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WP2 : Innovative plant-based dairy analogues : Fermented products

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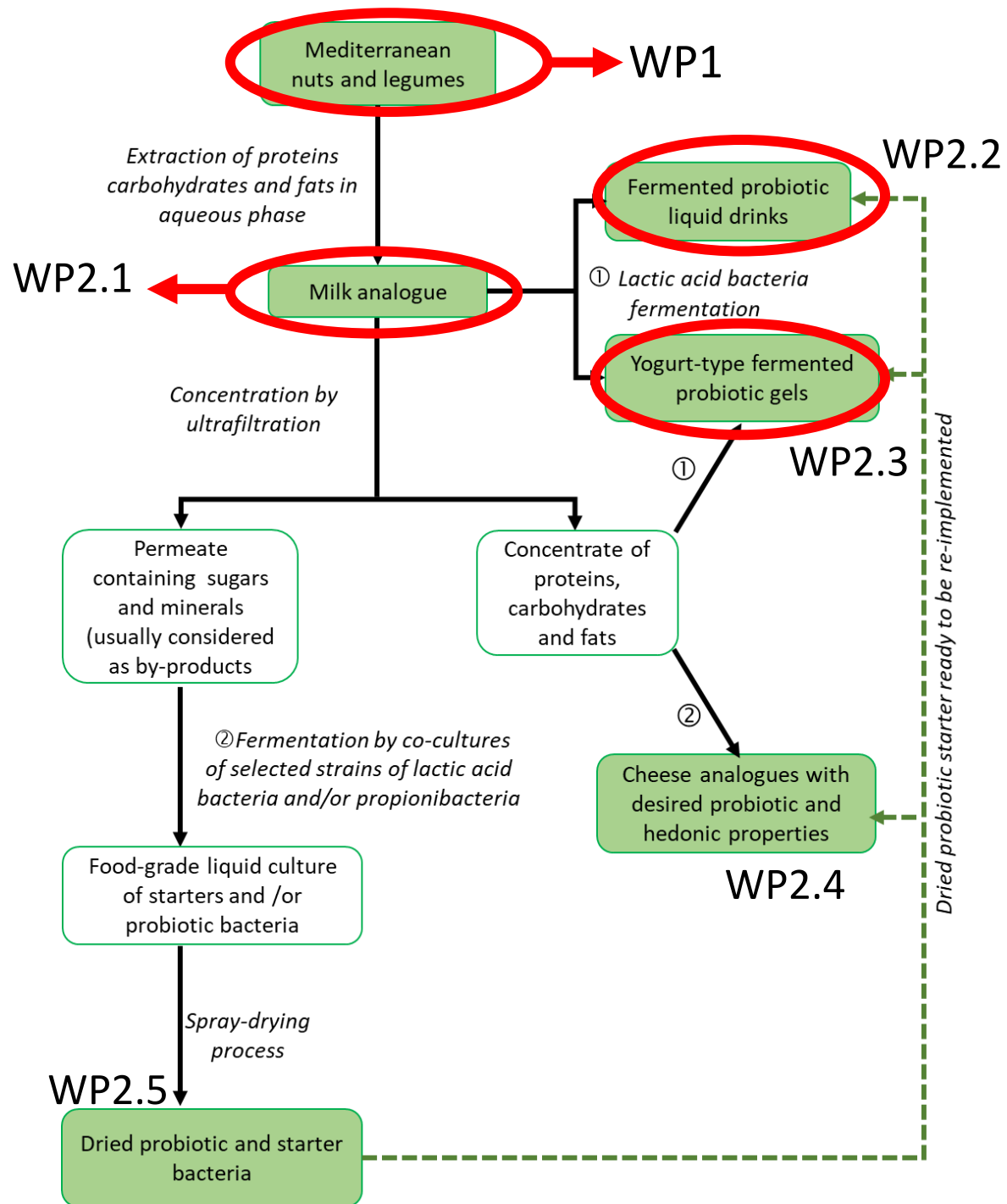


LOCALNUTLEG

WP2 : Innovative plant-based dairy analogues : Fermented products



LOCALNUTLEG



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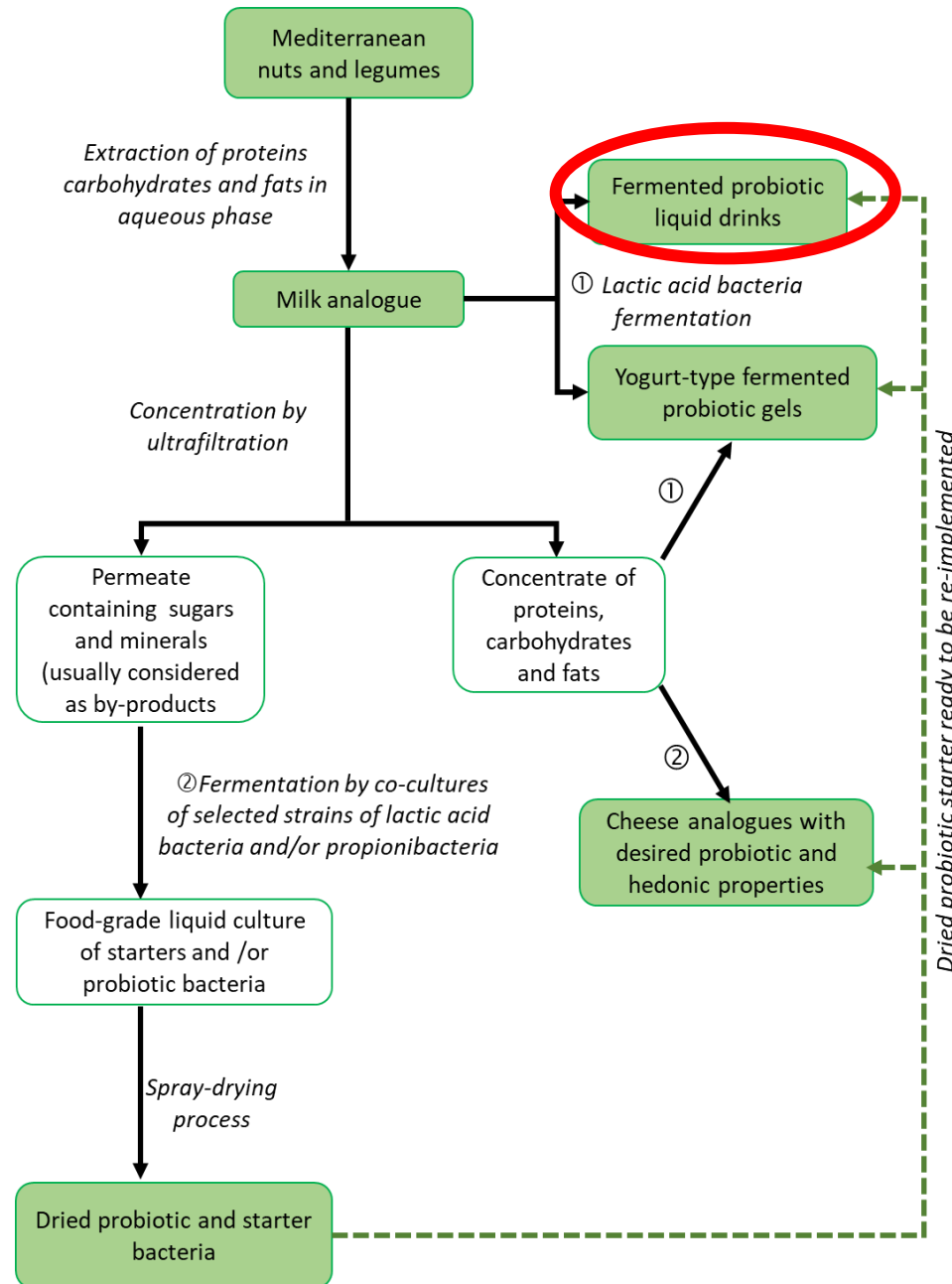
valerie.gagnaire@inrae.fr



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Task 2.2. Fermented probiotic plant-based beverages



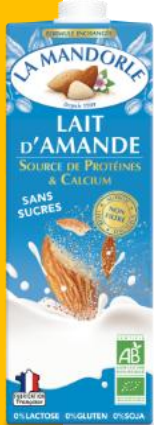
Experimental strategy :



Strains pre-selection (≈ 40 strains)

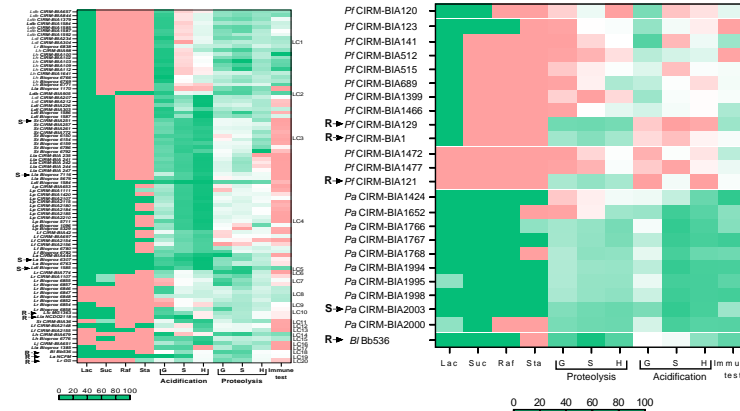
- Ability to use sucrose
- Anti-inflammatory properties

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)



Fundació Miquel Agustí



Lactic Acid Bacteria (LAB)

Gram +
Cocci or bacilli

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

Functions : Acidification,
coagulation, aromas/flavours,
proteolysis

Used in all fermented dairy
products (cheese, yogurt,
butter...)

Examples of LAB:

Streptococcus thermophilus

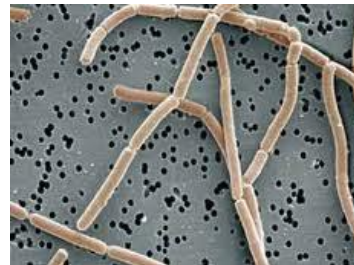
Lactococcus lactis

Lactobacillus delbrueckii subsp. *bulgaricus*

Lacticaseibacillus casei



S. thermophilus



L. delbrueckii subsp.
bulgaricus



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

Propionic Acid Bacteria (PAB)

Gram +
Cocobacilli (Short bacilli)

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

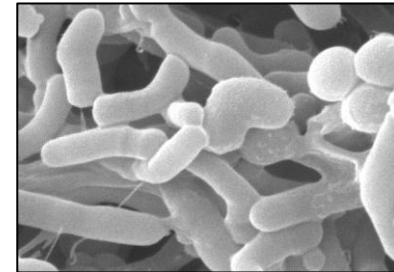
Functions : Aromas/flavours

Texture (Swiss-type cheese hole)
Used in cheeses

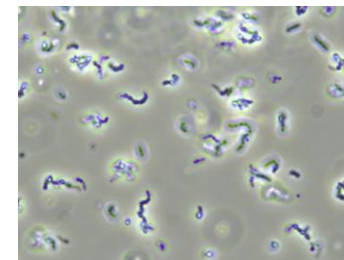
Examples of PAB:

Propionibacterium freudenreichii

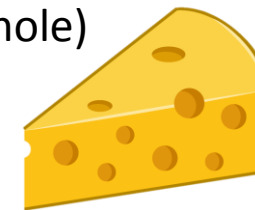
Acidipropionibacterium acidipropionici



P. freudenreichii



A. acidipropionici



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

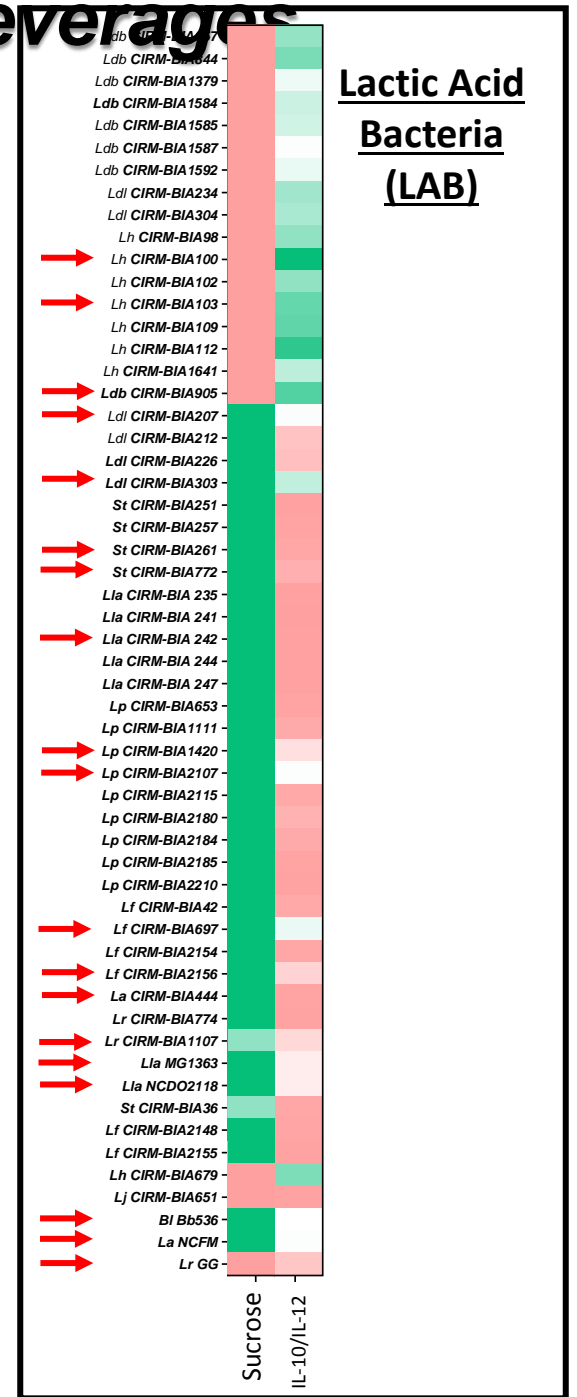
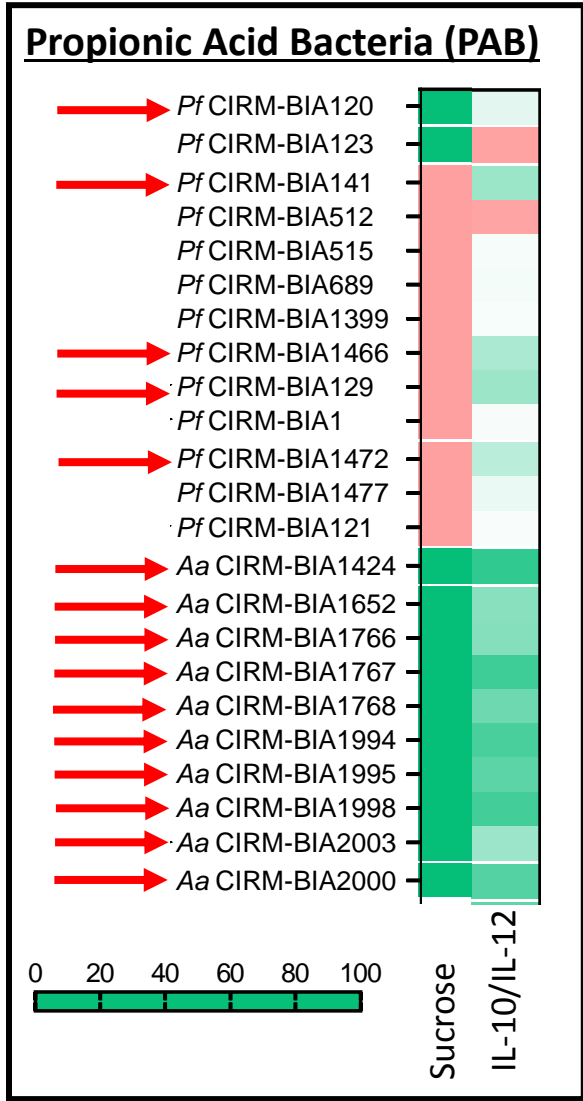


Task 2.2. Fermented probiotic plant-based beverages

Strains pre-selection

Pre-selection of 38 strains :

- 23 LAB
- 15 PAB



Screening of the pre-selected lactic acid bacteria strains



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* NCD02118
- *Lacticaseibacillus casei* CIRM-BIA1643

Thermophilic consortium (43°C) :

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

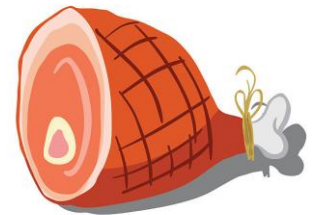
Species	Strain number	pH drop	Organoleptic assessment (appreciation converted into ratings)	Anti-inflammatory properties (IL10/IL12)
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Lla NCD02118	2,74	8	8
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Lla CIRM-BIA 242	2,42	6	0,02
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Lla Bioprox 5676	2,61	7	0,06
<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	Llc MG1363	1,55	3	8
<i>Lactiplantibacillus plantarum</i>	Lp CIRM-BIA1420	2,55	2	6,58
<i>Lactiplantibacillus plantarum</i>	Lp CIRM-BIA2107	2,57	2	11,18
<i>Lactiplantibacillus plantarum</i>	Lp Bioprox 1096	2,49	2	12,53
<i>Lacticaseibacillus casei</i>	Lc CIRM-BIA1643	1,04	7	Well-know probiotic effect
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	Ldl CIRM-BIA207	1,39	4	11,75
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	Ldl CIRM- BIA303	1,32	4	31,97
<i>Lactobacillus johnsonii</i>	Lj Bioprox 5467	2,52	4	Inconnu
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>	St CIRM-BIA772	1,5	10	1,5
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>	St CIRM-BIA251	2,88	9	0,1
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	Ldb CIRM-BIA905	0,46	8	71,98
<i>Limosilactobacillus fermentum</i>	Lf CIRM-BIA697	0,31	5	17,5
<i>Limosilactobacillus fermentum</i>	Lf CIRM-BIA2156	0,09	5	5,27
<i>Lacticaseibacillus rhamnosus</i>	Lr CIRM-BIA1107	0,98	7	5,86
<i>Lacticaseibacillus rhamnosus</i>	Lr GG	0,79	4	4
<i>Lactobacillus helveticus</i>	Lh CIRM-BIA100	0,38	5	100
<i>Lactobacillus helveticus</i>	Lh CIRM-BIA103	0,42	5	65,12
<i>Lactobacillus acidophilus</i>	La NCFM	0,86	3	11
<i>Lactobacillus acidophilus</i>	La CIRM-BIA444	2,05	5	0,29
<i>Bifidobacterium longum</i>	Bl Bb536	0,66	8	10

Task 2.2. Fermented probiotic plant-based beverages

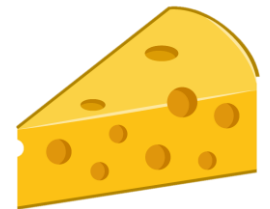
Screening of the pre-selected propionic acid bacteria strains

Species	Strain number	pH drop	Organoleptic assessment (appreciation converted into ratings)	Anti-inflammatory properties (IL10/IL12)
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA1652	2,21	3	52,24
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA1766	2,39	3	53,91
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA1767	1,74	3	79,32
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA1768	2,12	3	61,88
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA1994	1,7	3	75,82
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA1995	1,57	5	68,49
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA1998	2,17	3	77,57
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA2003	1,81	3	44,98
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA1424	0,95	5	84,46
<i>Acidipropionibacterium acidipropionici</i>	Aa CIRM-BIA2000	1,55	5	71,05
<i>Propionibacterium freudenreichii</i>	Pf CIRM-BIA120	0,77	8	19,97404906
<i>Propionibacterium freudenreichii</i>	Pf CIRM-BIA141	0,74	5	45,86368605
<i>Propionibacterium freudenreichii</i>	Pf CIRM-BIA129	0,7	5	45,62
<i>Propionibacterium freudenreichii</i>	Pf CIRM-BIA1466	1,15	5	39,88066655
<i>Propionibacterium freudenreichii</i>	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	<i>Streptococcus thermophilus</i>	St CIRM-NA772	2,65	4
	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	Ldb CIRM-BIA905		
Thermophilic consortium : Lla NCDO2118 + Lc 1643	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Lla NCDO2118	2,4	3,00
	<i>Lacticaseibacillus casei</i>	Lc CIRM-PK1643		

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

rancid and fatty notes

Lactic and fresh notes

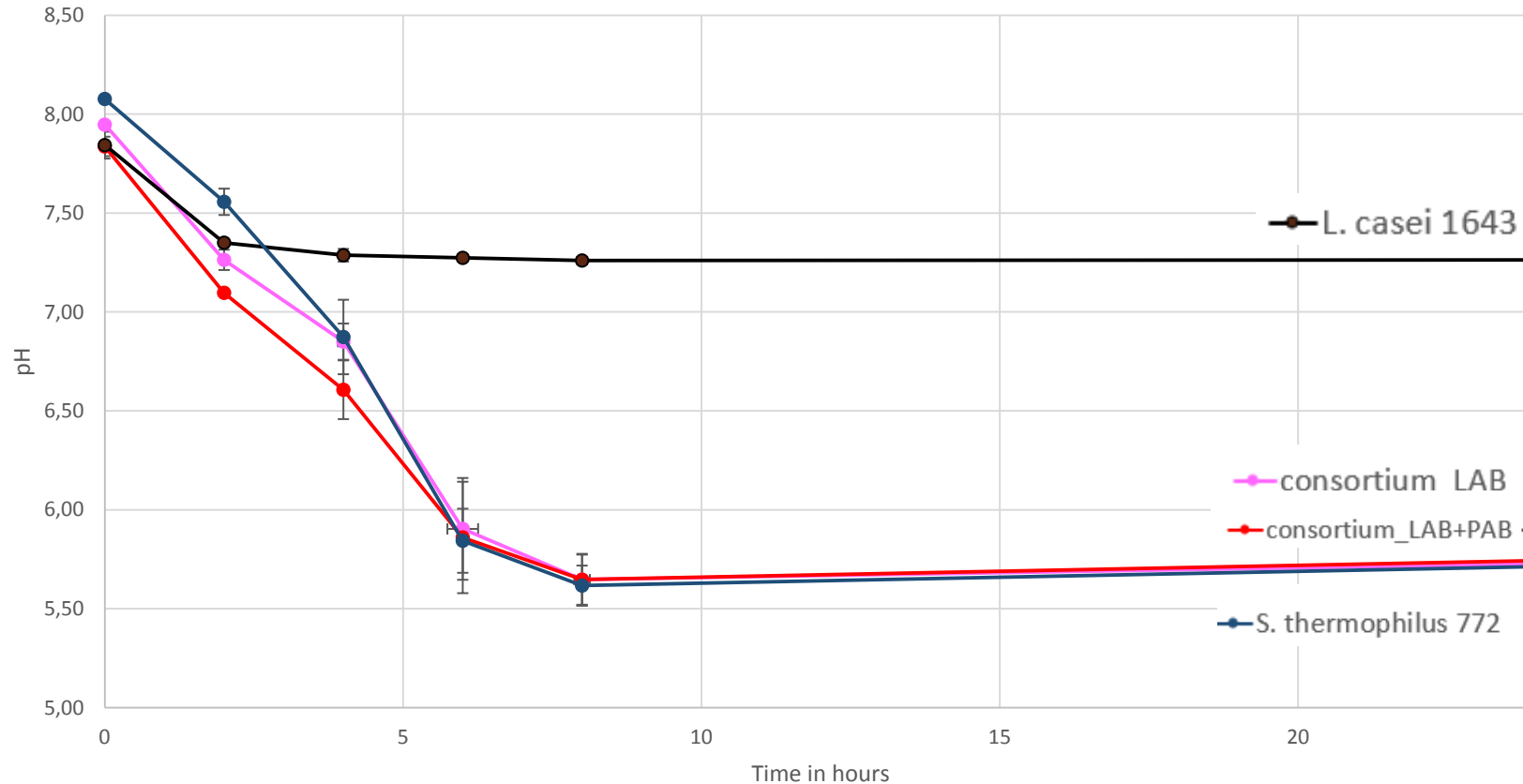
Selection of two consortium for the following study :

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



Growth kinetics – pH results

Strain/consortia acidification curves at 37°C in almond milk from La Mandorle



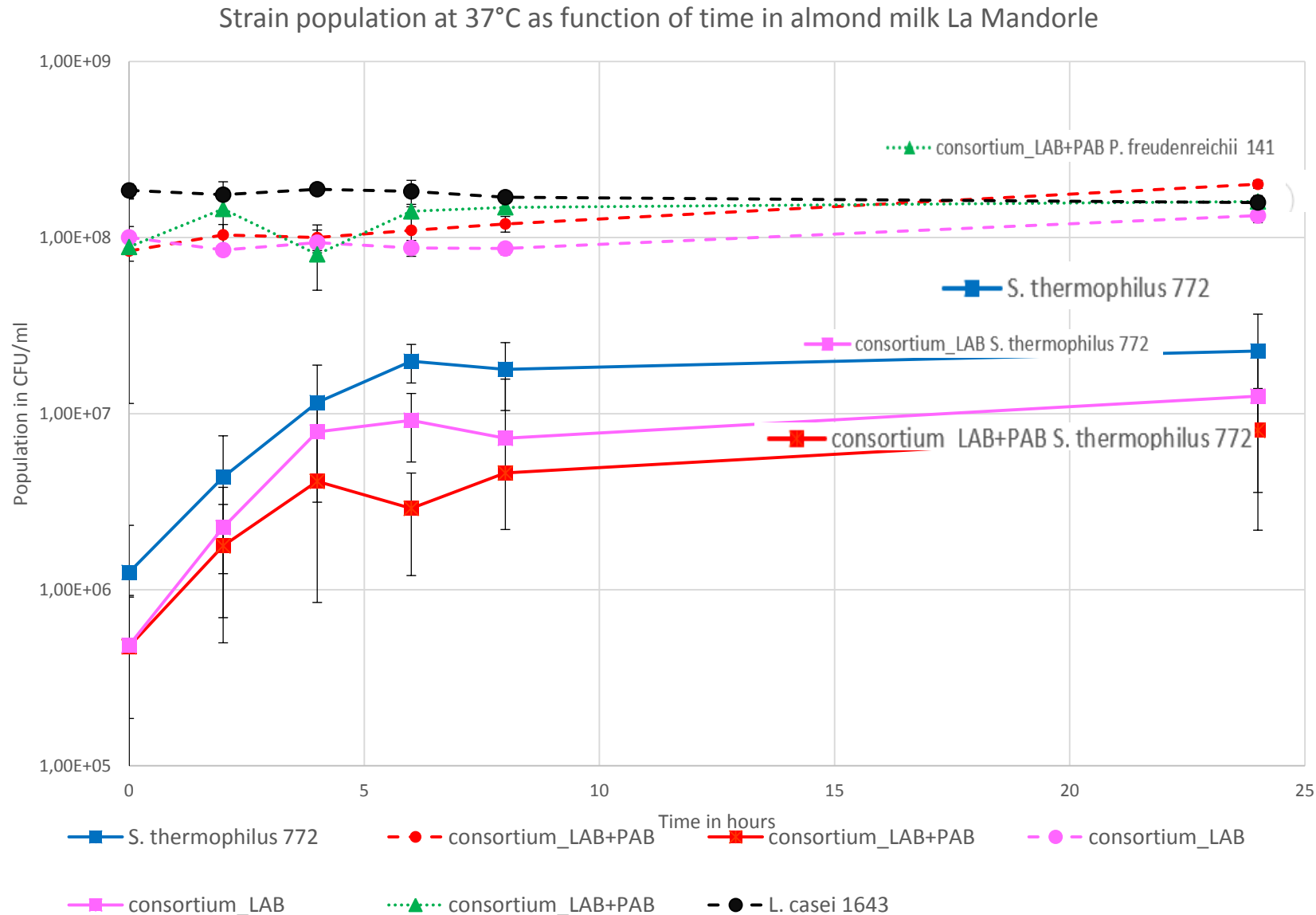
S. thermophilus is responsible for pH drop

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

consortium_LAB consortium_LAB+PAB L. casei 1643 S. thermophilus 772



Growth kinetics – bacterial count results

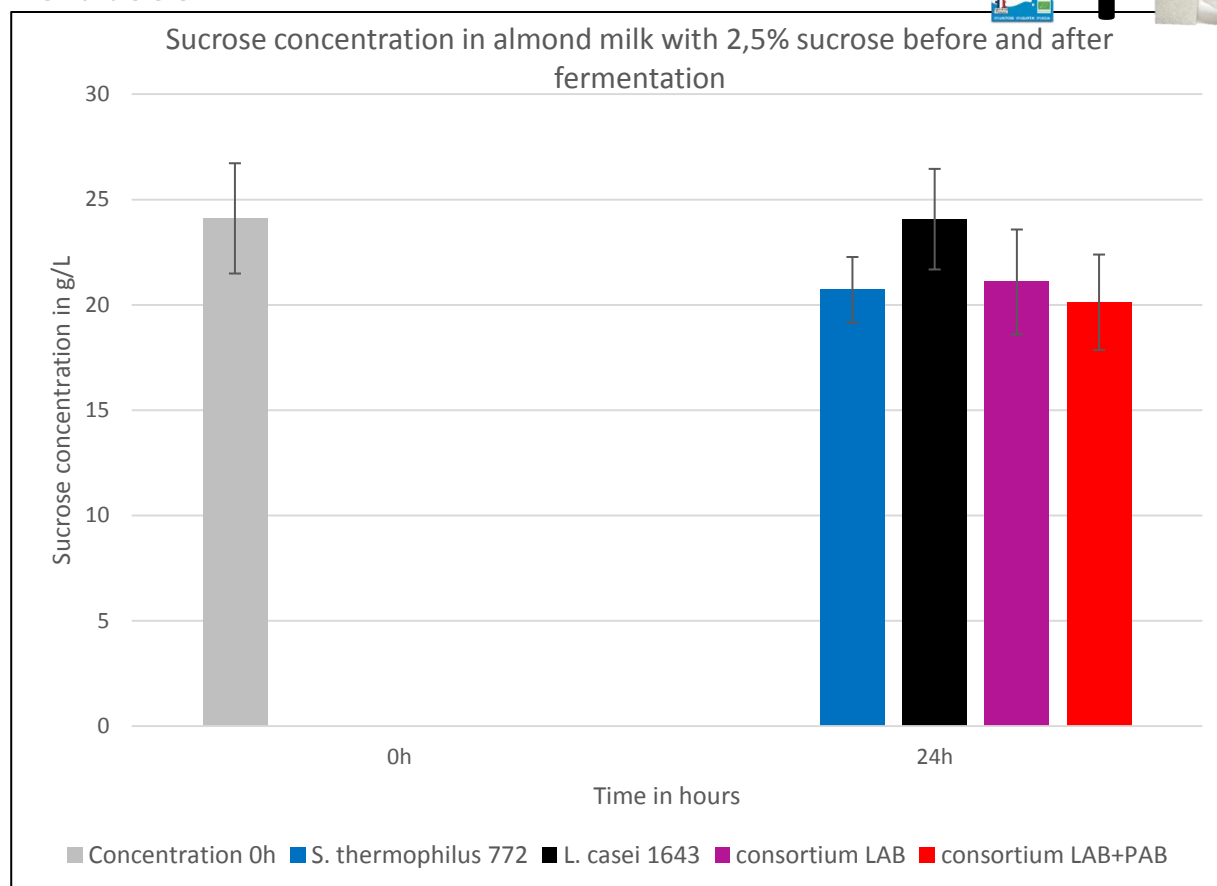
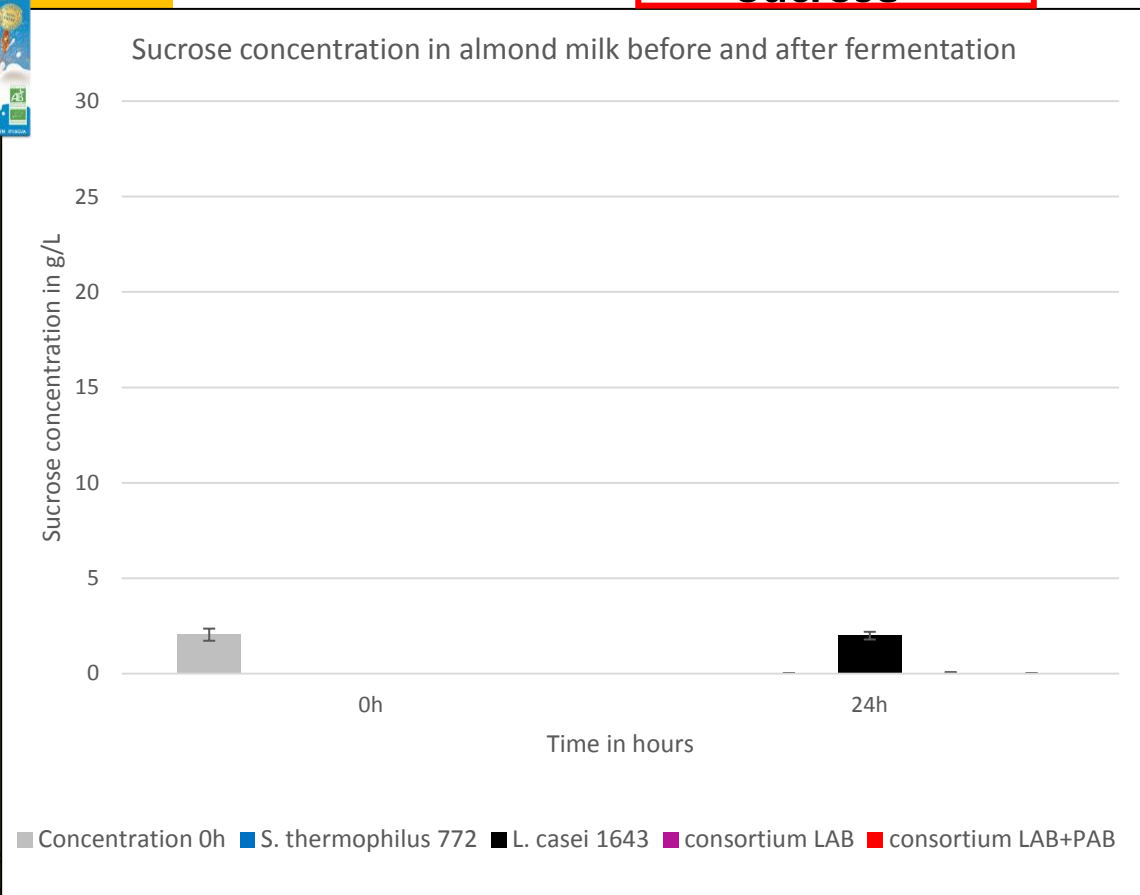
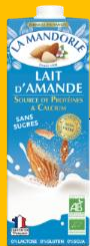
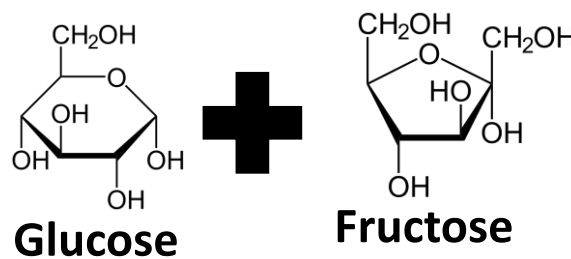
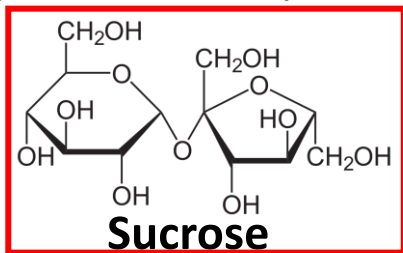


Growth of *S. thermophilus* (1 log)
 Lower growth in the LAB+PAB consortium (competition for free amino acids)

consortium LAB+PAB L. casei 1643
 L. casei 1643
 consortium_LAB L. casei 1643

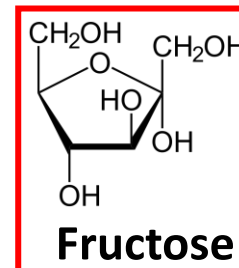
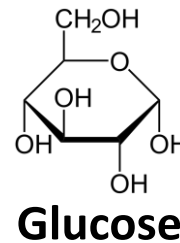
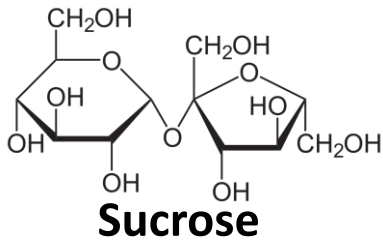
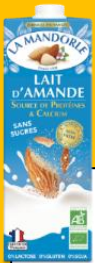
Population of *L. casei* needed to induce a probiotic effect : 10⁸ CFU/ml
 Slight growth of *L. casei* in both consortia (use of fructose/glucose provided by *S. thermophilus* which degrades sucrose)

Population of *P. freudenreichii* stable (10⁸ CFU/ml)
 Enough to induce a probiotic effect

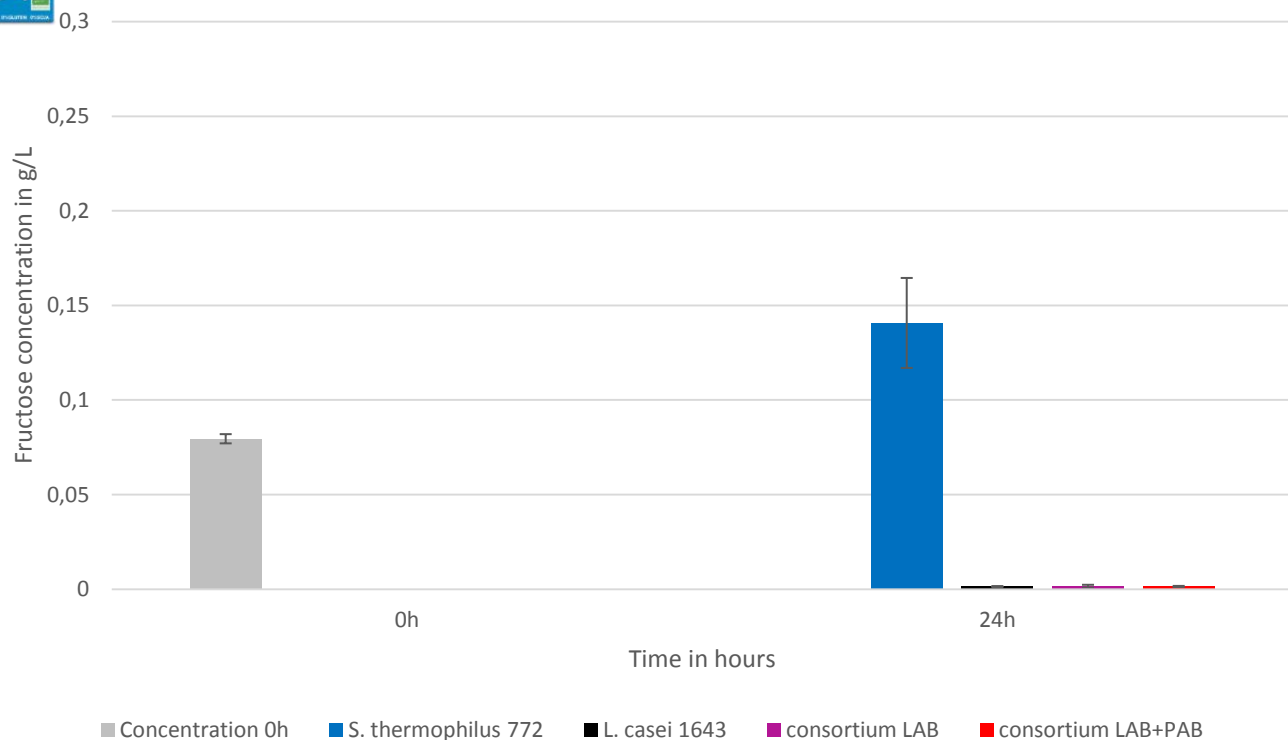


No consumption of the sucrose by *L. casei* (sucrose -)
 Consumption of all the sucrose (2 g/L) by *S. thermophilus* (sucrose +)

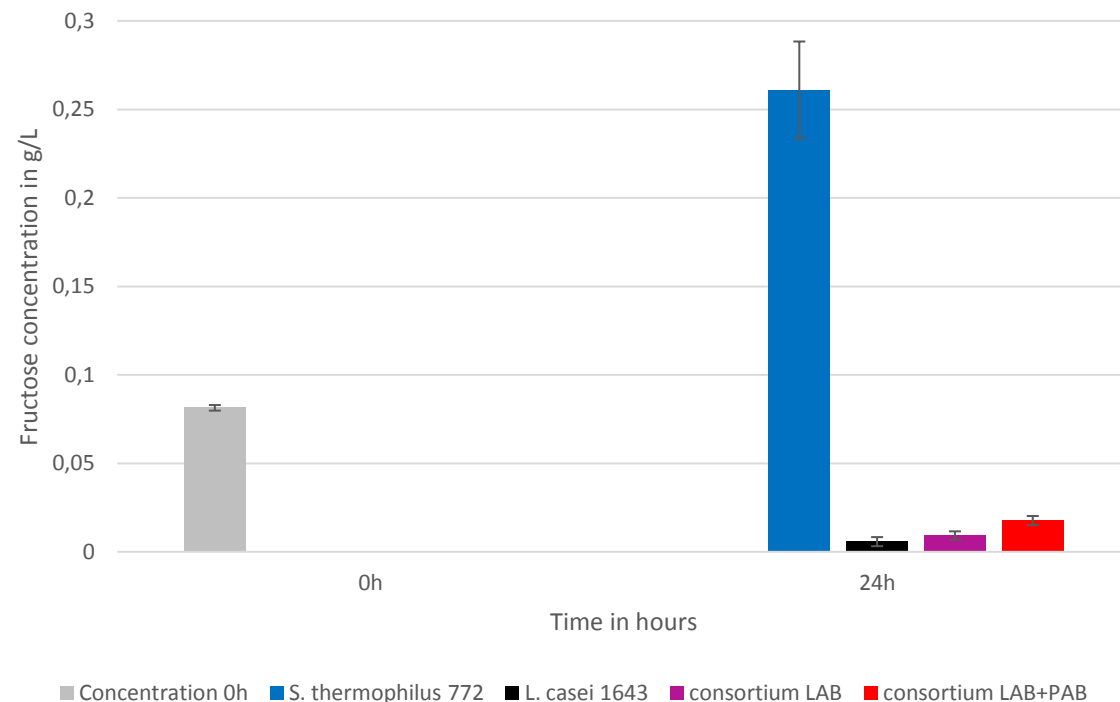
Consumption of sucrose is slightly more important (3 g/L) in almond milk with 25 g/L sucrose



Fructose concentration in almond milk before and after fermentation

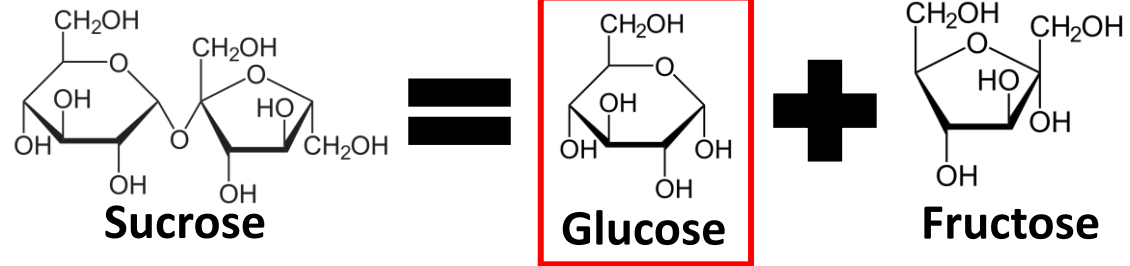


Fructose concentration in almond milk with 2,5% sucrose before and after fermentation

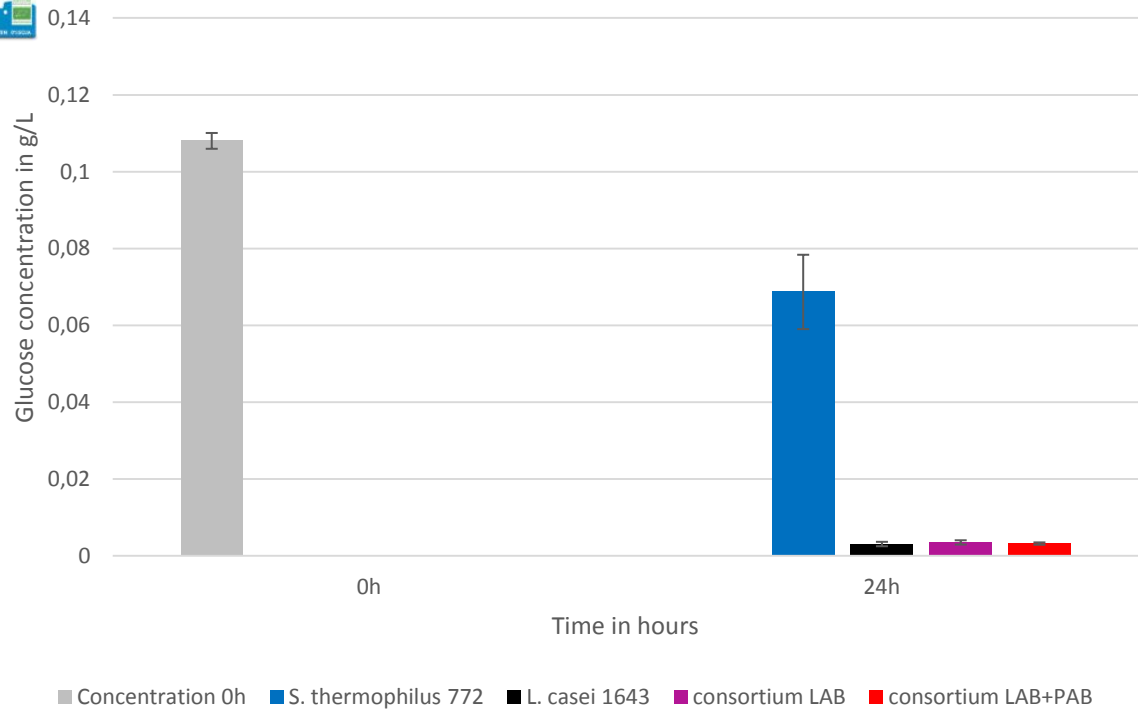


Fructose release by *S. thermophilus* (sucrose + and fructose +/-)
Fructose consumption by *L. casei* (fructose +)

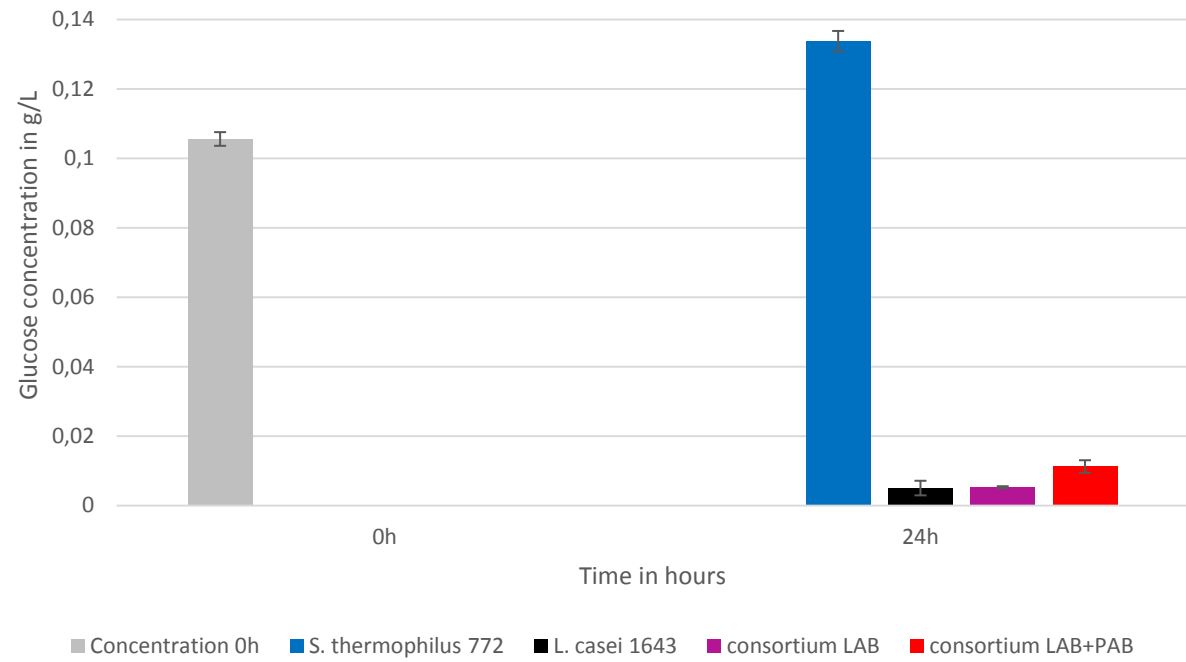
Same conclusion but with more fructose released (because more sucrose were used by *S. thermophilus*)



Glucose concentration in almond milk before and after fermentation



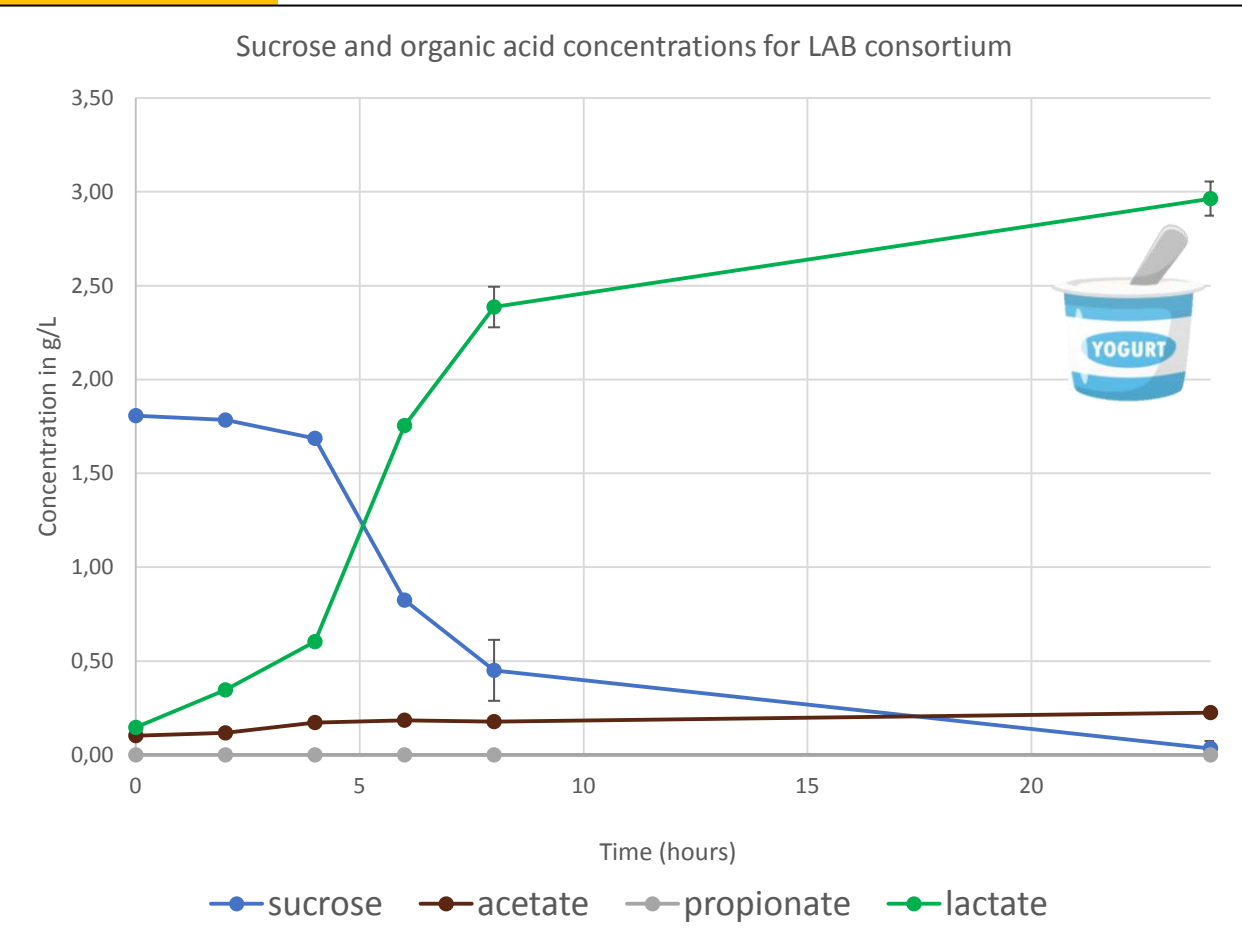
Glucose concentration in almond milk with 2,5% sucrose before and after fermentation



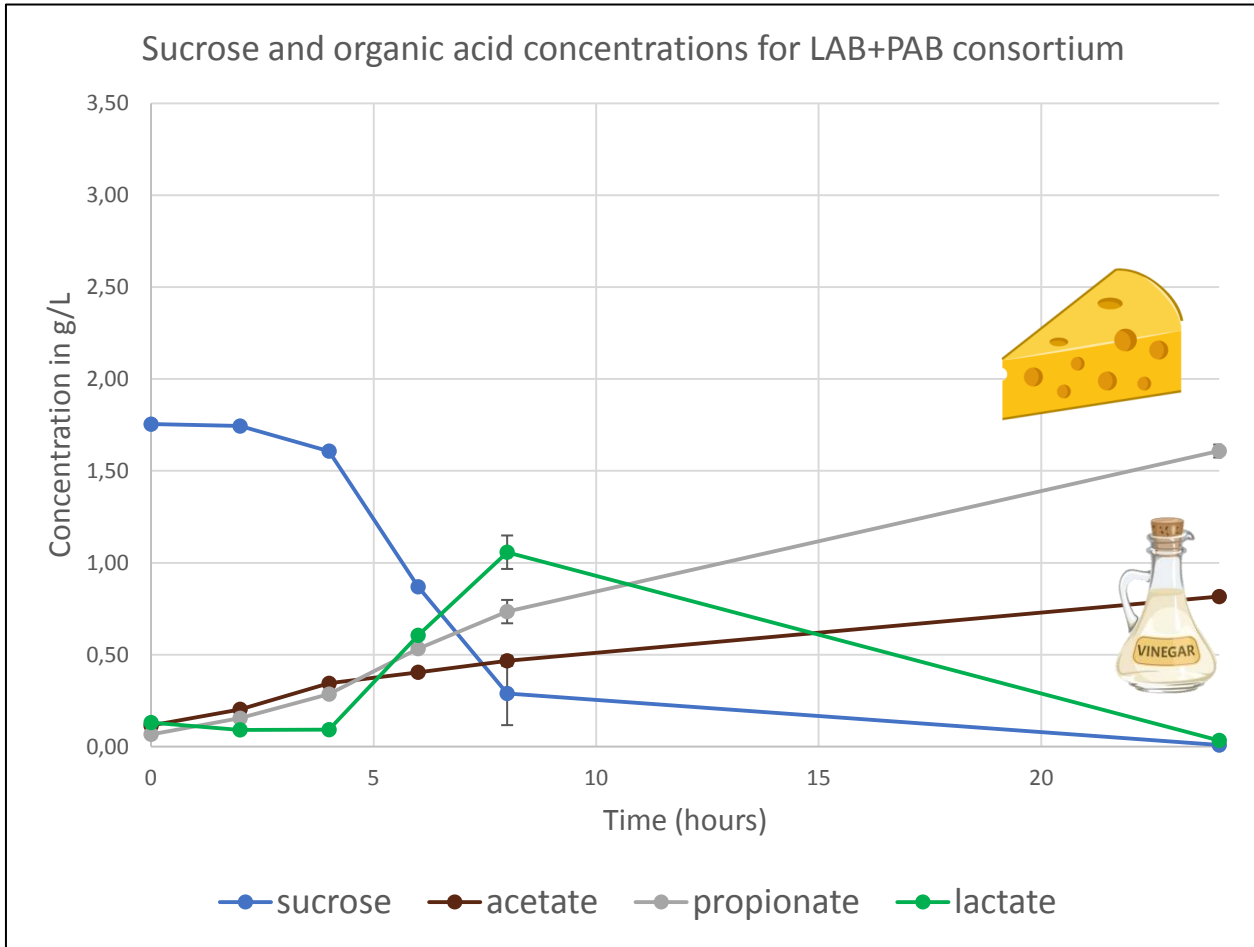
Glucose consumption by all strains (glucose +)
Residual glucose because *S. thermophilus* releases some (sucrose +)

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

Growth kinetics



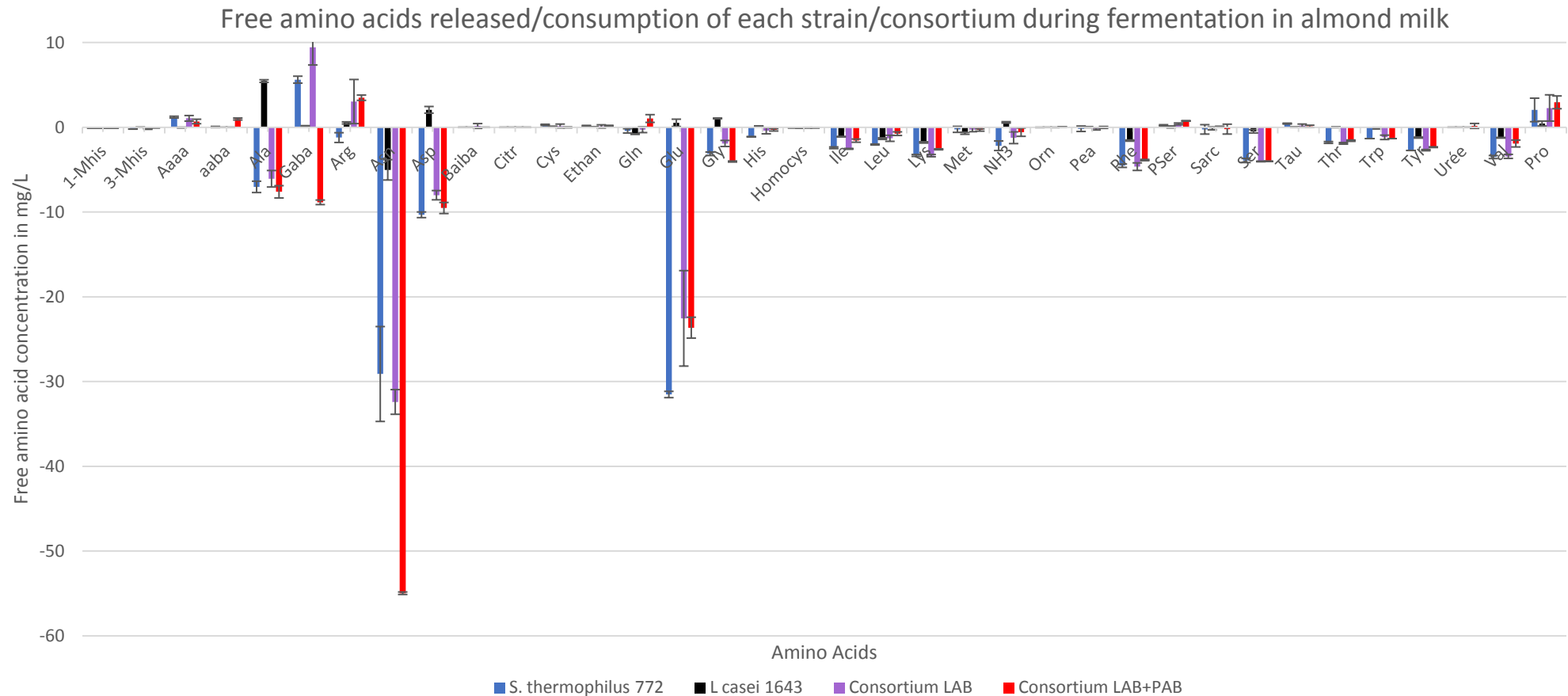
Sucrose consumption and lactate production by LAB



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)

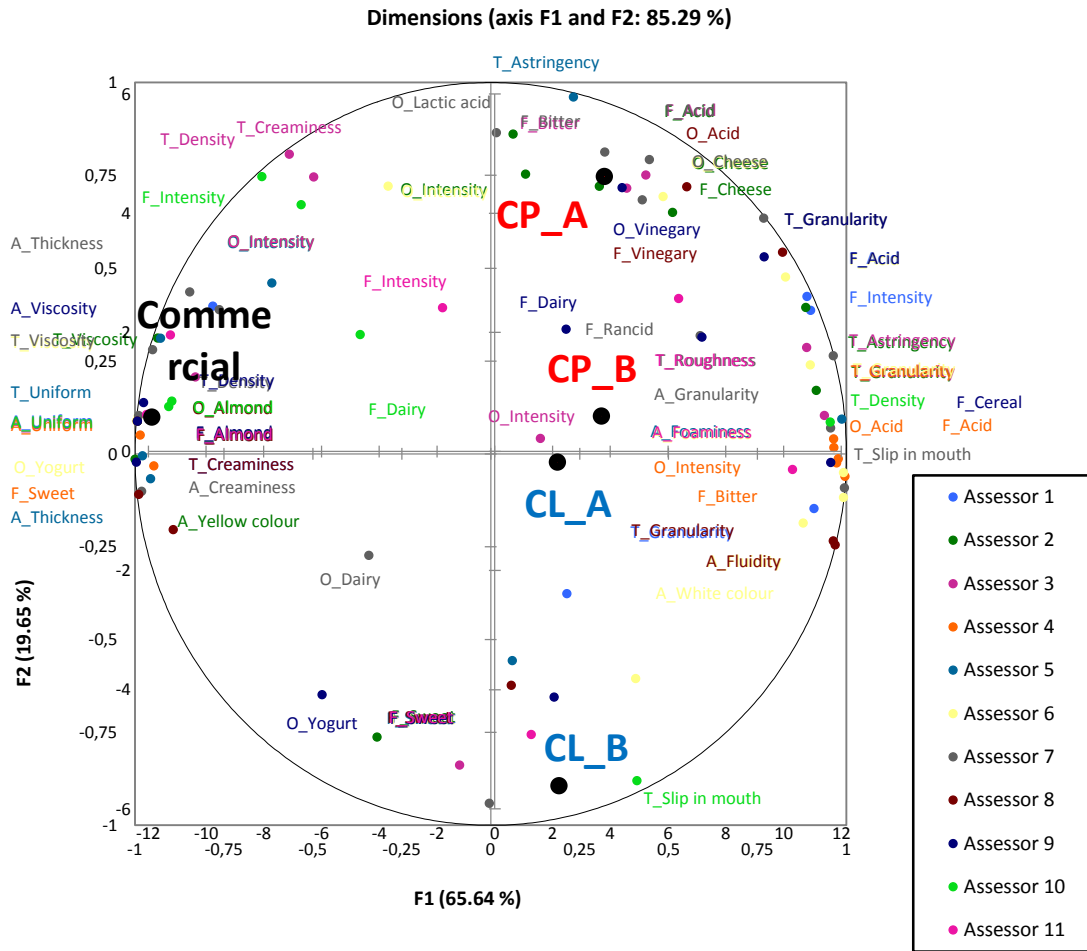
⇒ **Same results with or without 2.5% sucrose**



Task 2.2. Fermented probiotic plant-based beverages

Sensory analysis perform in WP5

Main results of the sensory analysis



PCA of the sensory analysis perform in WP5



	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



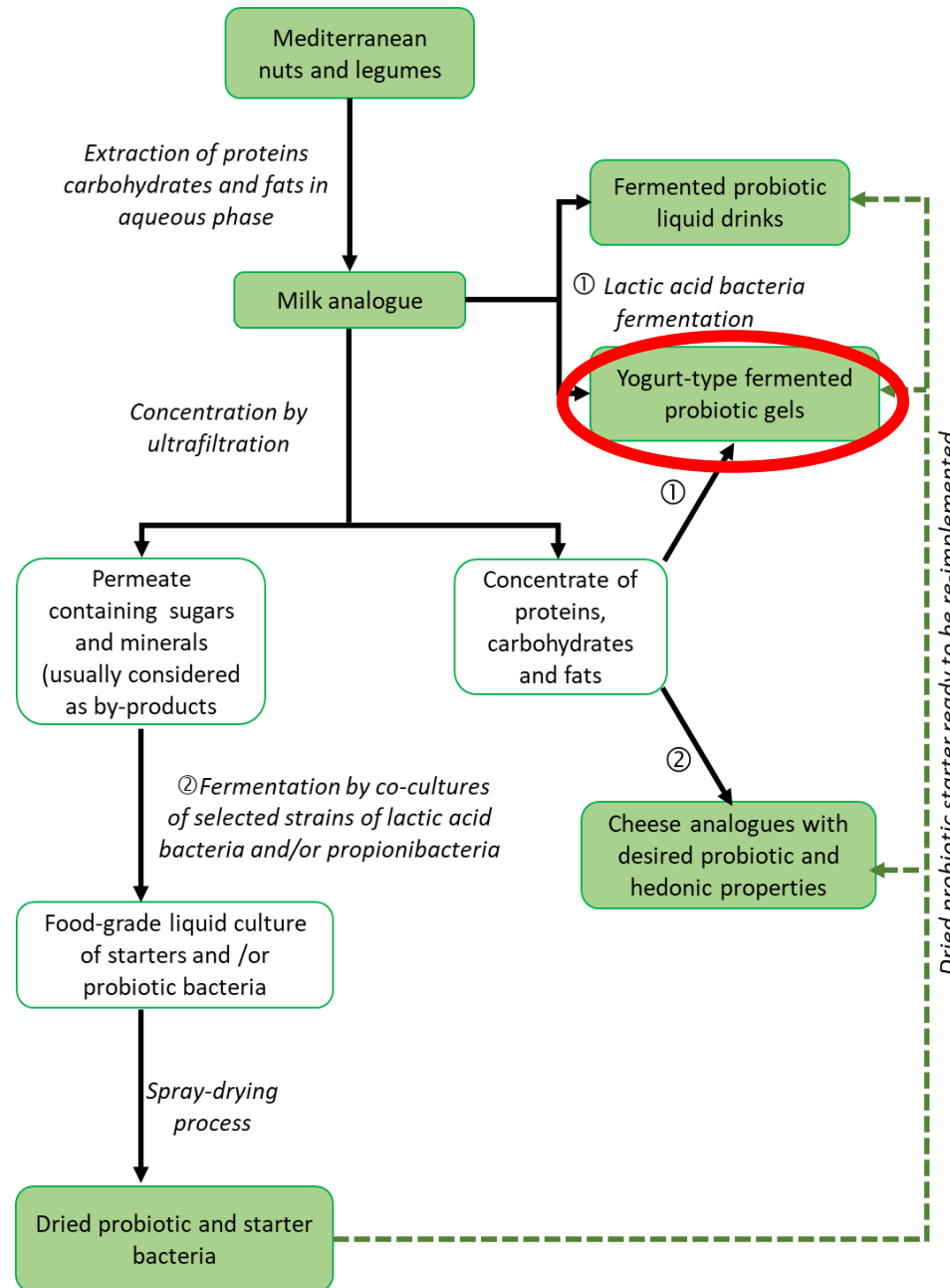
Fermented milk before shaking



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)



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

- 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

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

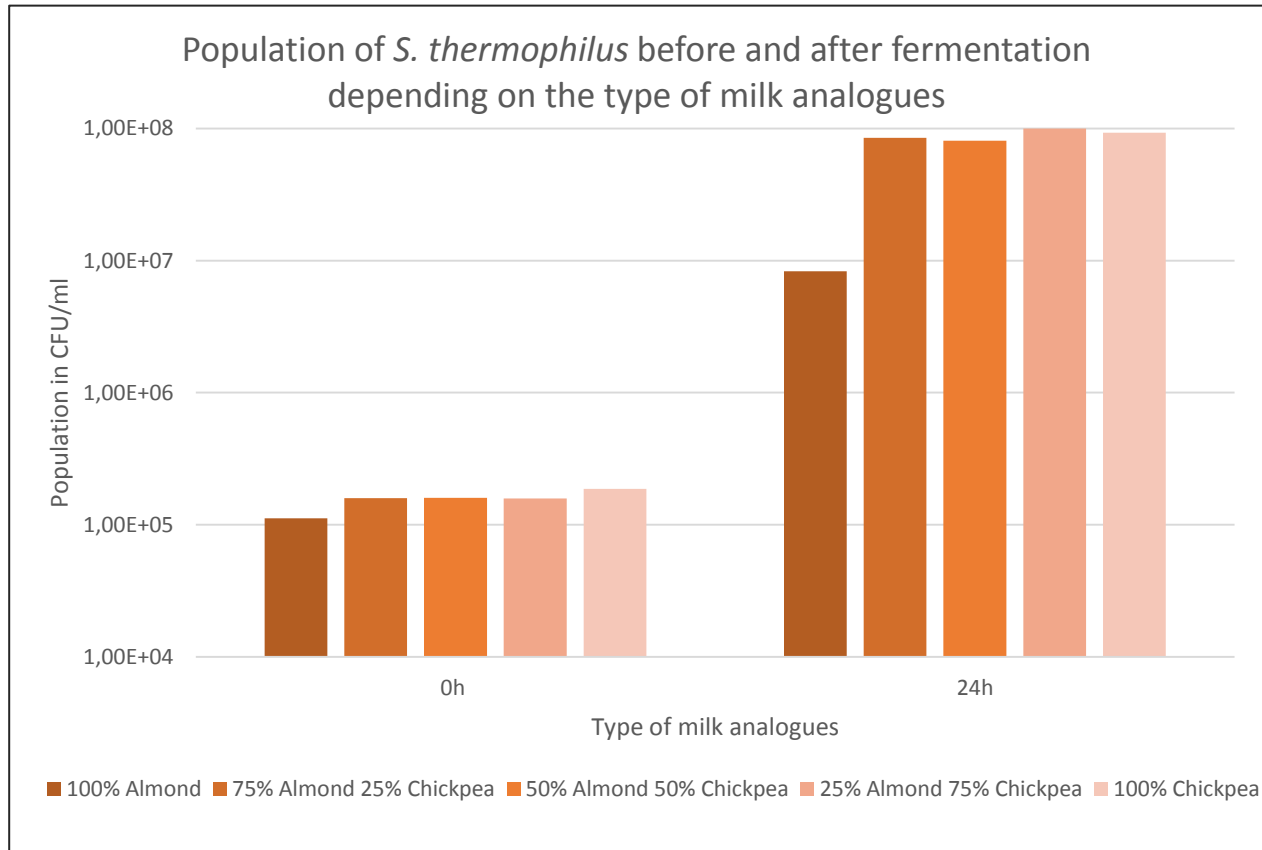
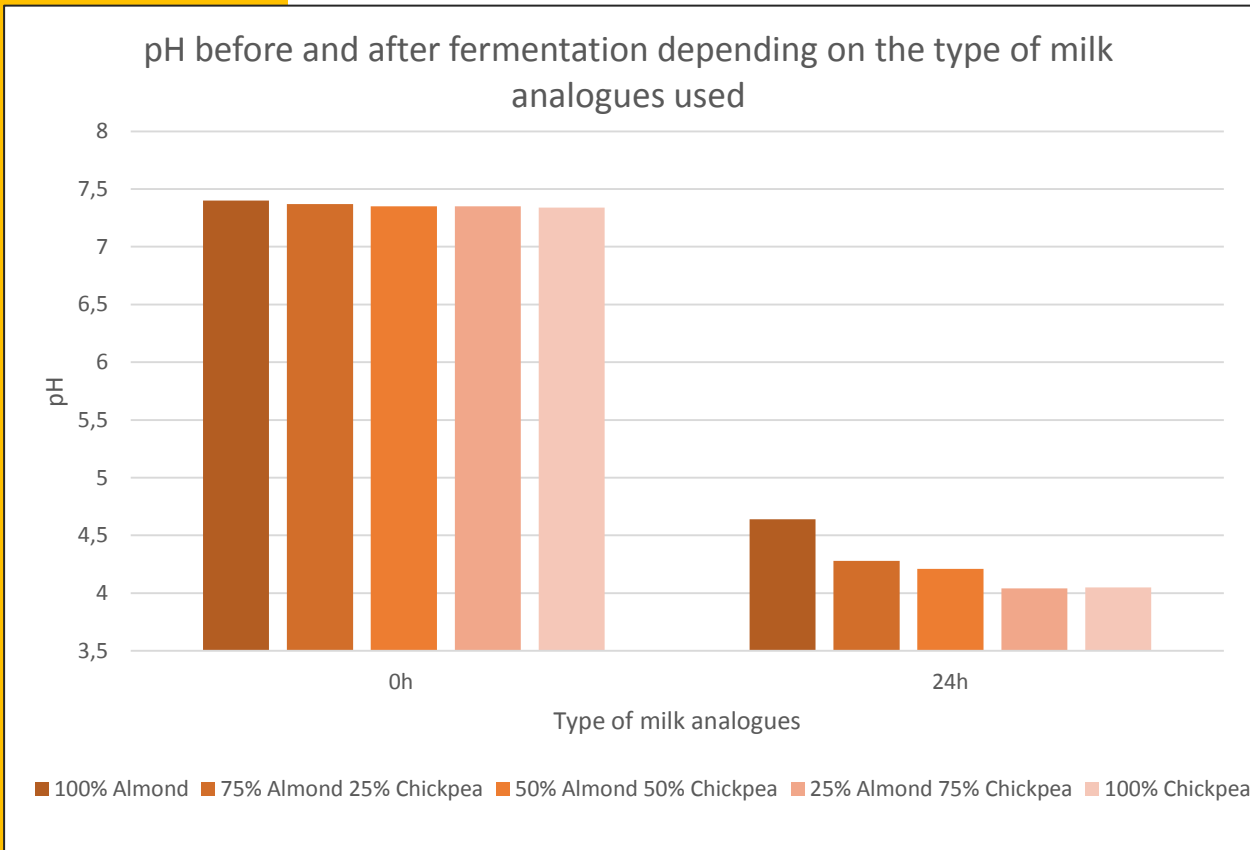
- Best result obtain with ChickP G910
 - No protein precipitation after heat treatment
 - Firm gel with low syneresis after fermentation
 - Need to add sucrose (2.5%) so that the strain can acidify enough ($\text{pH} \approx 4,5$)
 - Sweet, slightly acidic, milky/yogurt taste



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

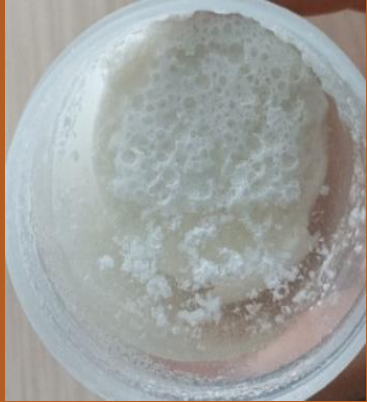











Good acidification of milk analogues
Higher pH drop with chickpea (Higher carbohydrate concentration with the hydrolysis phase ? More free amino acid ?)

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.

