WP2 : Innovative plant-based dairy analogues : Fermented products

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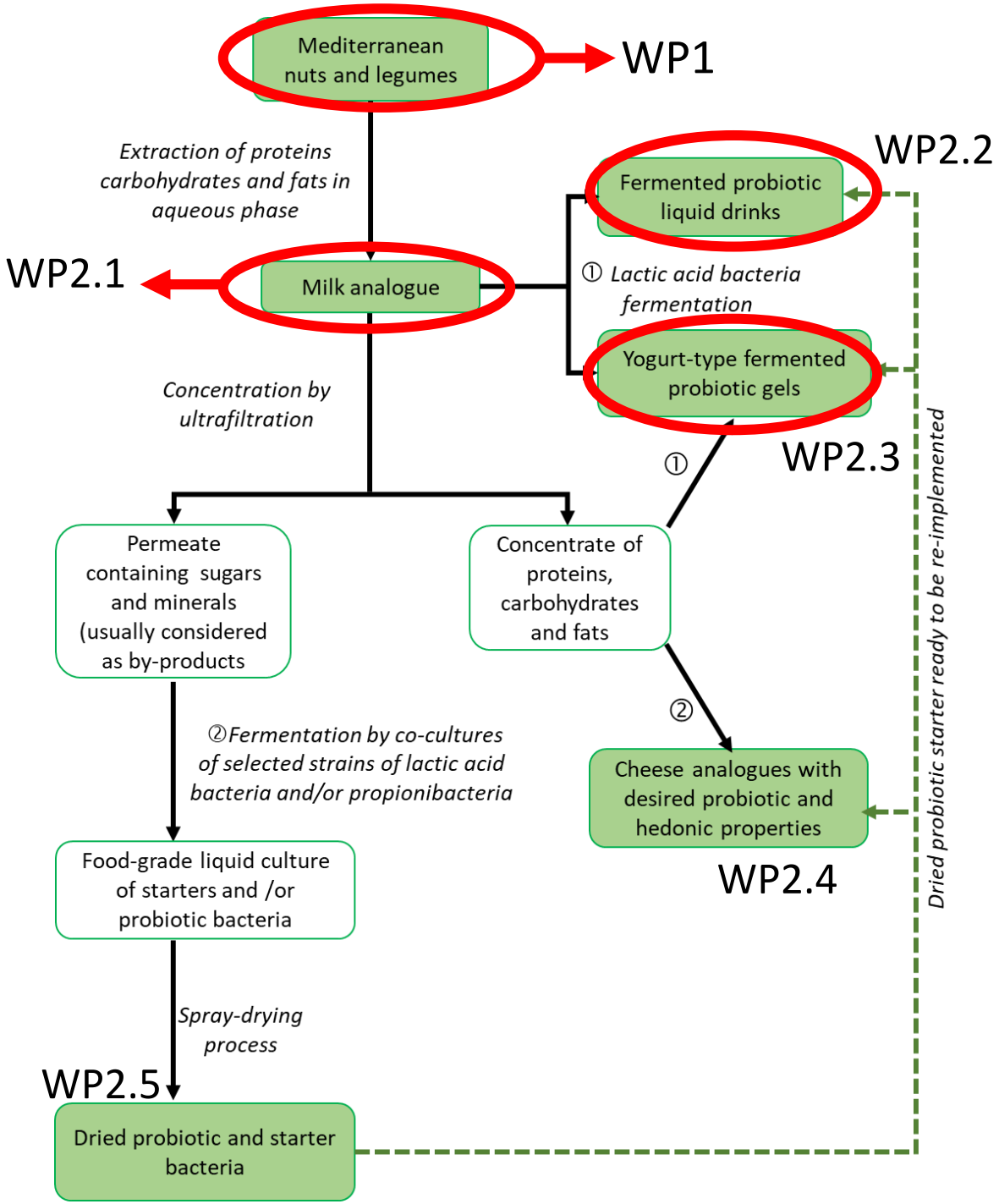
LOCALNUTLEG

WP2 : Innovative plant-based dairy analogues : Fermented products

Rennes, May 03, 2023



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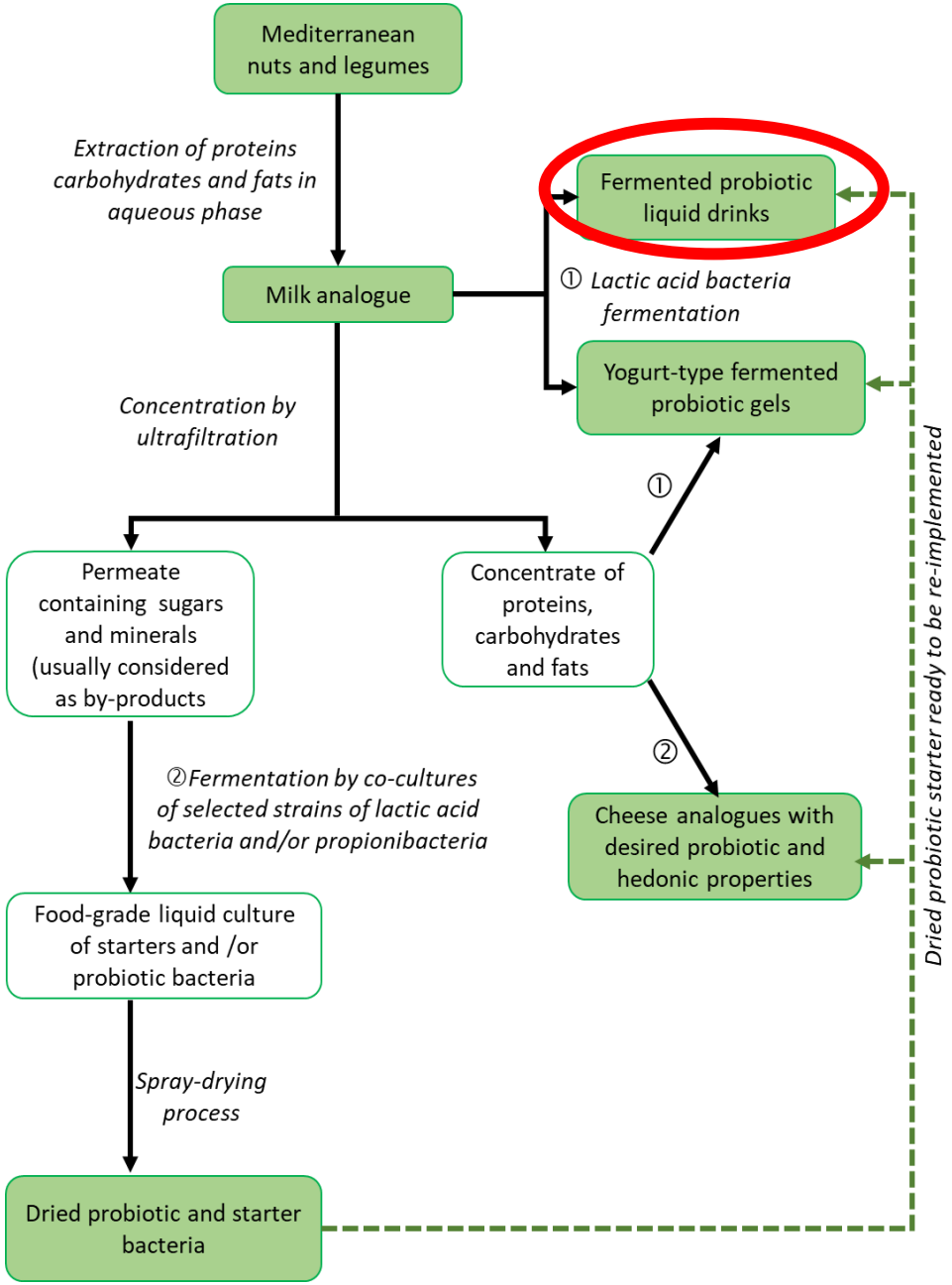
Task 2.2. Fermented probiotic plant-based beverages

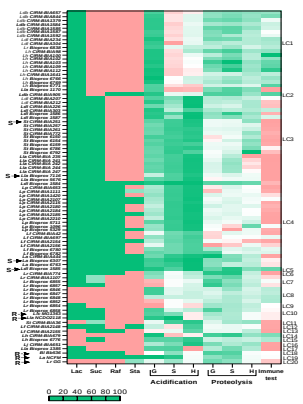


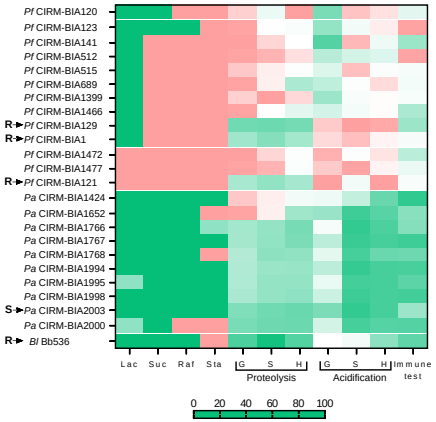
Task 2.2. Fermented probiotic plant-based beverages

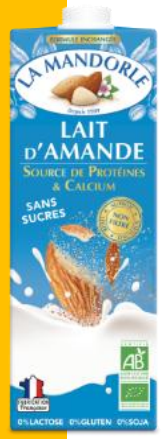
Experimental strategy :

Strains pre-selection (≈ 40 strains)
- Ability to use sucrose
- Anti-inflammatory properties

Use of the work of Illikoud et al., 2023







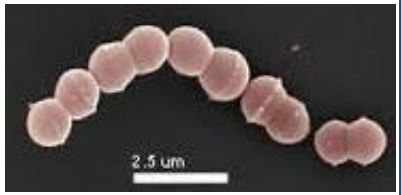
Screening of the pre-selected strains on commercial UHT almond milk
- Strain growth assessment (pH, bacterial count)
- Organoleptic properties assessment (lab test (5-6 tasters))

Creation and testing of consortia with the selected strains

Study of selected consortia in almond milk with and without 2,5% sucrose
- Growth kinetics (bacterial count, pH, consumption/production of carbohydrate, organic acid, amino acid, volatile compounds…)
- Anti-inflammatory properties evaluation (with cell culture) **in progress**
- Sensory analysis with WP5 (UPC & FMA in Barcelona)

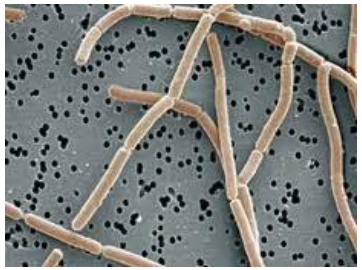
Lactic Acid Bacteria (LAB)

Gram +
Cocci or bacilli



S. thermophilus

Feature : **Acid lactic** production from different carbohydrates


L. delbrueckii subsp. *bulgaricus*

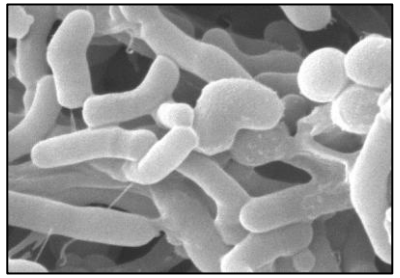
Functions : Acidification, coagulation, aromas/flavours, proteolysis
Used in all fermented dairy products (cheese, yogurt, butter…)

Examples of LAB:
Streptococcus thermophilus
Lactococcus lactis
Lactobacillus delbrueckii subsp. *bulgaricus*
Lacticaseibacillus casei

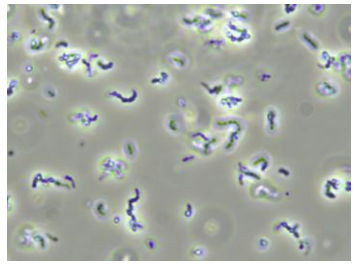
In the WP2 Localnutleg :
Use for the fermented milk, the yogurt-type and the cheese analogue

Propionic Acid Bacteria (PAB)

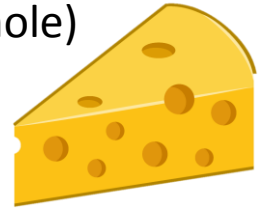
Gram +
Cocobacilli (Short bacilli)


P. freudenreichii

Feature : **Propionic acid** (involved in the test and smell of cheese) production from **acid lactic**


A. acidipropionici

Functions : Aromas/flavours
Texture (Swiss-type cheese hole)
Used in cheeses



Examples of PAB:
Propionibacterium freudenreichii
Acidipropionibacterium acidipropionici

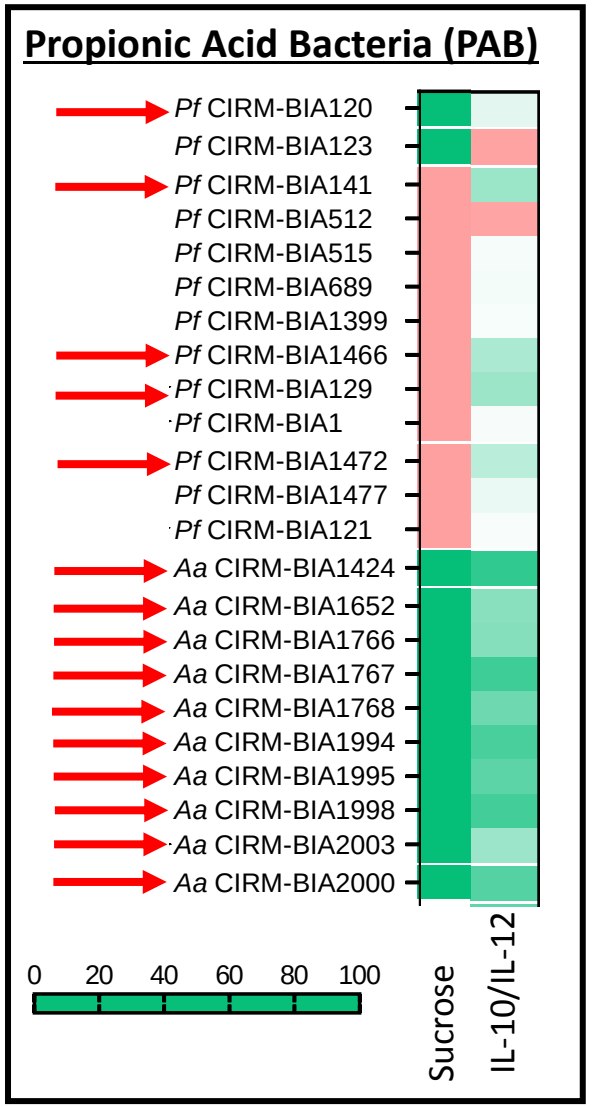
In the WP2 Localnutleg :
Use for the cheese analogue with the LAB

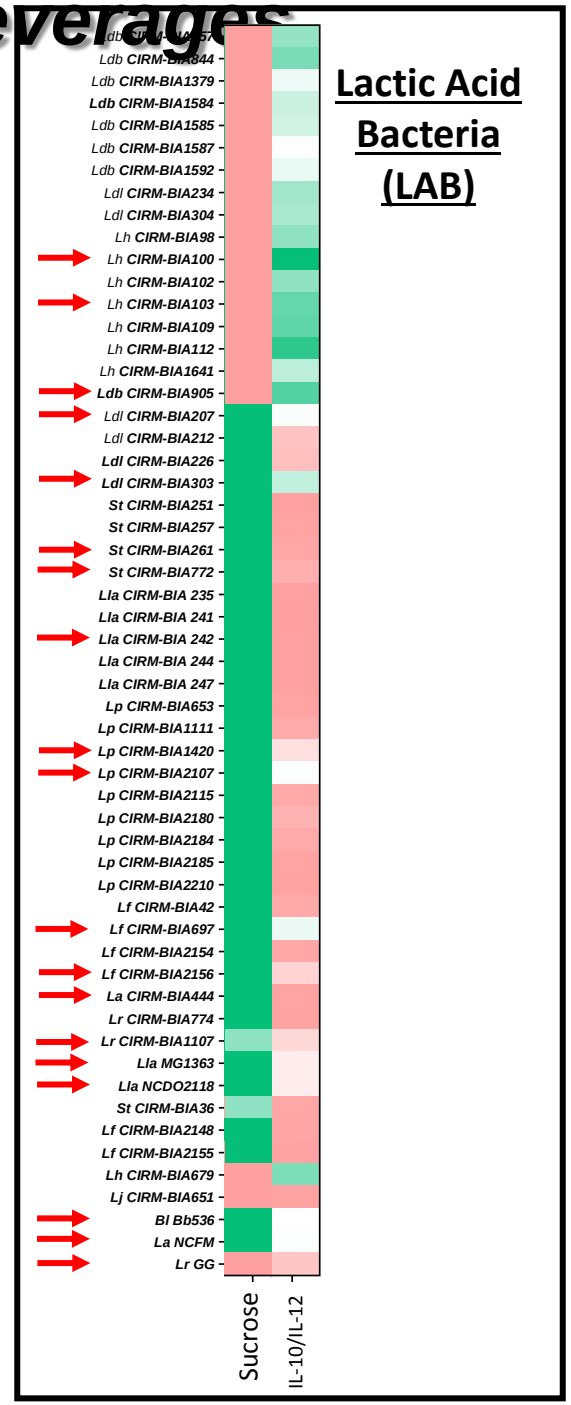
Task 2.2. Fermented probiotic plant-based beverages

Strains pre-selection

Pre-selection of 38 strains :

- 23 LAB
- 15 PAB





Illikoud et al., 2023

Task 2.2. Fermented probiotic plant-based beverages

Screening of the pre-selected lactic acid bacteria strains

Species	Strain number	pH drop	Organoleptic assessment (appreciation converted into ratings)	Anti-inflammatory properties (IL10/IL12)
Lactococcus lactis subsp. *lactis*	Lla NCDO2118	2,74	8	8
Lactococcus lactis subsp. *lactis*	Lla CIRM-BIA 242	2,42	6	0,02
Lactococcus lactis subsp. *lactis*	Lla Bioprox 5676	2,61	7	0,06
Lactococcus lactis subsp. *cremoris*	Llc MG1363	1,55	3	8
Lactiplantibacillus plantarum	Lp CIRM-BIA1420	2,55	2	6,58
Lactiplantibacillus plantarum	Lp CIRM-BIA2107	2,57	2	11,18
Lactiplantibacillus plantarum	Lp Bioprox 1096	2,49	2	12,53
Lacticaseibacillus casei	Lc CIRM-BIA1643	1,04	7	Well-know probiotic effect
Lactobacillus delbrueckii subsp. *lactis*	Ldl CIRM-BIA207	1,39	4	11,75
Lactobacillus delbrueckii subsp. *lactis*	Ldl CIRM- BIA303	1,32	4	31,97
Lactobacillus johnsonii	Lj Bioprox 5467	2,52	4	Inconnu
Streptococcus salivarius subsp. *thermophilus*	St CIRM-BIA772	1,5	10	1,5
Streptococcus salivarius subsp. *thermophilus*	St CIRM-BIA251	2,88	9	0,1
Lactobacillus delbrueckii subsp. *bulgaricus*	Ldb CIRM-BIA905	0,46	8	71,98
Limosilactobacillus fermentum	Lf CIRM-BIA697	0,31	5	17,5
Limosilactobacillus fermentum	Lf CIRM-BIA2156	0,09	5	5,27
Lacticaseibacillus rhamnosus	Lr CIRM-BIA1107	0,98	7	5,86
Lacticaseibacillus rhamnosus	Lr GG	0,79	4	4
Lactobacillus helveticus	Lh CIRM-BIA100	0,38	5	100
Lactobacillus helveticus	Lh CIRM-BIA103	0,42	5	65,12
Lactobacillus acidophilus	La NCFM	0,86	3	11
Lactobacillus acidophilus	La CIRM-BIA444	2,05	5	0,29
Bifidobacterium longun	Bl Bb536	0,66	8	10

Some strains give a lactic (yogurt) note, promising for fermented milk making

Selection of strains to design consortia :

Mesophilic consortium (30°C) :

- *Lactococcus lactis* subsp. *lactis* NCDO2118
- *Lacticaseibacillus casei* CIRM-BIA1643

Thermophilic consortium (43°C) :

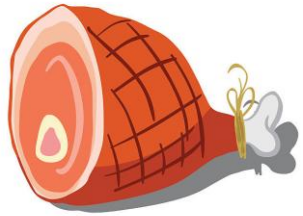
- *Streptococcus thermophilus* CIRM-BIA772
- *Lactobacillus delbrueckii* subsp. *bulgaricus* CIRM-BIA905

Task 2.2. Fermented probiotic plant-based beverages

Screening of the pre-selected propionic acid bacteria strains

Species	Strain number	pH drop	Organoleptic assessment (appreciation converted into ratings)	Anti-inflammatory properties (IL10/IL12)
Acidipropionibacterium acidipropionici	Aa CIRM-BIA1652	2,21	3	52,24
Acidipropionibacterium acidipropionici	Aa CIRM-BIA1766	2,39	3	53,91
Acidipropionibacterium acidipropionici	Aa CIRM-BIA1767	1,74	3	79,32
Acidipropionibacterium acidipropionici	Aa CIRM-BIA1768	2,12	3	61,88
Acidipropionibacterium acidipropionici	Aa CIRM-BIA1994	1,7	3	75,82
Acidipropionibacterium acidipropionici	Aa CIRM-BIA1995	1,57	5	68,49
Acidipropionibacterium acidipropionici	Aa CIRM-BIA1998	2,17	3	77,57
Acidipropionibacterium acidipropionici	Aa CIRM-BIA2003	1,81	3	44,98
Acidipropionibacterium acidipropionici	Aa CIRM-BIA1424	0,95	5	84,46
Acidipropionibacterium acidipropionici	Aa CIRM-BIA2000	1,55	5	71,05
Propionibacterium freudenreichii	Pf CIRM-BIA120	0,77	8	19,97404906
Propionibacterium freudenreichii	Pf CIRM-BIA141	0,74	5	45,86368605
Propionibacterium freudenreichii	Pf CIRM-BIA129	0,7	5	45,62
Propionibacterium freudenreichii	Pf CIRM-BIA1466	1,15	5	39,88066655
Propionibacterium freudenreichii	Pf CIRM-BIA1472	0,72	8	34,60363517

Meat notes



Cheesy notes

Promising for vegetable cheese making

Task 2.2. Fermented probiotic plant-based beverages

Creation and testing of consortia with the selected strains

Code	Species	Strain number	pH drop	Organoleptic assessment (appreciation converted into ratings)
Mesophilic consortium : St 772 + Ldb 905	*Streptococcus thermophilus*	St CIRM-BIA772	2,65	4
	Lactobacillus delbrueckii subsp. *bulgaricus*	Ldb CIRM-BIA905		
Thermophilic consortium : Lla NCDO2118 + Lc 1643	*Lactococcus lactis* subsp. *lactis*	Lla NCDO2118	2,4	3,00
	Lacticaseibacillus casei	Lc CIRM-BIA1643		

Strains can give good flavours alone but once in consortium give bad ones

rancid and fatty notes

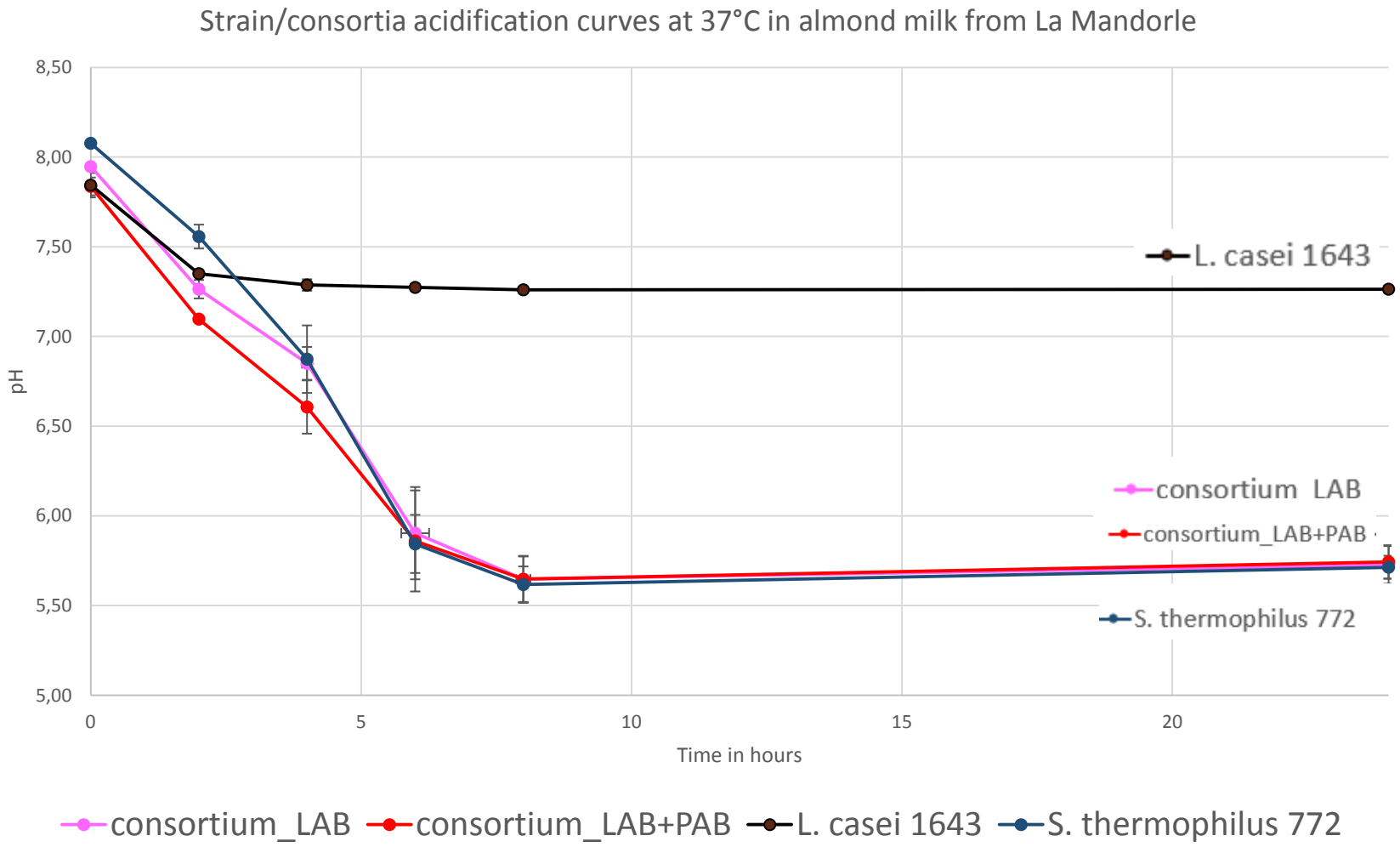
Lactic and fresh notes

Selection of two consortium for the following study :

- LAB consortium
- LAB+PAB consortium (addition of a PAB strain (Pf CIRM-141)) to improve the probiotic effect

Task 2.2. Fermented probiotic plant-based beverages

Growth kinetics – pH results

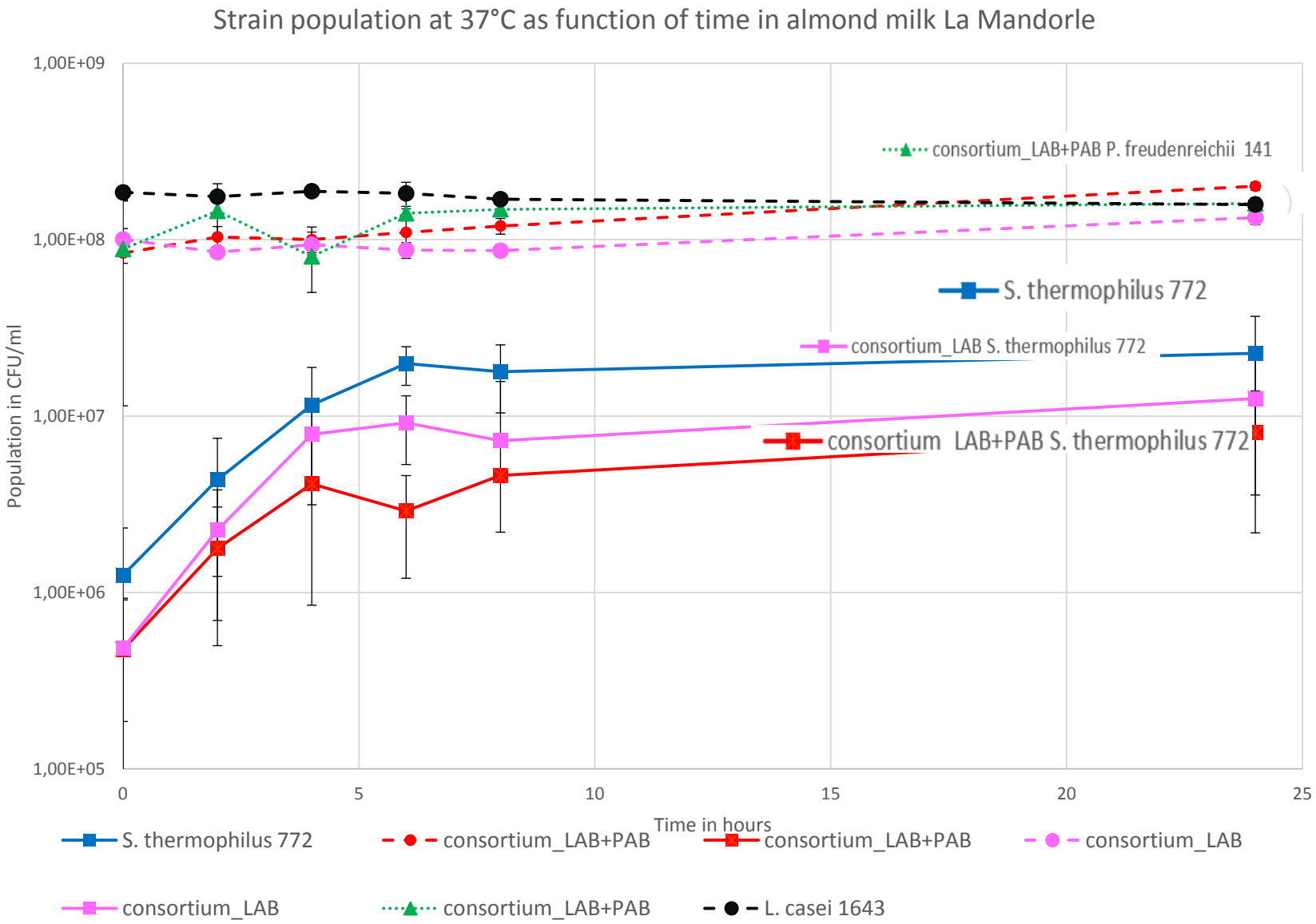


S. thermophilus is responsible for pH drop

Similar results with 2,5% sucrose
Slightly stronger acidification (Final pH of 5,2)

Task 2.2. Fermented probiotic plant-based beverages

Growth kinetics – bacterial count results



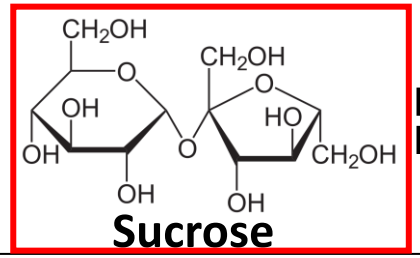
Growth of *S. thermophilus* (1 log)
Lower growth in the LAB+PAB consortium (competition for free amino acids)

Population of *L. casei* needed to induce a probiotic effect : 10^8 CFU/ml
Slight growth of *L. casei* in both consortia (use of fructose/glucose provided by *S. thermophilus* which degrades sucrose)

Population of *P. freudenreichii* stable (10^8 CFU/ml)
Enough to induce a probiotic effect

Task 2.2. Fermented probiotic plant-based beverages

Growth kinetics – carbohydrates consumption



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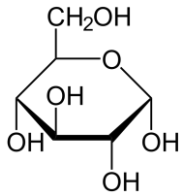

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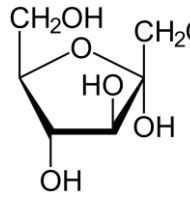
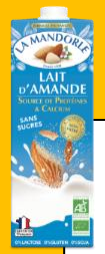
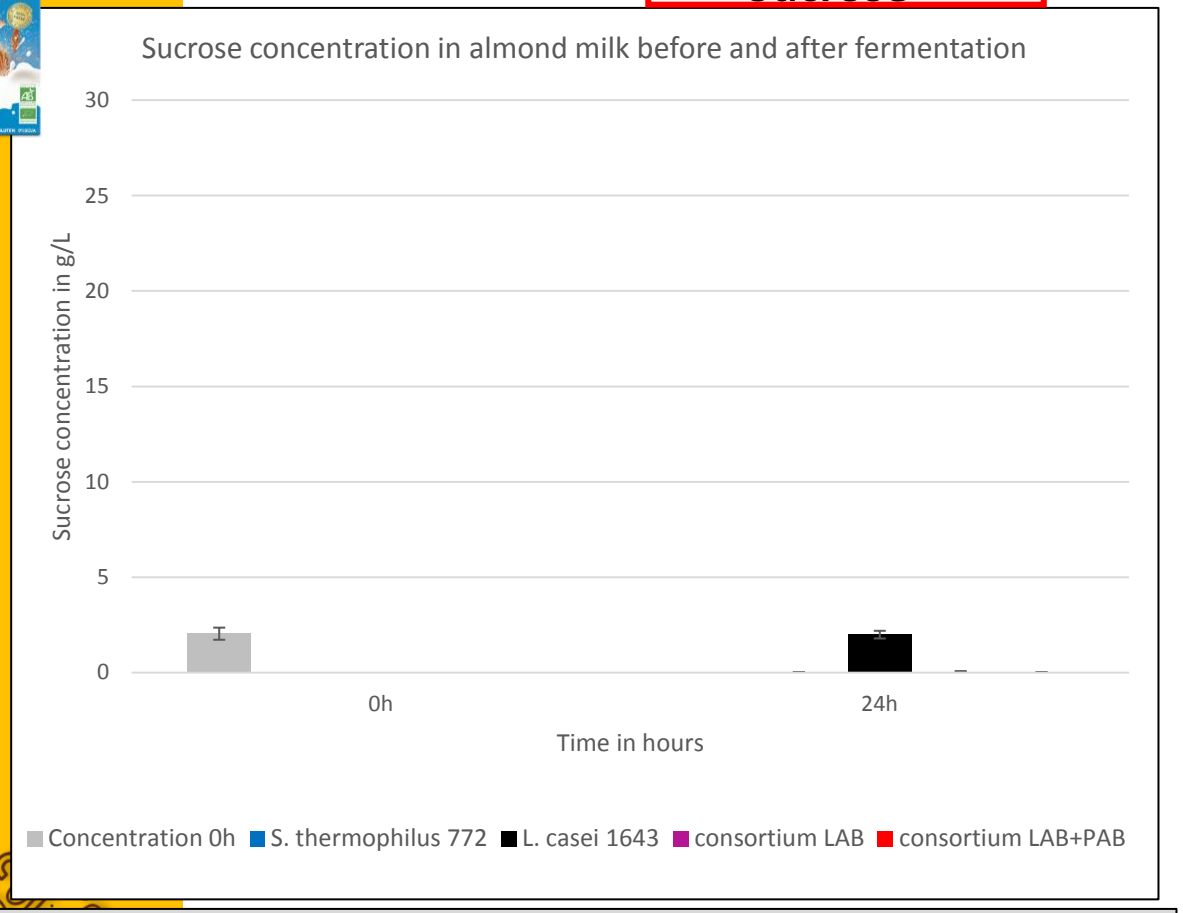
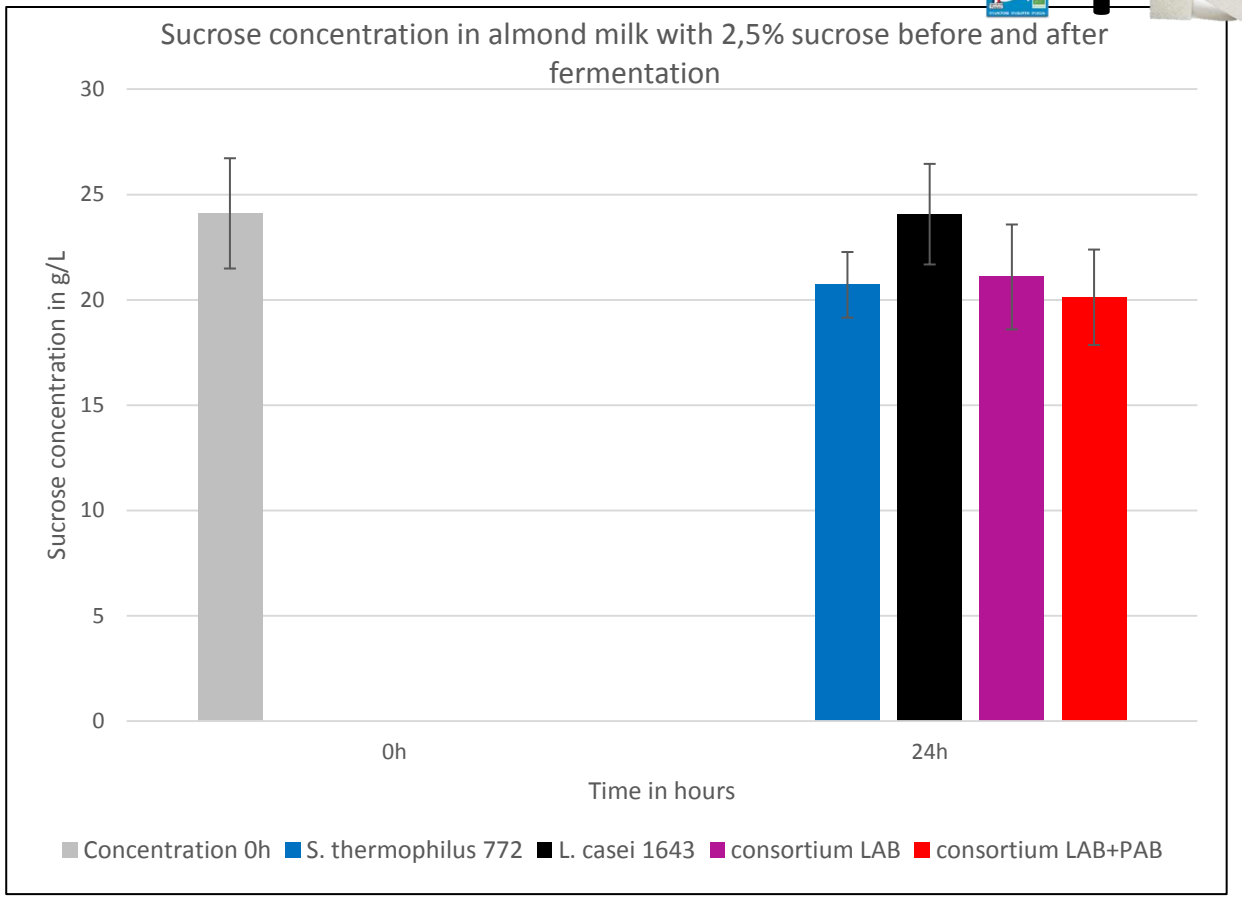






No consumption of the sucrose by *L. casei* (sucrose -)
Consumption of all the sucrose (2 g/L) by *S. thermophilus* (sucrose +)

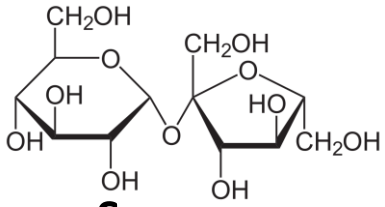
+



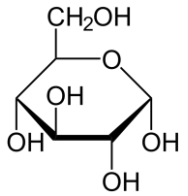
Consumption of sucrose is slightly more important (3 g/L) in almond milk with 25 g/L sucrose

Task 2.2. Fermented probiotic plant-based beverages

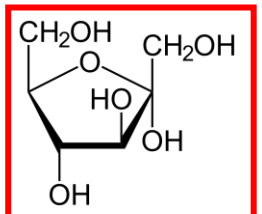
Growth kinetics – carbohydrates consumption



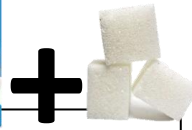
Sucrose

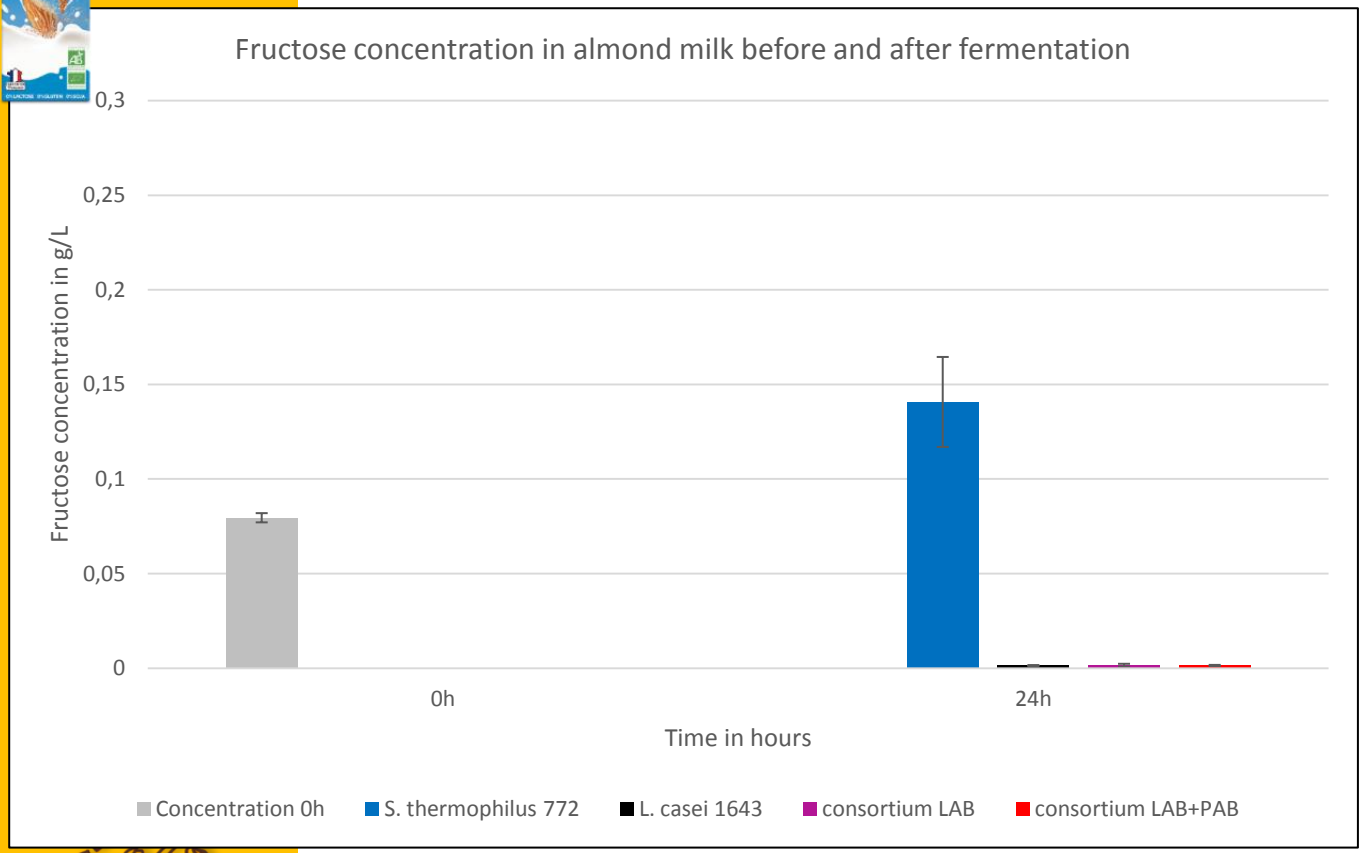


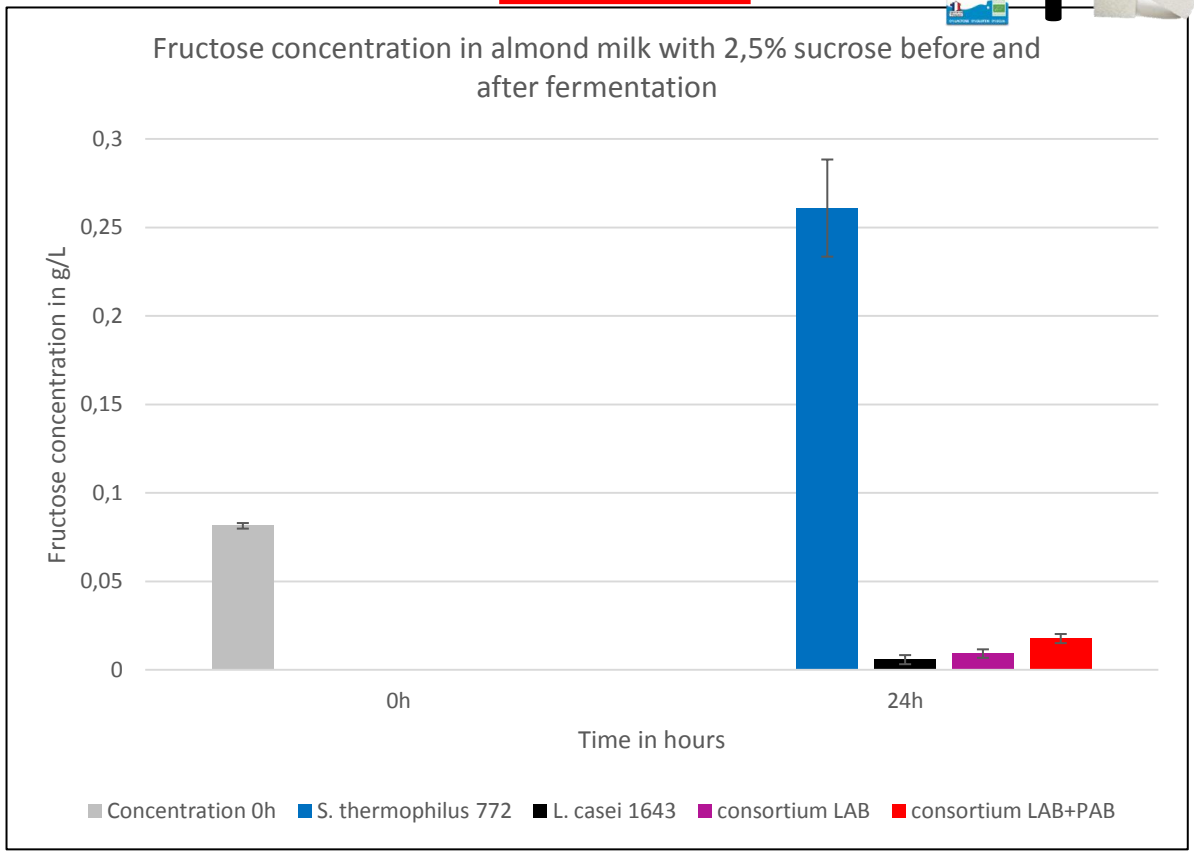
Glucose



Fructose





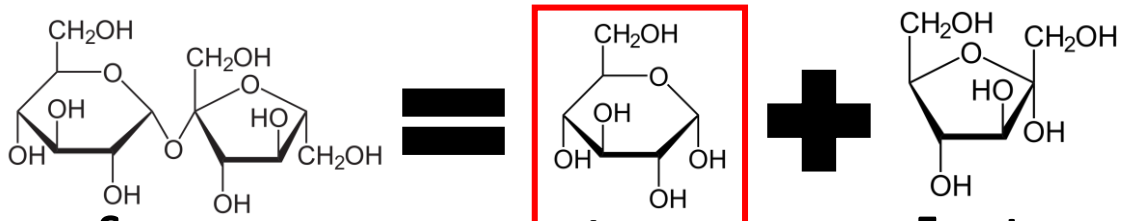


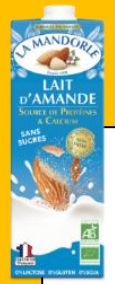
Fructose release by *S. thermophilus* (sucrose + and fructose +/-)
Fructose consumption by *L. casei* (fructose +)

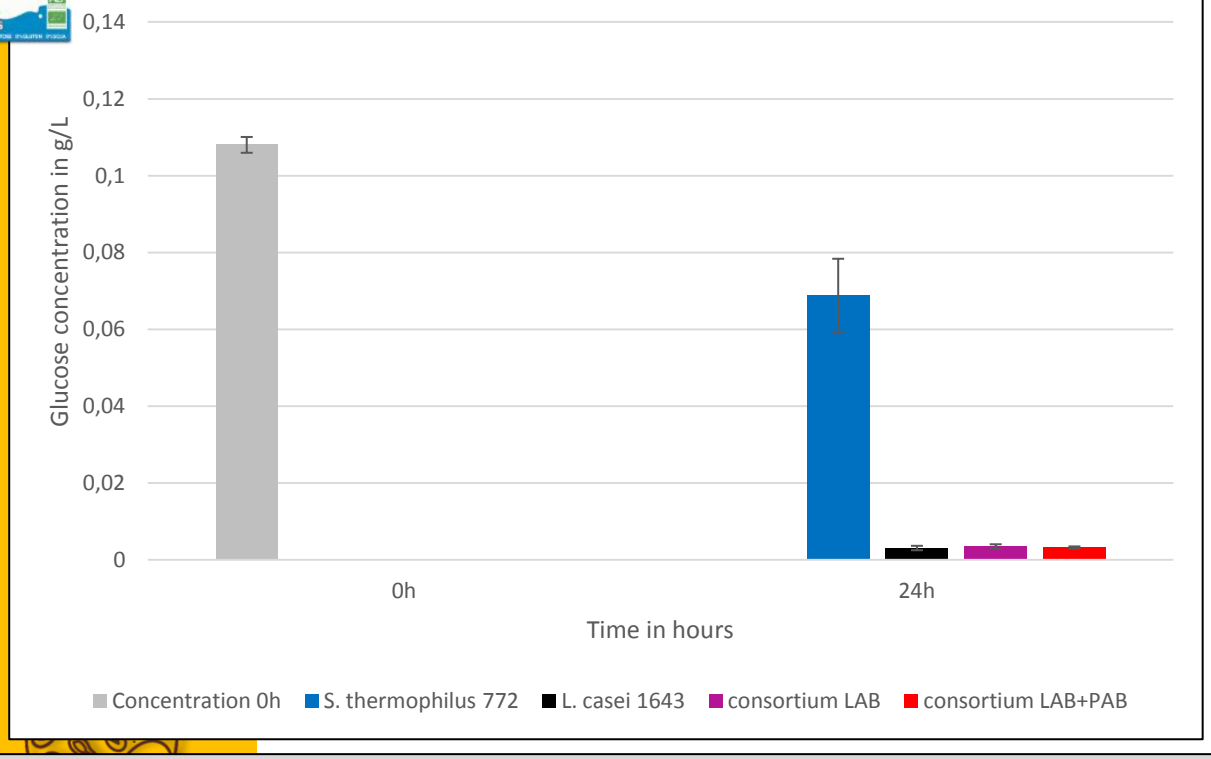
Same conclusion but with more fructose released (because more sucrose were used by *S. thermophilus*)

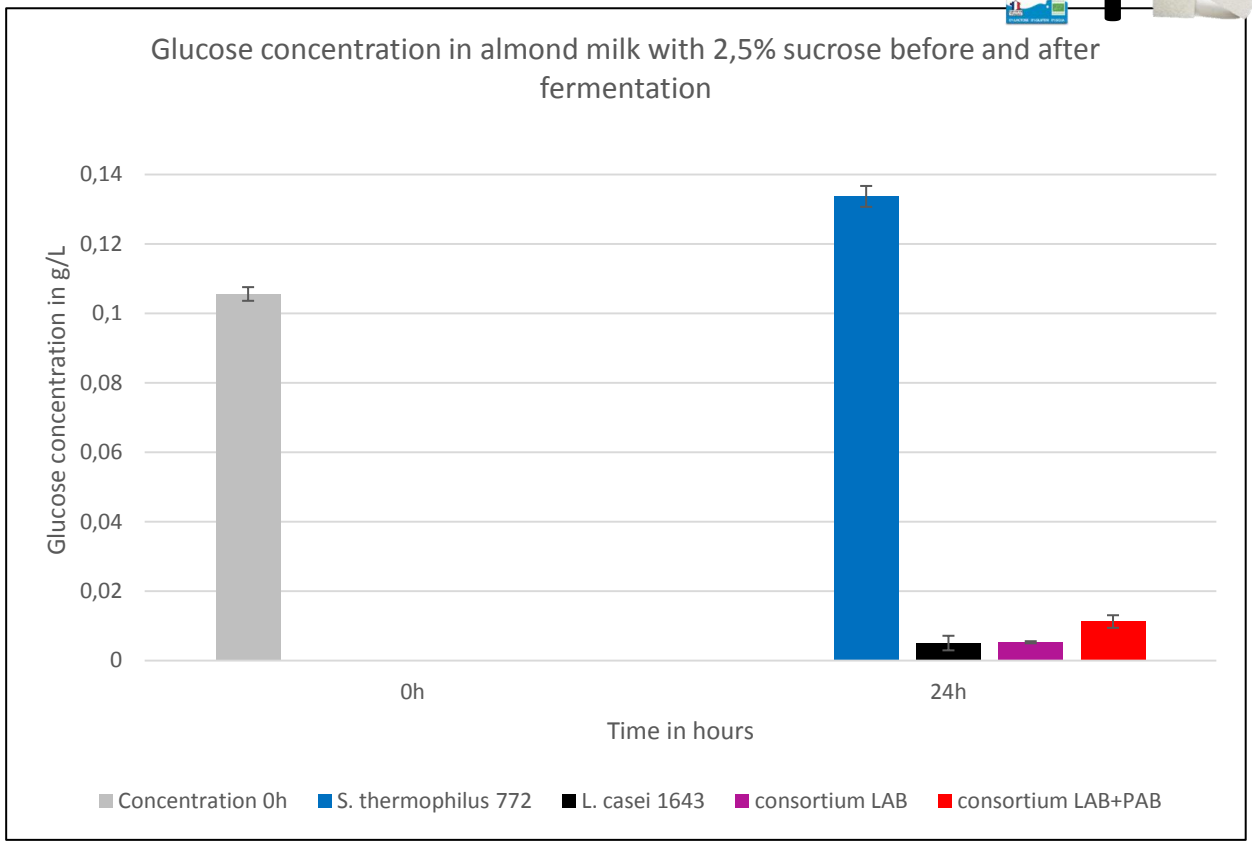
Task 2.2. Fermented probiotic plant-based beverages

Growth kinetics – carbohydrates consumption







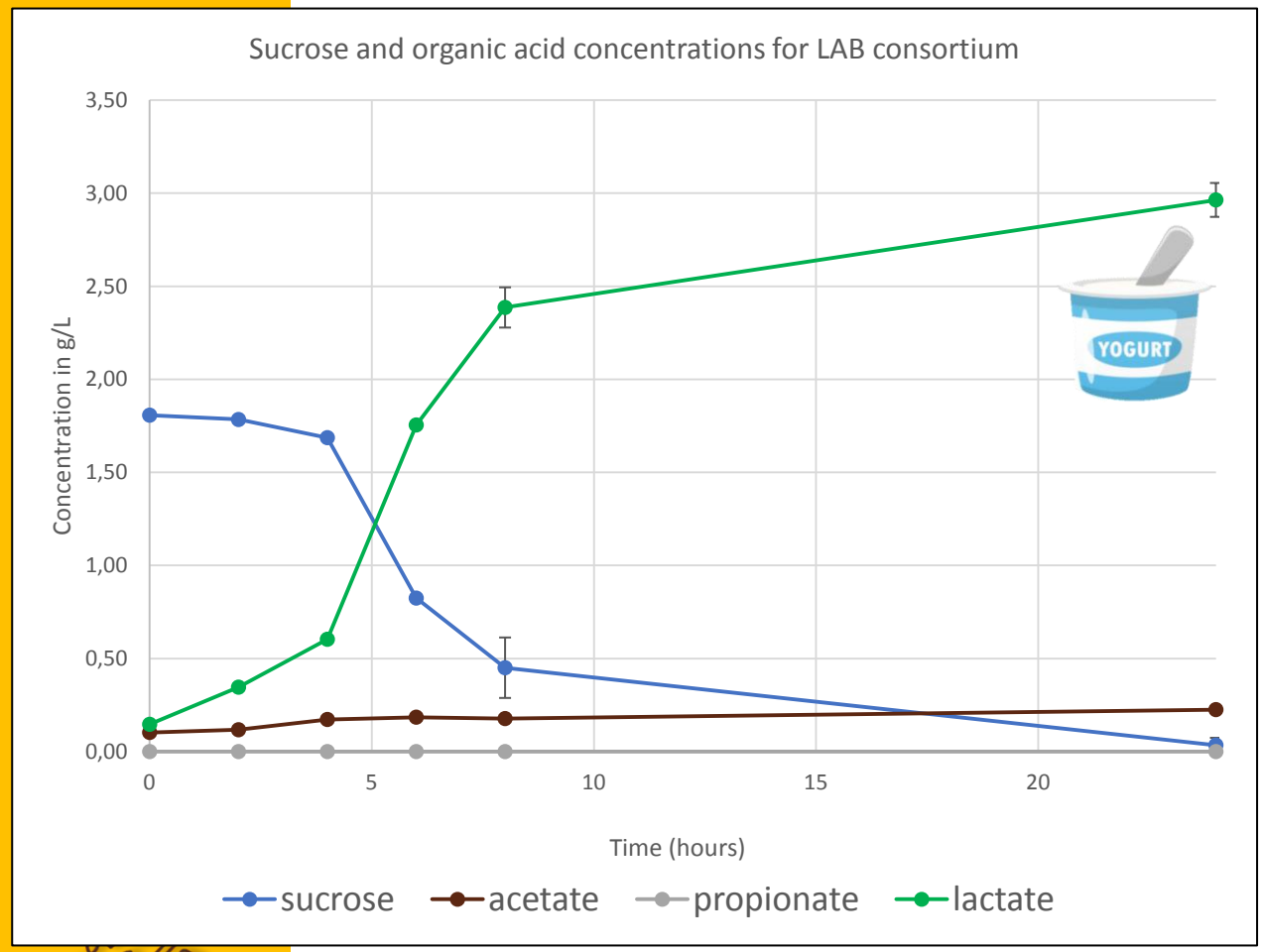


Glucose consumption by all strains (glucose +)
Residual glucose because *S. thermophilus* releases some (sucrose +)

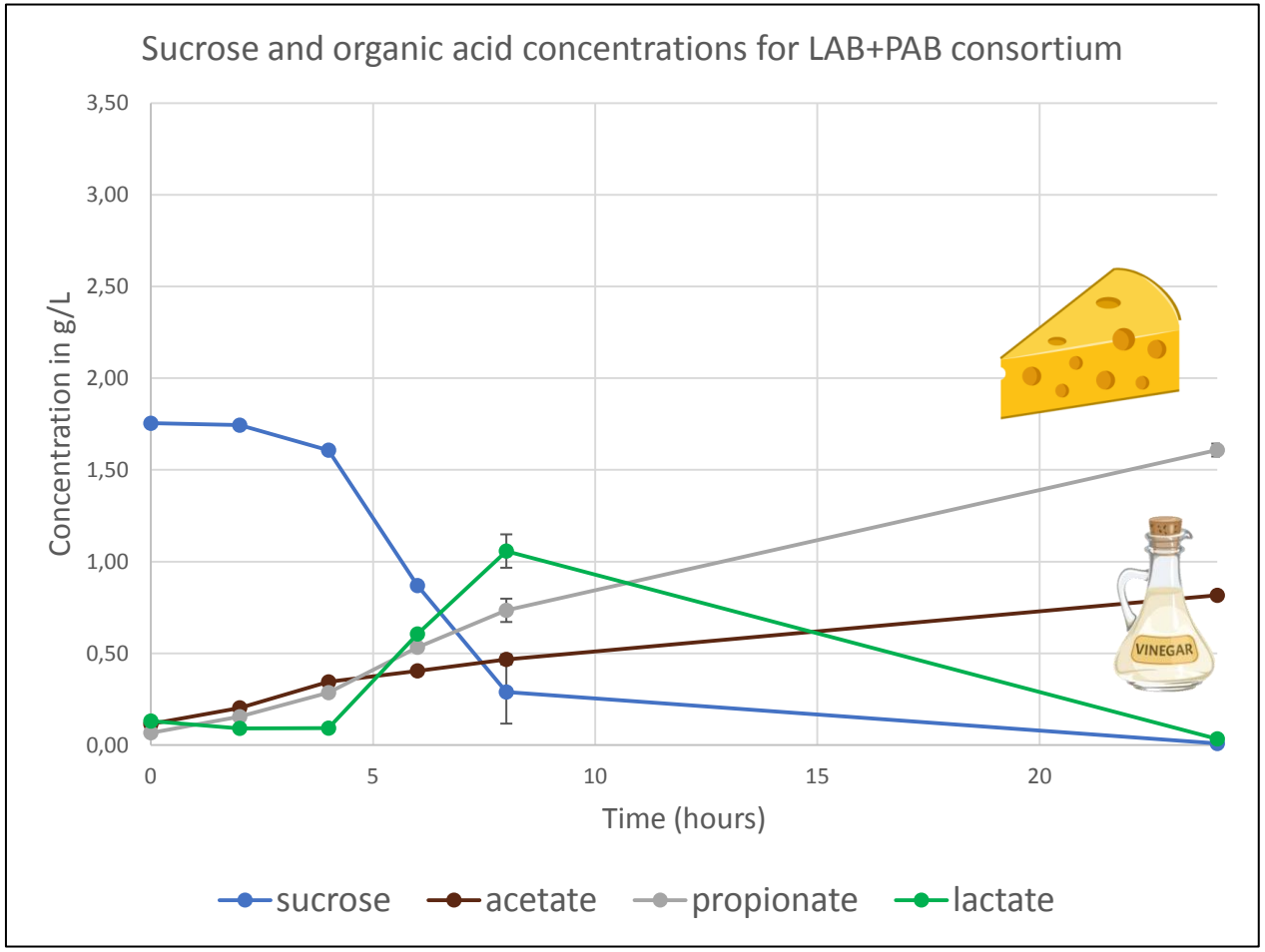
Same conclusion but *S. thermophilus* metabolizes more sucrose than it consumes glucose

Task 2.2. Fermented probiotic plant-based beverages

Growth kinetics



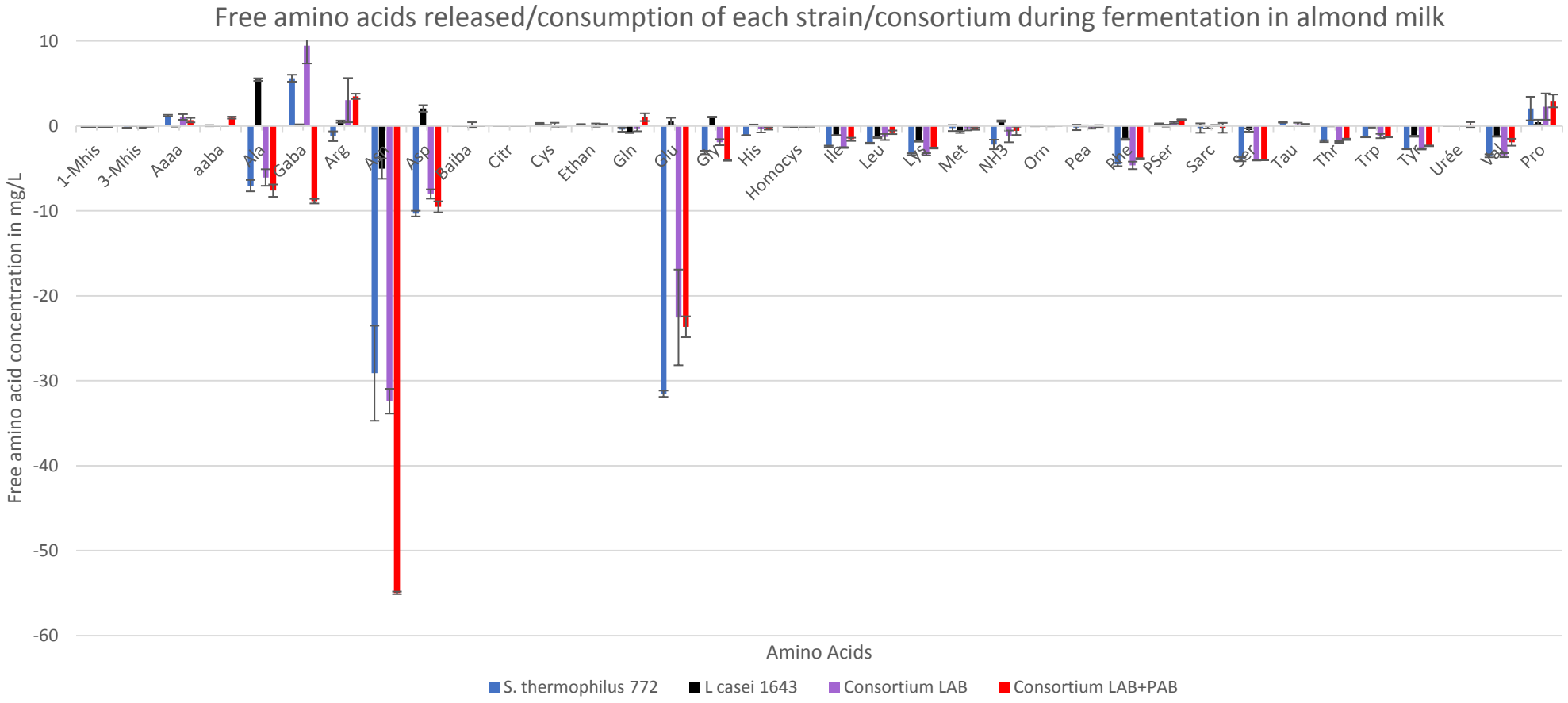
Sucrose consumption and lactate production by LAB



Sucrose consumption and lactate production by LAB
Lactate consumption and propionate/acetate production by PAB

Task 2.2. Fermented probiotic plant-based beverages

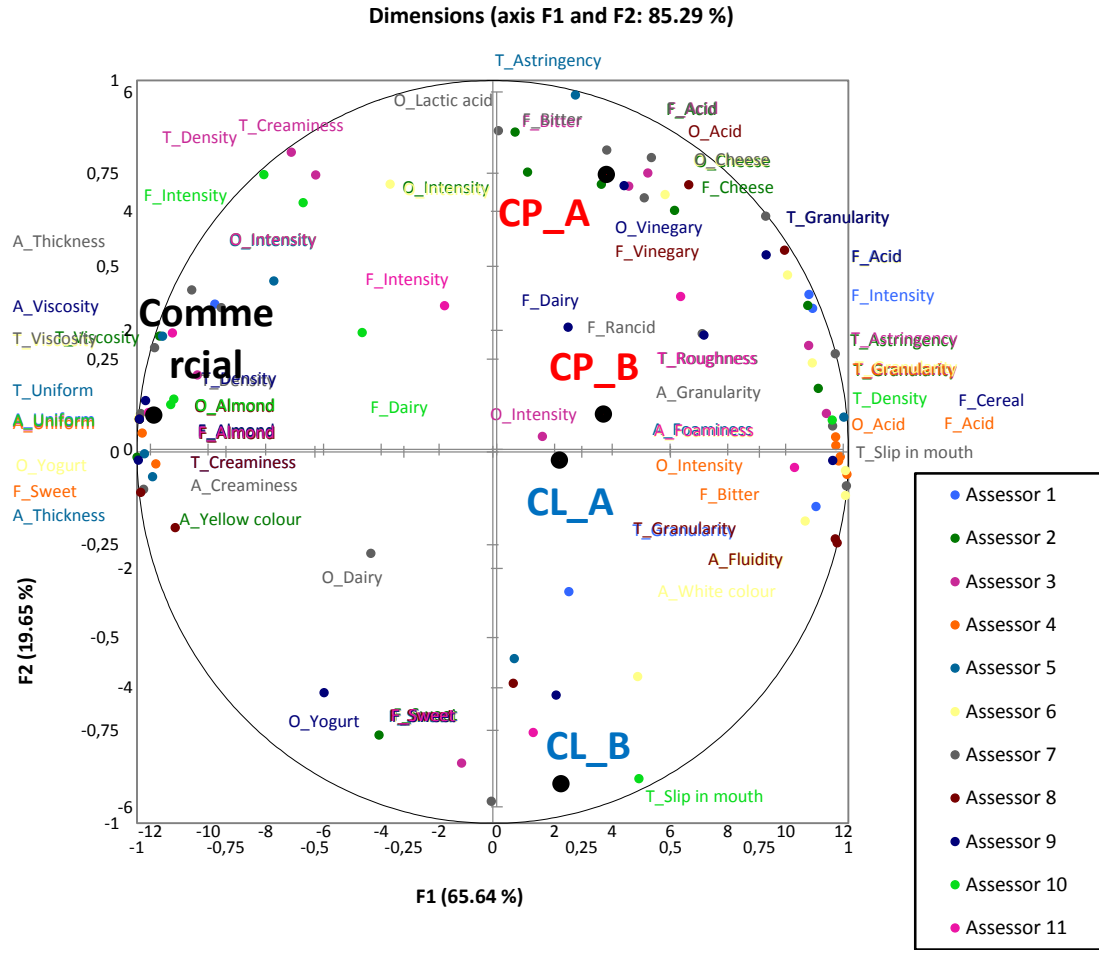
Growth kinetics – Amino acids



Very low amino acid released
High consumption of free amino acids
⇒ The strains are not proteolytic and used the free amino acids in almond milk (to be confirmed with an SDS-PAGE gel and OPA assay)
⇒ Could explain the limited growth of *S. thermophilus* in the presence PAB (competition for free amino acids)
⇒ **Same results with or without 2.5% sucrose**

Task 2.2. Fermented probiotic plant-based beverages

Sensory analysis perform in WP5



PCA of the sensory analysis perform in WP5

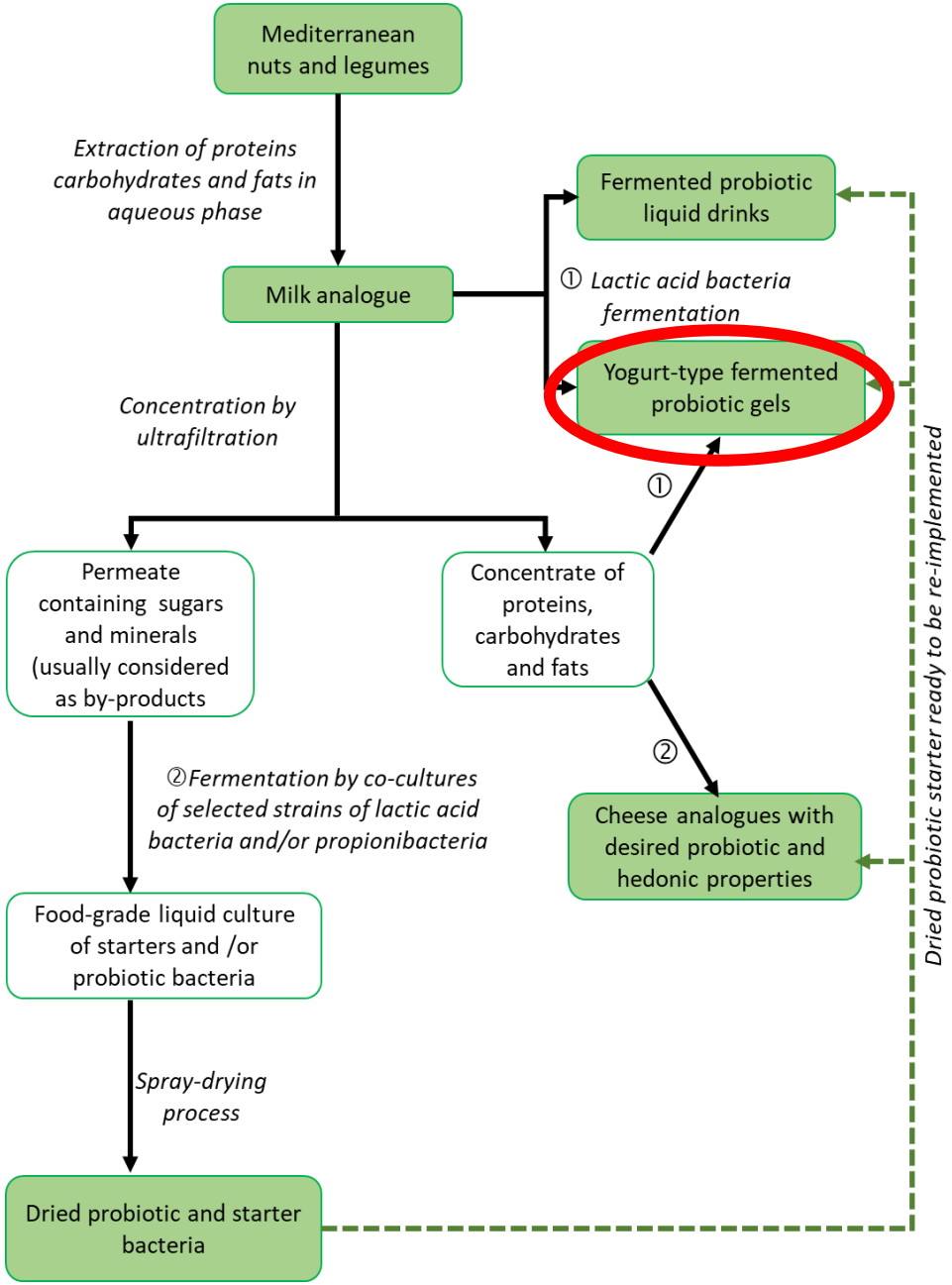
Main results of the sensory analysis

	Almond Milk 	Almond milk with 2,5% sucrose added 
Consortium LAB	Foaminess Astringent Granularity Roughness	Sweet Yogurt Slip in mouth
Consortium LAB+PAB	Cheese Intensity Acid Bitter Granularity	Foaminess Astringent Granularity Roughness

Fermented milk before shaking

Fermented milk after shaking

Task 2.3. Yogurt-type fermented probiotic gels



Task 2.3. Yogurt-type fermented probiotic gels

Experimental strategy

Need a higher protein concentration to obtain a firmer gel

2 methods explored :

- Enrichment of almond milk with powder
- Use of protein-enriched plant-based milk supplied by Fraunhofer

Almond/chickpea milk mixes

Almond	Chickpea
100%	0%
75%	25%
50%	50%
25%	75%
0%	100%

Use of protein-enriched plant-based milk (mix almond/chickpea) supplied by Fraunhofer (≈3% of protein)

Fraunhofer

Enrichment of almond milk with powder (target of 4% of protein)

ChickP

- Use of multiple powders (fatty and defatted almond flour, almond powder, ChickP powder (G910 & S930))
- Heat treatment to sterilize the mixture (115°C – 20 min)

Fermentation of these plant-based milk enriched in protein with the *S. thermophilus* previously selected

- Growth assessment (pH, bacterial count)
- Organoleptic properties assessment (lab test (5-6 tasters))

Study of selected strains on Fraunhofer's milks (in discussion)

- Addition of a probiotic bacteria (*L. casei* or *L. delbrueckii* subsp. *bulgaricus*)
- Overall composition of the milks and target metabolites (carbohydrates, organic acid…)
- Rheological measurements
- Sensory analysis

Task 2.3. Yogurt-type fermented probiotic gels

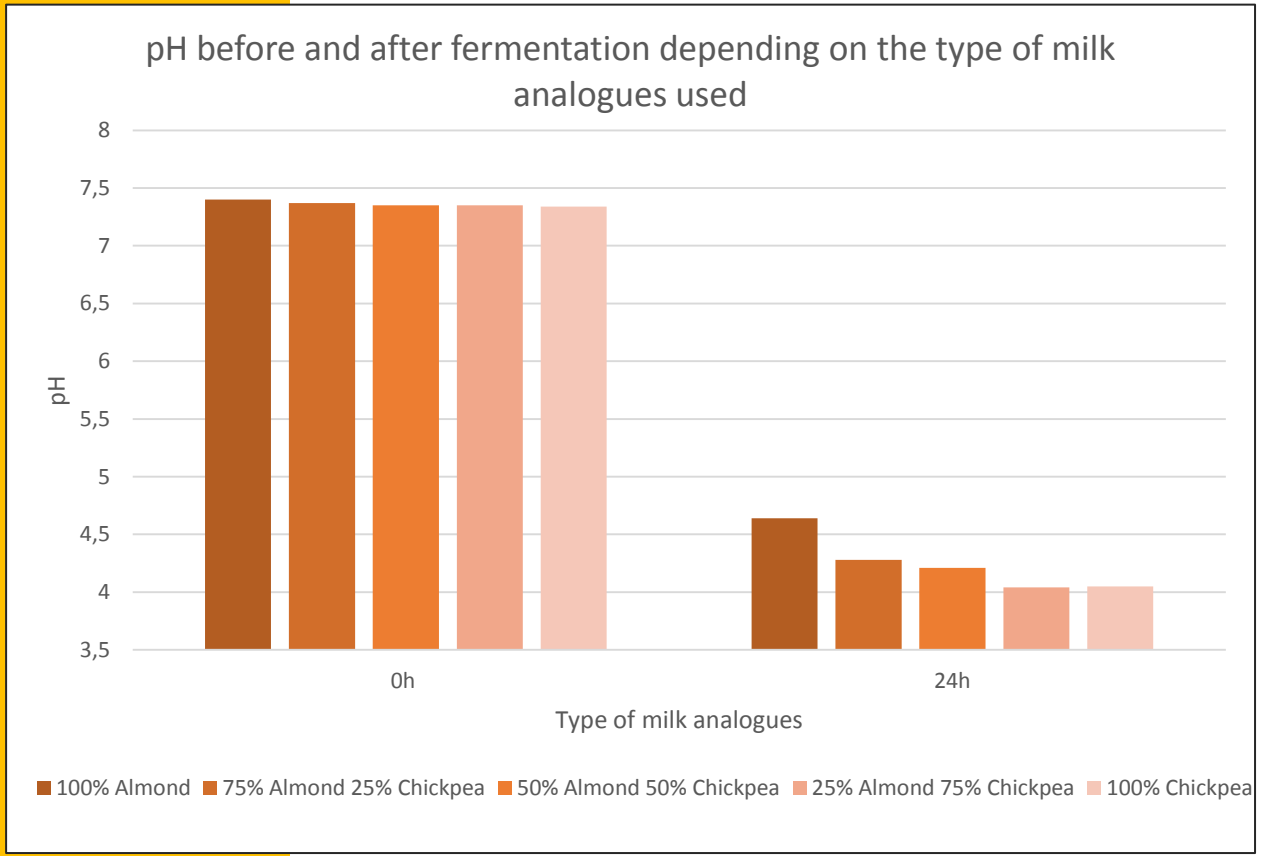
Enrichment of almond milk with powder (target of 4% of protein)

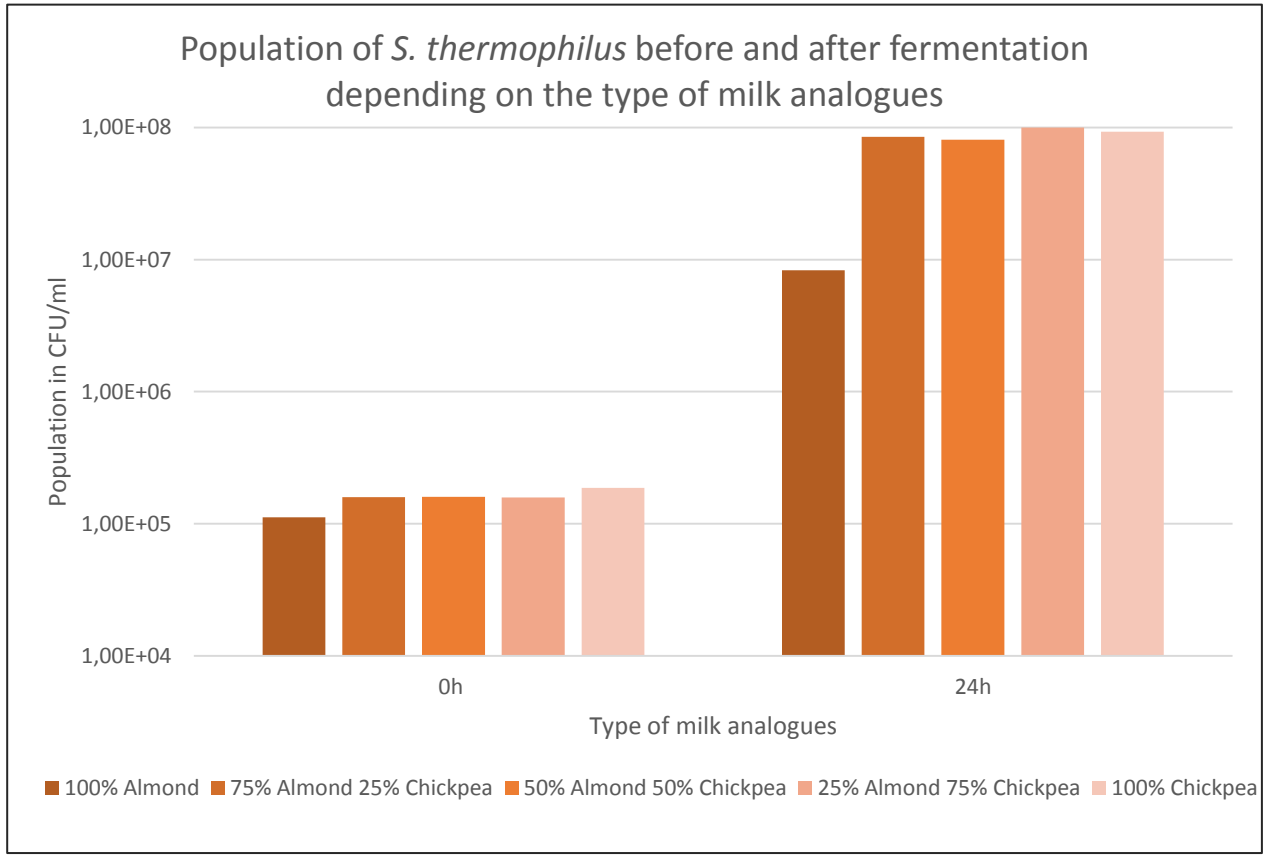
- Best result obtain with ChickP G910
  - No protein precipitation after heat treatment
  - Firm gel with low syneresis after fermentation
  - Need to add sucrose (2.5%) so that the strain can acidify enough (pH≈4,5)
  - Sweet, slightly acidic, milky/yogurt taste

Task 2.3. Yogurt-type fermented probiotic gels

Use of protein-enriched plant-based milk (almond/chickpea) supplied by Fraunhofer (3% of protein)

Growth assessment



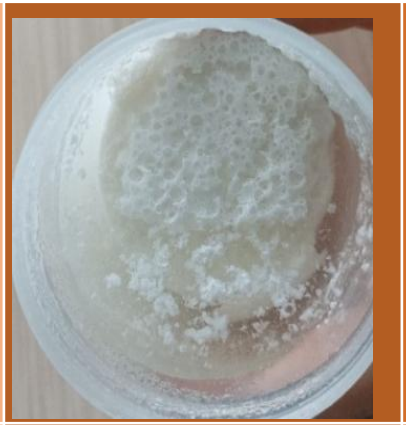


Good acidification of milk analogues
Higher pH drop with chickpea (Higher carbohydrate concentration with the hydrolysis phase ? More free amino acid ?)

Growth of *S. thermophilus* (2-3 log)
Better growth with chickpea (Higher carbohydrate concentration with the hydrolysis phase ? More free amino acid ?)

Task 2.3. Yogurt-type fermented probiotic gels

Use of protein-enriched plant-based milk (almond/chickpea) supplied by Fraunhofer (3% of protein)

	100% Almond	75% Almond 25% Chickpea	50% Almond 50% Chickpea	25% Almond 75% Chickpea	100% Chickpea
Appearance of the gel					
Appearance of the gel in the spoon					

Not very firm gel and strong syneresis with 100% almond
Firm gel and low syneresis from 50% to 100% chickpea

Task 2.3. Yogurt-type fermented probiotic gels

Use of protein-enriched plant-based milk (almond/chickpea) supplied by Fraunhofer (3% of protein)

"Sensory analysis" :

- **100% Almond :** Milky, fresh, acid notes, appreciated
- **100% Chickpea :** Notes of chickpea and fat, not appreciated
- **Mixture almond/chickpea :** Milky, fresh and acid notes, appreciated

Globally :

- Mixture (50/50) of the two milk analogues seems to be a good compromise : **good smell and taste with good texture**
- *S. thermophilus* was compared to an industrial yogurt starter (VegaClassic from Chr. Hansen) and was more appreciated (Better covers the legumes and roasted notes which were not appreciated)

Thank you for your attention !

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References

Illikoud N, Do Carmo FLR, Daniel N, Jan G, Gagnaire V. **2023**. Development of innovative fermented products by exploiting the diversity of immunomodulatory properties and fermentative activity of lactic and propionic acid bacteria. *Food Research International* **166**: 112557.