^a ± 1.80	57.44 ^b ± 3.61
	48	5.0	47.73 ^c ± 0.58	12.16 ^b ± 0.81	10.10 ^b ± 0.73	69.99 ^b ± 1.68
Inulin	5	6.1	11.42 ^b ± 1.50	3.38 ^b ± 0.77	2.20 ^b ± 0.57	16.99 ^b ± 2.38
	10	5.1	19.67 ^b ± 4.31	6.25 ^b ± 2.34	5.36 ^b ± 1.87	31.28 ^b ± 8.06
	24	4.8	33.86 ^b ± 3.72	9.87 ^b ± 4.41	12.35 ^b ± 2.20	56.10 ^b ± 6.09
	48	4.8	40.46 ^b ± 4.75	10.90 ^b ± 5.10	18.87 ^c ± 2.78	70.24 ^b ± 3.67
Control	5	6.8	3.91 ^a ± 1.56	1.62 ^a ± 0.64	0.94 ^a ± 0.24	6.47 ^a ± 2.15
	10	6.7	9.38 ^a ± 2.24	1.21 ^a ± 0.56	1.69 ^a ± 0.94	12.29 ^a ± 3.04
	24	6.9	19.26 ^a ± 2.03	2.63 ^a ± 1.41	3.75 ^a ± 1.03	25.64 ^a ± 1.49
	48	6.8	25.58 ^a ± 2.22	3.99 ^a ± 1.06	4.88 ^a ± 1.09	34.45 ^a ± 1.61

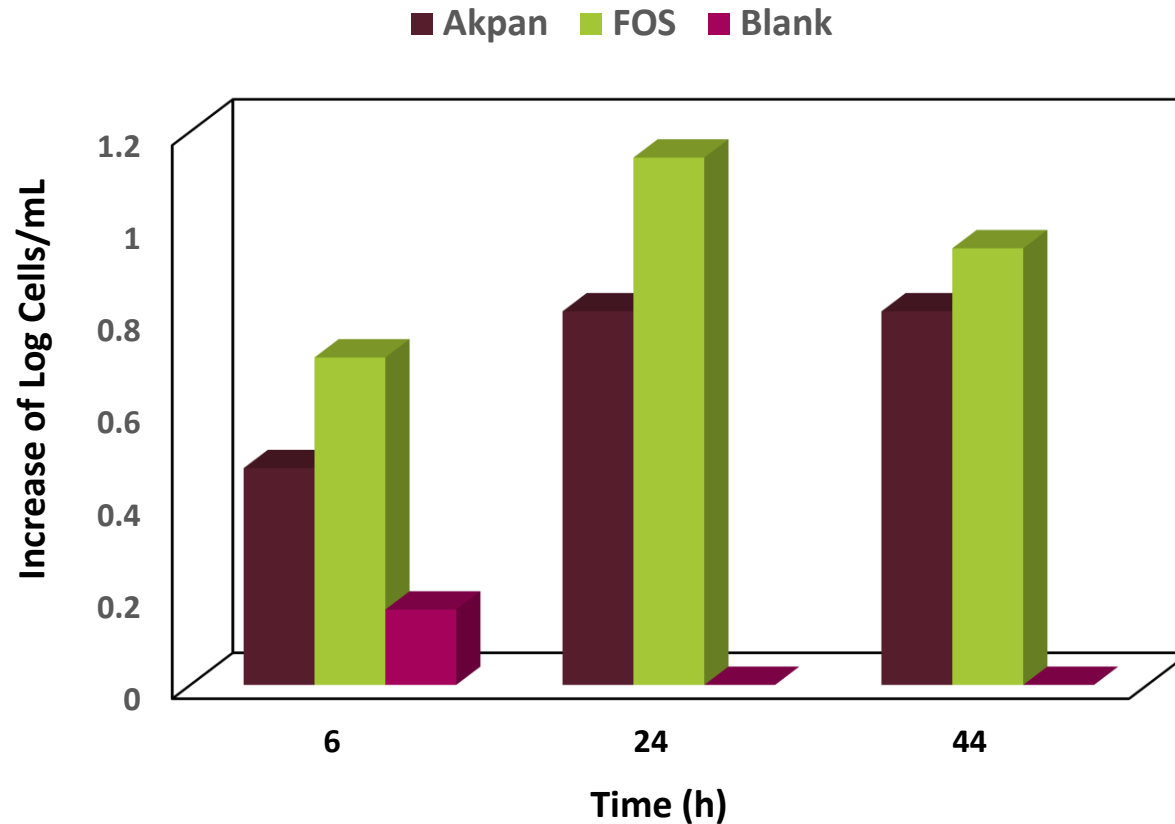


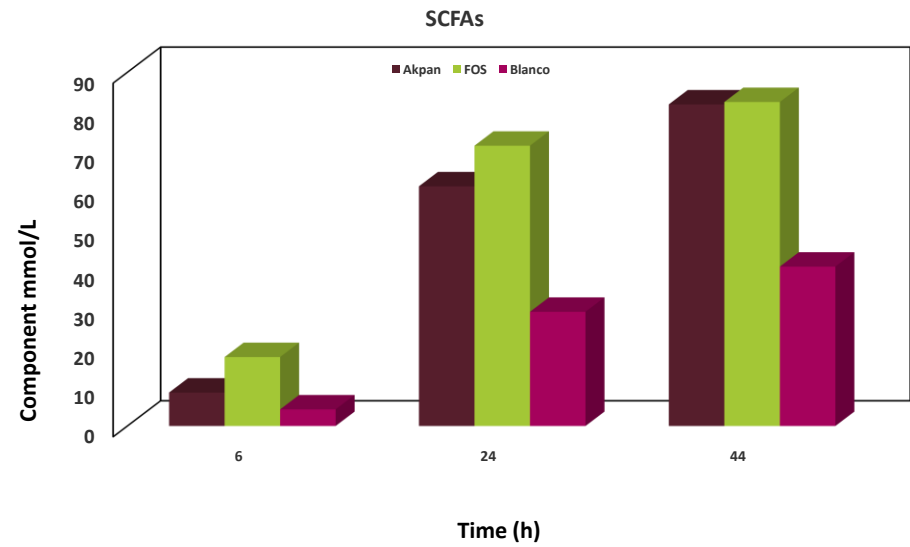
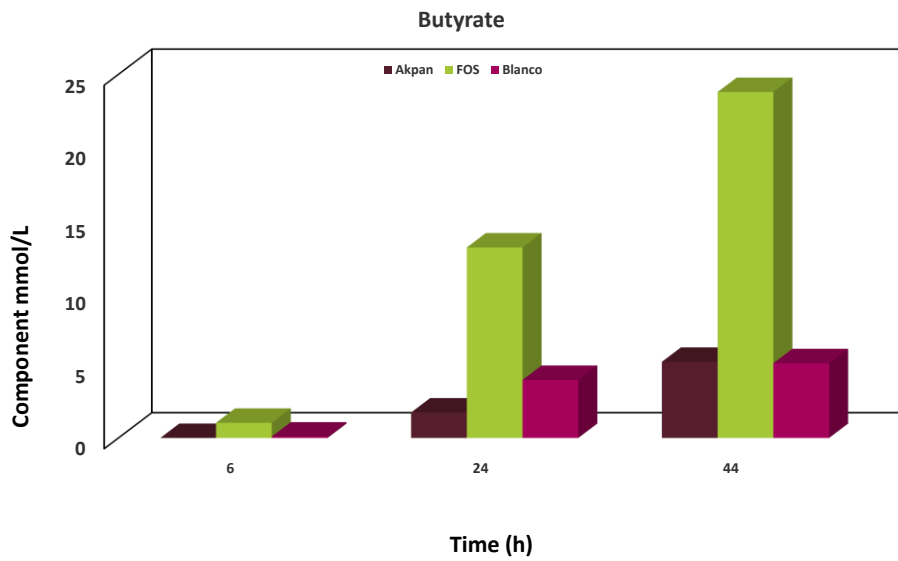
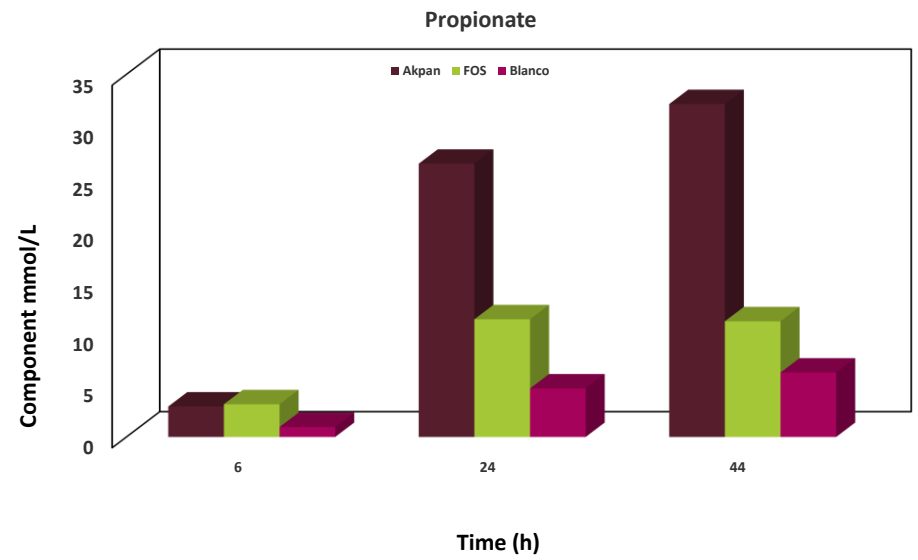
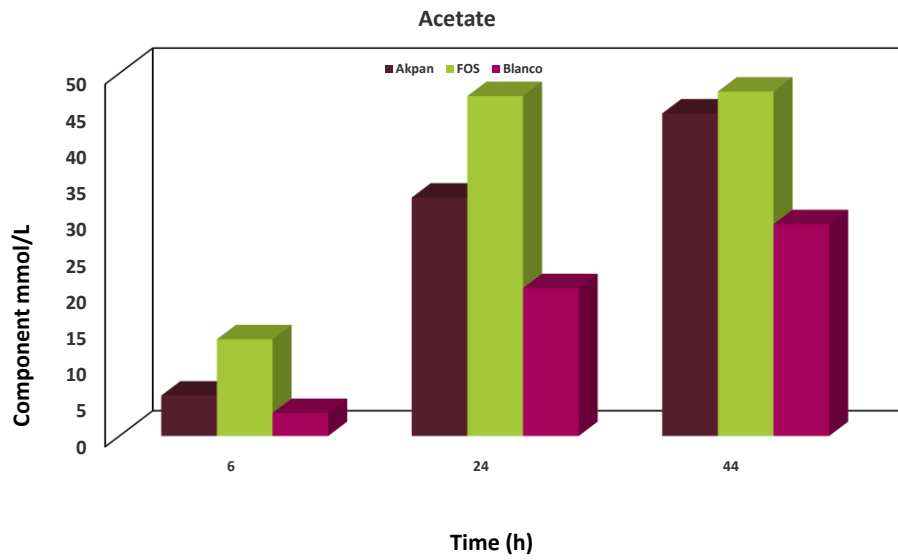
Assessment of prebiotic potential of Akpan and effects on the human intestinal microbiota

Beatriz Gullón^{a,b,#}, Miguel Pereira^{a,#}, Christian Mestres^c, Joseph Hounhouigan^d, Dominique Pallet^c, José Luis Alonso^e, Manuela Pintado^{a*}

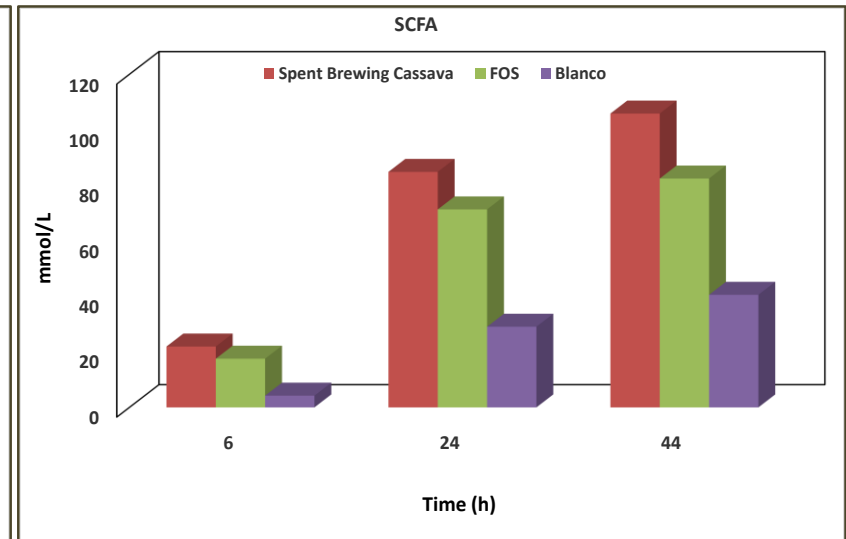
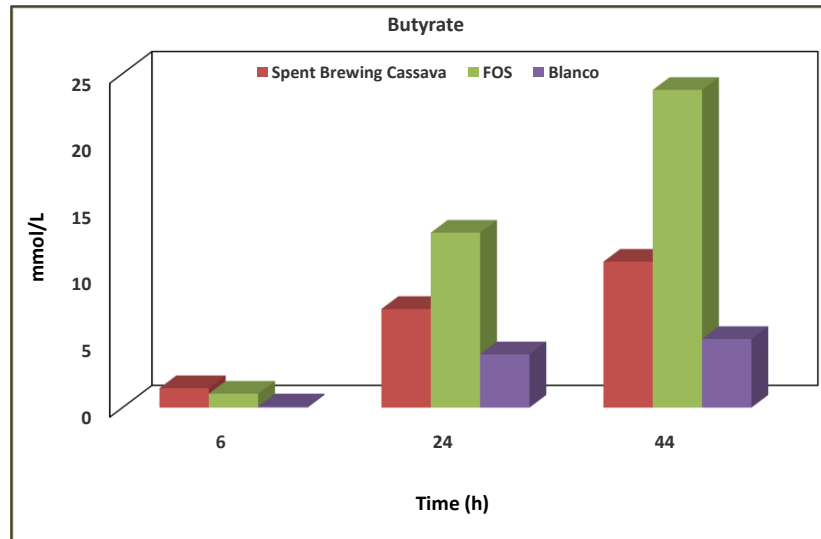
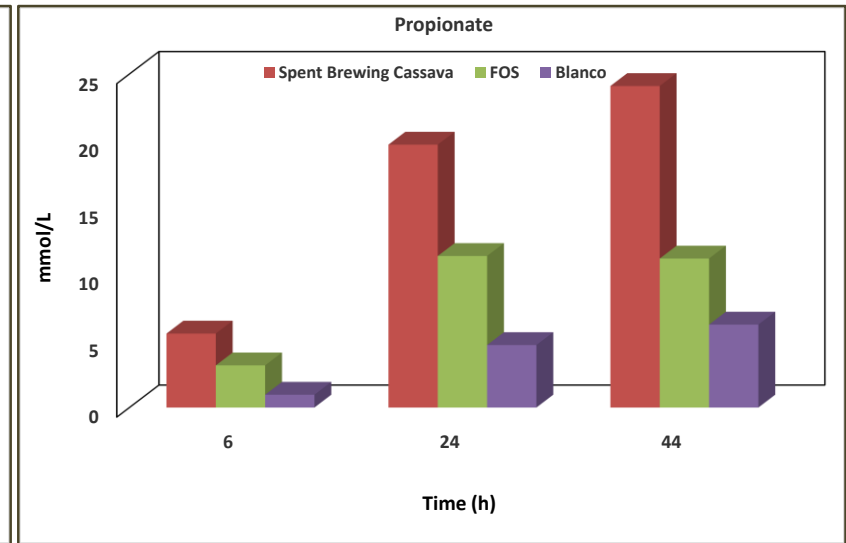
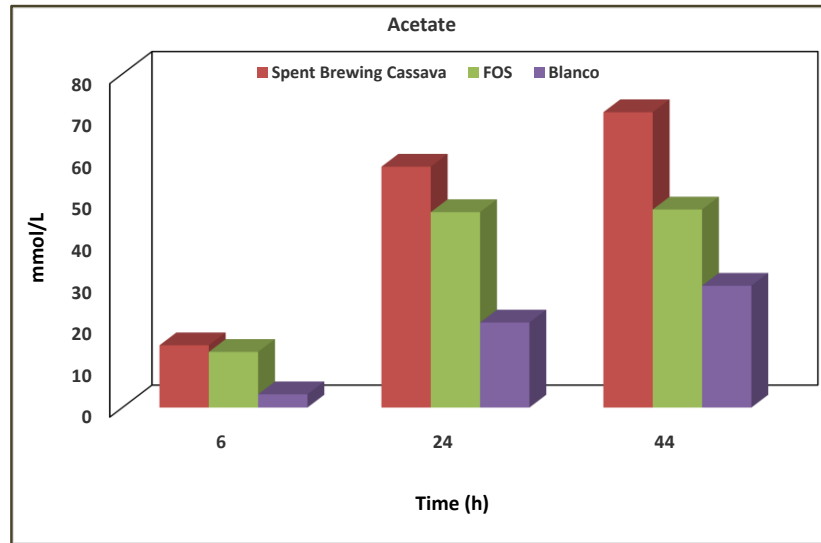
Journal of Functional Foods (accepted)

Bifidobacterium

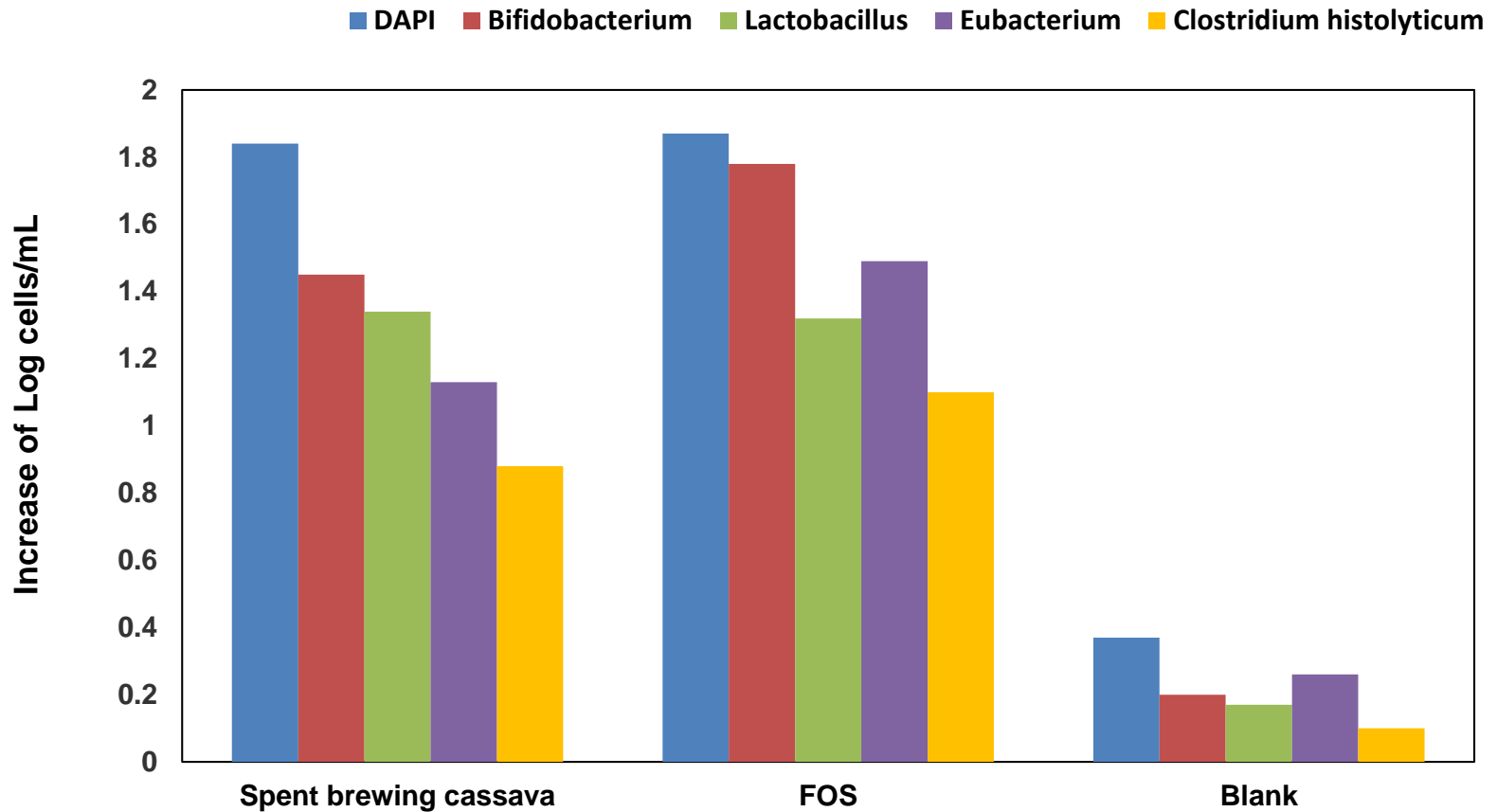




Atividade Prebiótica de resíduo de cerveja de mandioca

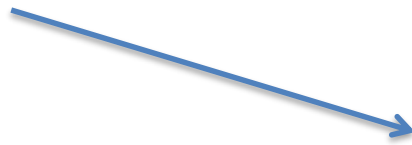


Atividade Prebiótica de resíduo de cerveja de mandioca



Actividades Prebiótica – *In vivo*

Modelos animais

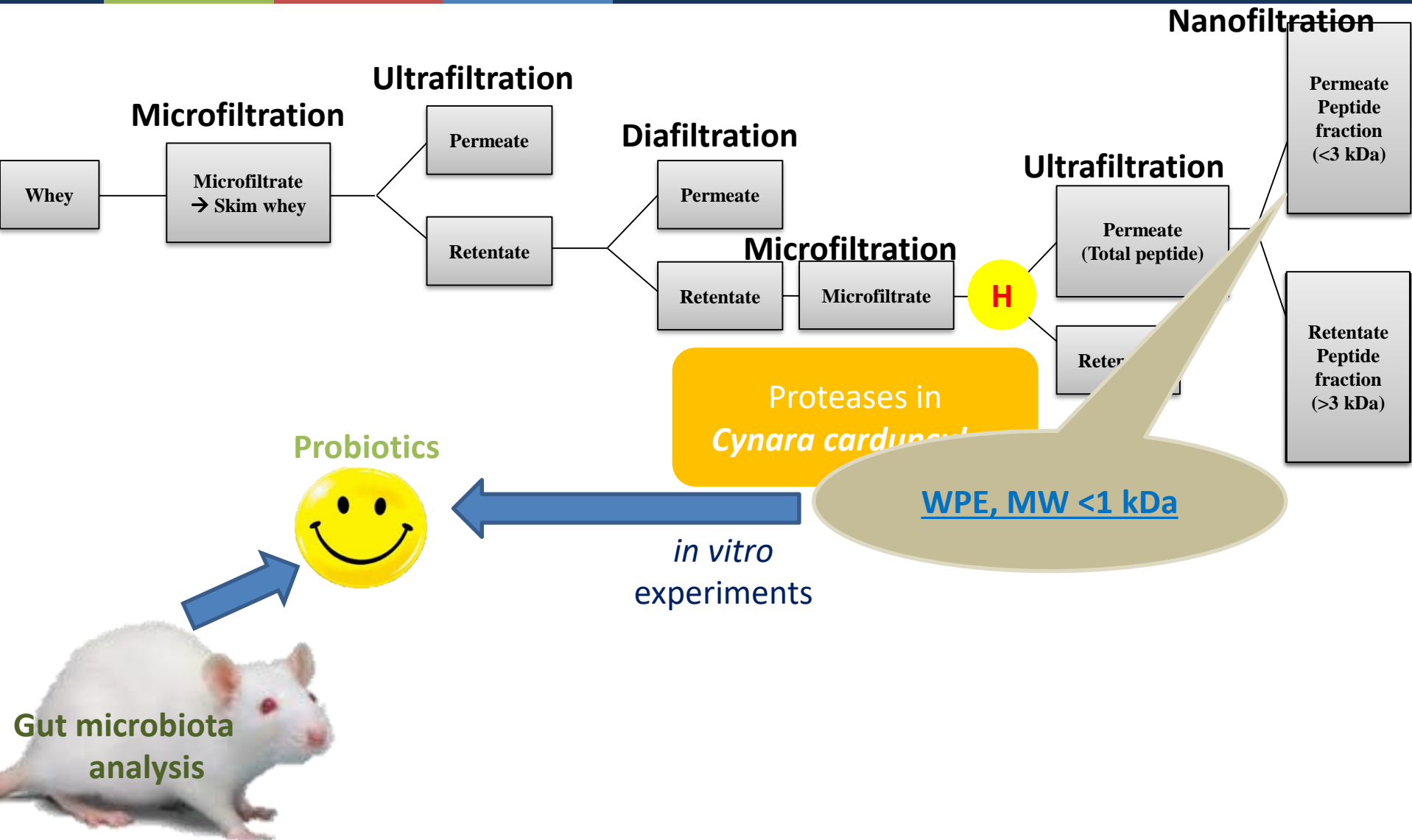


Síndrome Metabólico



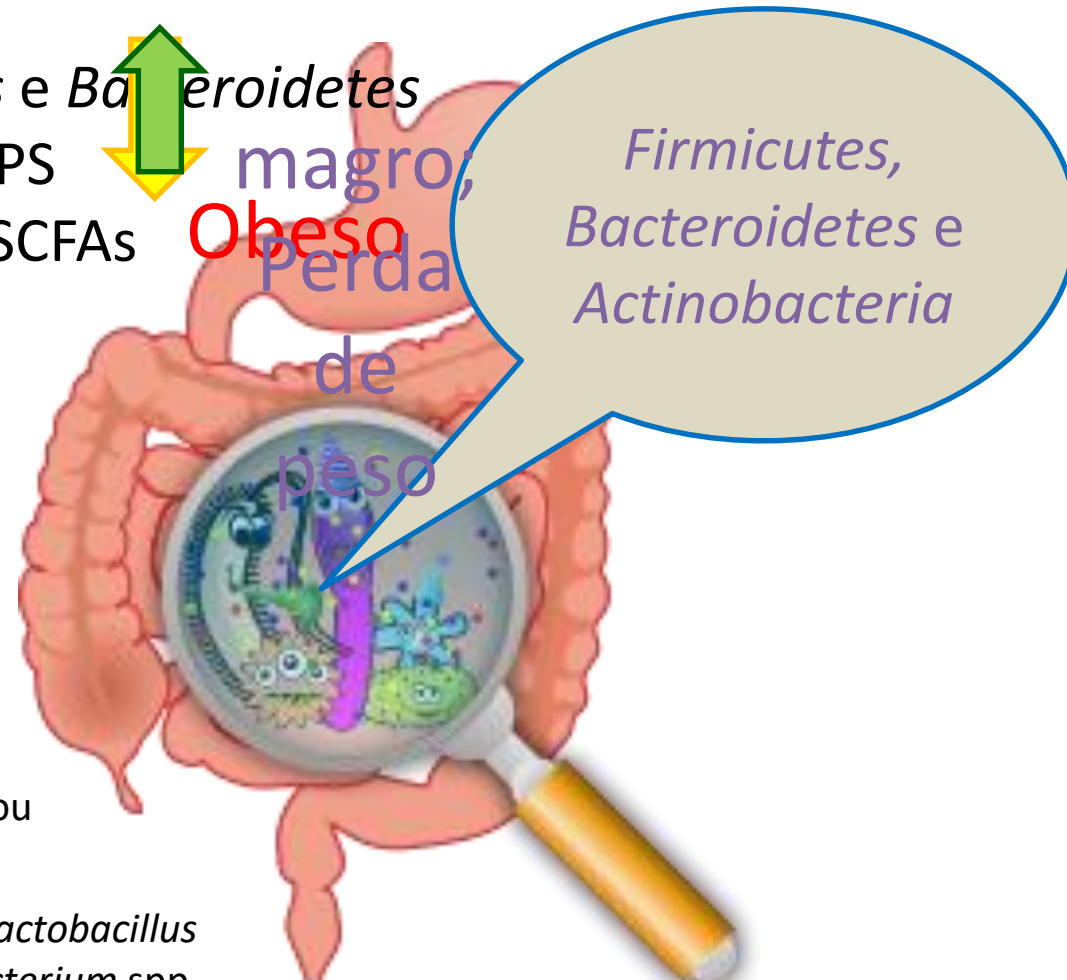
Financiamento: QREN/COMPETE – ACTIPEP Nº 11531

OBJECTIVES



✓ Associada com a **OBESIDADE**

- Quantidade de *Firmicutes* e *Bacteroidetes*
- Inflamação causada por LPS
- Metabolitos bacterianos: SCFAs



Prebióticos

Polissacáridos, oligosacáridos, ou monosacáridos

Probióticos

Bacteroides, *Lactobacillus* spp., *Bifidobacterium* spp.

Lactato, acetato

Firmicutes

Firmicutes
Clostridium cluster IX

Firmicutes
Clostridium cluster IV e XIVa

Propionato

Butirato

LPS: Lipopolisacárido

SCFAs: Short-chain fatty acids

Gut microbiota analysis

- 16S rRNA expression (qPCR)



DNA
extraction



Realtime qPCR:
Quantification of
16S rRNA expression

Group	Phyla	Genera
St	<i>Actinobacteria</i> <i>Firmicutes</i> <i>Bacteroidetes</i>	<i>Lactobacillus</i> spp. <i>Bifidobacterium</i> spp.
St + <u>WPE 350 mg/kg/day</u>		
HF		
HF + <u>WPE 350 mg/kg/day</u>		

Group	Phyla	Genera
St	<i>Actinobacteria</i> <i>Firmicutes</i> <i>Bacteroidetes</i>	<i>Lactobacillus</i> spp. <i>Bifidobacterium</i> spp.
St + <u>P 5 mg/kg/day</u>		
HF		
HF + <u>P 5 mg/kg/day</u>		

St: Standard diet

HF: High-fat diet

WPE: Whey peptide extract

P: Pure peptide

Under the standard diet:

Intake of WPE

Significantly ↑



Bifidobacterium spp.
Lactobacillus spp.
Bacteroidetes



Actinobacteria
Firmicutes



F/B ratio

Under the HF diet:

Intake of WPE

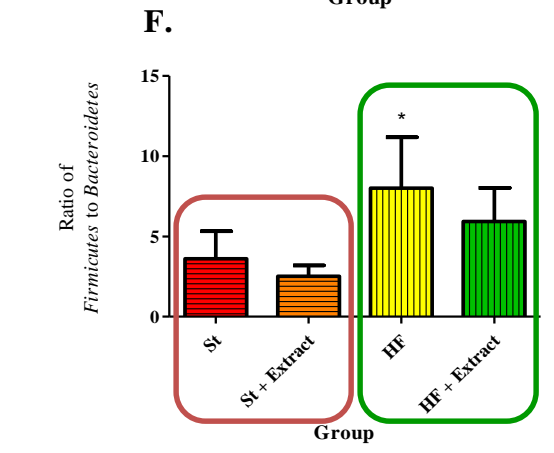
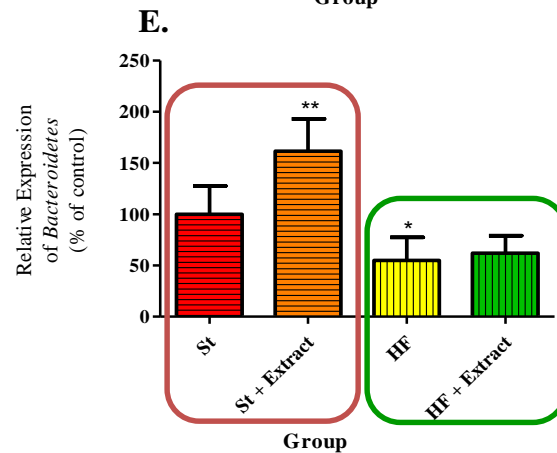
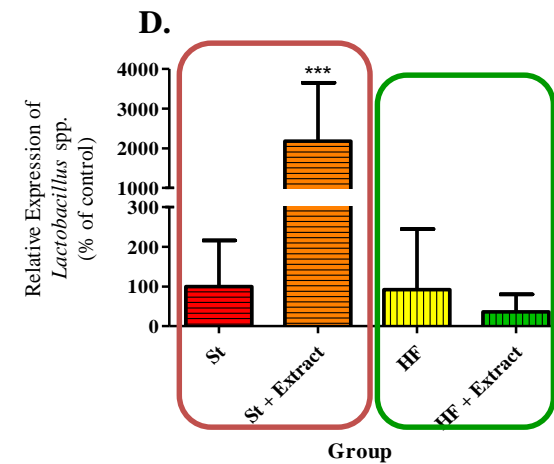
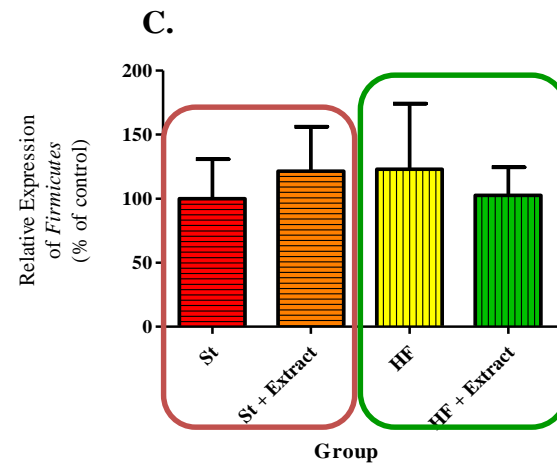
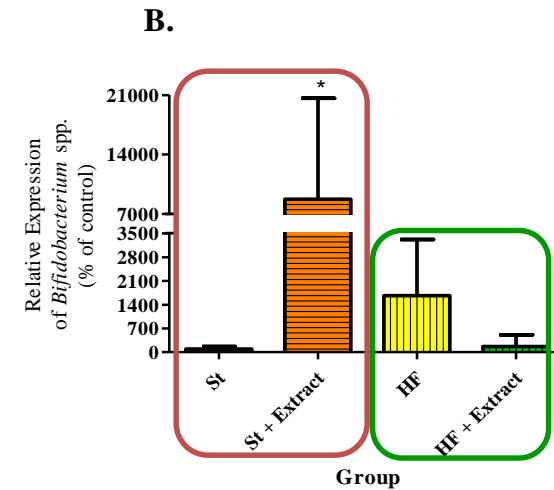
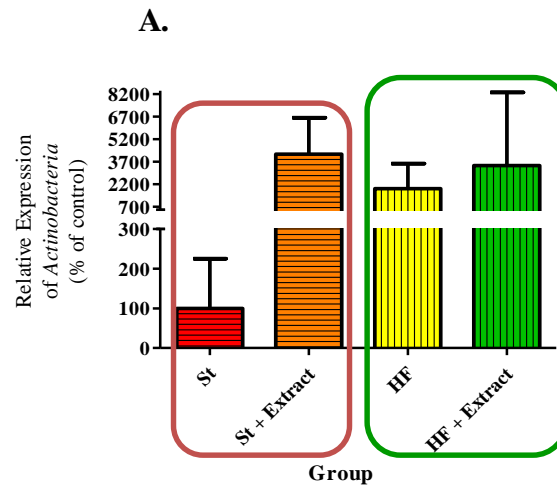
No significant change



Actinobacteria



Bifidobacterium spp.
Lactobacillus spp.
Firmicutes
F/B ratio



Effects of Spent Yeast Peptides on Gut Microbiota



Manuela Amorim

Prof^a. Doutora Maria Manuela Estevez Pintado

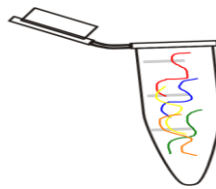
Prof^a. Doutora Maria da Conceição Costa Pinho Calhau

Síndrome Metabólica – Efeito prébiotico de péptidos de levedura

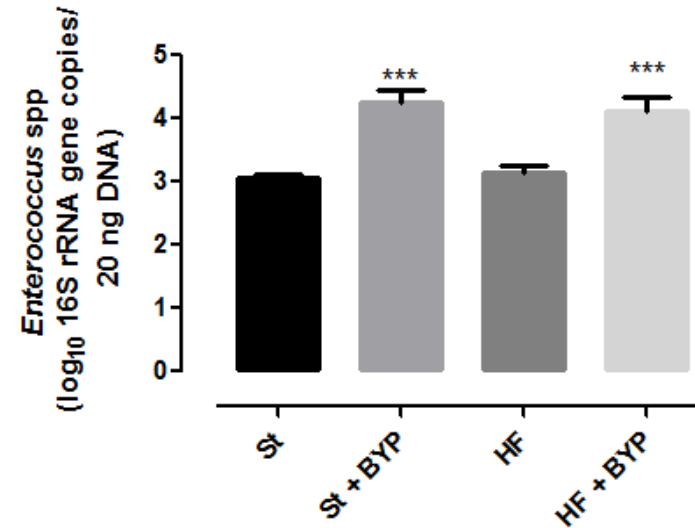
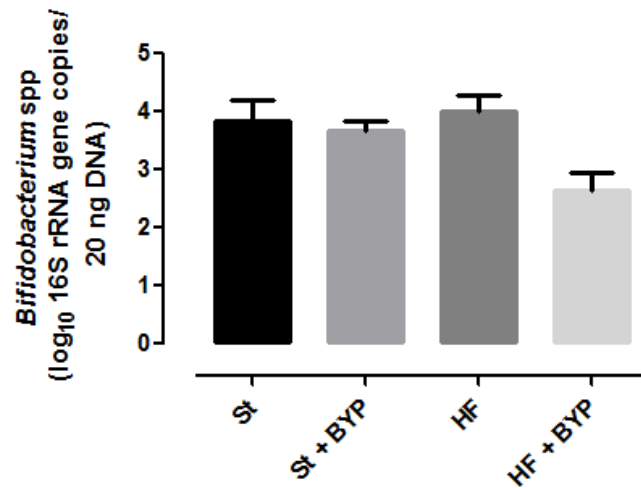
Amostras fecais
directamente colhidas do
colon dos animais



Extração DNA
Genómico



Análise RNA 16S – PCR Tempo real
Bifidobacterium spp *Enterococcus* spp
Filos:
Firmicutes, Bacteroidetes



Project team

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Governo da República Portuguesa



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Obrigado pela vossa atenção!