Nut-based fermented products as cheese-alternatives: exploitation of lactic acid bacterial consortia

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INTRODUCTION

The increasing interest in plant-based products has stimulated the development of cheese surrogates obtained through Lactic Acid Bacteria (LAB) fermentation of nuts or legumes. These products can pose safety (pathogens, spoilage microbiota) and quality (texture, aroma) challenges[1]. This study, part of the InnoSol4Med project, evaluated the potential of autochthonous LAB strains, isolated from artisanal spontaneously fermented products, as starter cultures for the fermentation of different nuts (cashews, almonds, hazelnuts, Macadamia nuts) to obtain spreadable cheese-like food.

PHASE 1 – SINGLE STRAIN FERMENTATIONS

Five LAB strains (Tab.1) were inoculated in 4 raw materials during soaking phase (Fig.1) and growth performances were tested monitoring acidification kinetics (pH measure) and microbial counts on selective media (MRS for LAB and VRBGA to monitor enterobacteria, as main spoilage microflora).

Strain	Species	Isolation source	Country
FFt$_0$I	*Leuconostoc lactis*	Fermented cashew nuts	Italy
BPF2	*Lactiplantibacillus paraplantarum*	Salchichónes	Spain
ST6	*Pediococcus acidilactici*	Salchichónes	
ISM1	*Latilactobacillus sakei*	Fermented vegetables	
KC11	*Lactiplantibacillus plantarum*	Daikon	Croatia

Tab.1 LAB strains used as potential starter cultures for nut fermentation

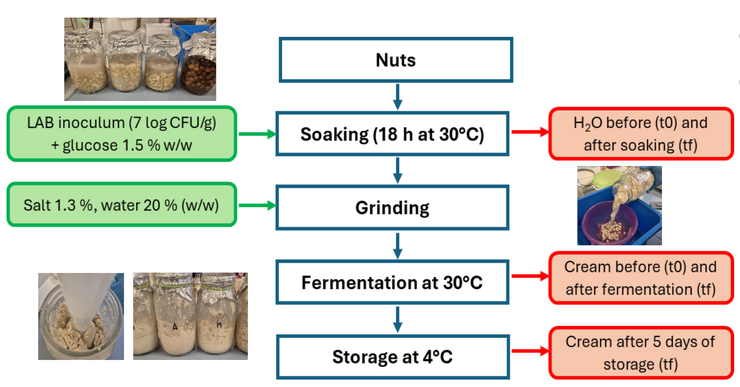

Fig.1 Flowchart of the process for nut fermentation with single LAB strains (Red boxes: sampling points)

- Strain ISM1 failed to outcompete the wild microbial population: slower acidification kinetics and microbial counts showed that this strain was replace by other LAB species.
- Hazelnut were unsuitable for obtaining a spreadable cream and did not allow fast acidification (Fig. 2) → Presence of relevant counts of enterobacteria in fermented cream

MAIN CONCLUSIONS

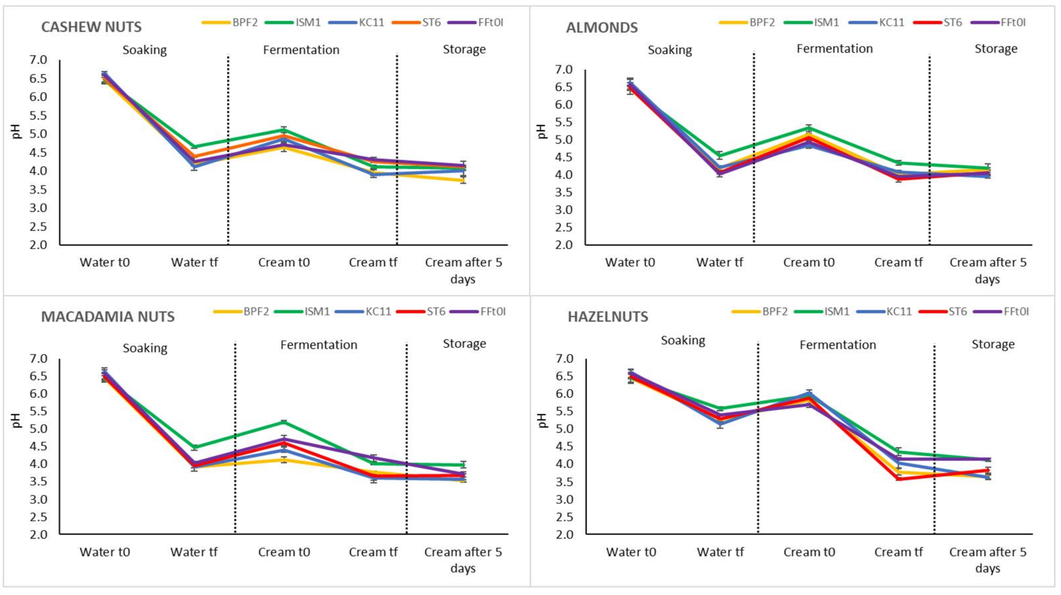

Fig.2 Acidification kinetics of the five LAB strains tested in different raw materials

- *Lat. sakei* ISM1 and hazelnuts were excluded from further analyses because unsuitable for this process
- The presence of enterobacteria highlighted the need to revise the process → a water bath pre-treatment of nuts was introduced to reduce potential contamination
- The combination of raw materials and the use of LAB consortia (one heterofermentative strain + one homofermentative strain) were tested to enrich aroma profile and increase acidification rates.

PHASE 2 – FERMENTATIONS WITH LAB CONSORTIA

Based on the results of phase 1, the process was modified (Fig. 3). Besides heat treatments, the LAB starter cultures were added scalarly, to set up a two-phase fermentation, reducing potential competition among them (previous *in vitro* tests had showed inhibiting activity of strain BPF2 and ST6 against FFt$_0$I). The combinations of raw materials (60 % cashew + 40 % macadamia or almonds) were optimized to obtain a spreadable cream using amounts of nuts also able to impart peculiar aromatic notes.

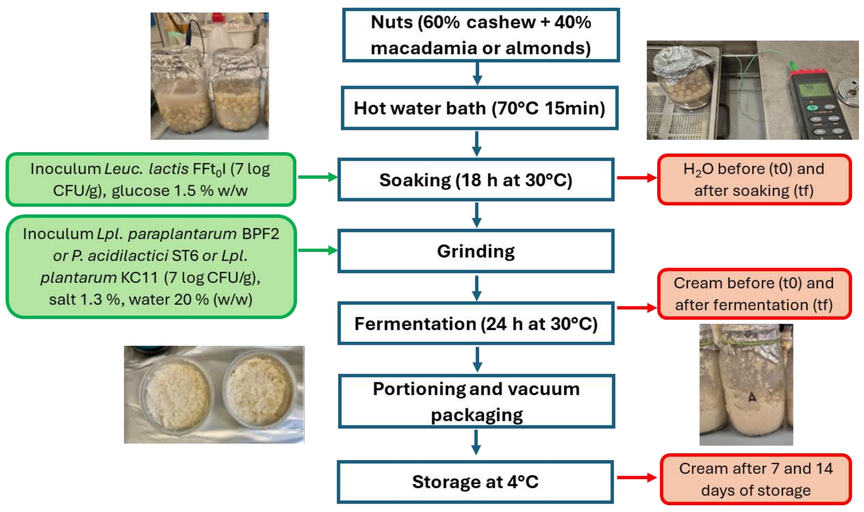

Fig.3 Flowchart of the two-phase nut fermentation process with LAB consortia (Red boxes: sampling points)

Analyses performed:

- Microbial counts of lactobacilli and enterobacteria, acidification kinetics (Tab. 2)
- Aroma profile through SPME-GC-MS, according to Tabanelli et al.[2] (Fig. 4)

LAB	Sample	CASHEW+MACADAMIA			CASHEW+ALMOND		
		Lactobacilli	Enterobacteria	pH	Lactobacilli	Enterobacteria	pH
FFt$_0$I + BPF2	Water t0	6.84 ± 0.06	<1	5.81 ± 0.11	7.06 ± 0.13	<1	5.92 ± 0.04
	Water tf	9.16 ± 0.12	<1	4.49 ± 0.05	9.10 ± 0.09	<1	4.26 ± 0.01
	Cream t0	7.95 ± 0.09	3.48 ± 0.32	4.68 ± 0.04	8.09 ± 0.10	2.41 ± 0.21	4.80 ± 0.01
	Cream tf	9.15 ± 0.13	<1	3.95 ± 0.08	9.27 ± 0.02	<1	4.23 ± 0.01
	Cream 7 days	8.98 ± 0.10	<1	3.73 ± 0.06	9.15 ± 0.10	<1	4.35 ± 0.01
	Cream 14 days	8.92 ± 0.14	<1	3.75 ± 0.01	8.83 ± 0.04	<1	3.87 ± 0.07
FFt$_0$I + ST6	Water t0	6.84 ± 0.06	<1	5.81 ± 0.11	7.06 ± 0.13	<1	5.92 ± 0.04
	Water tf	9.18 ± 0.05	<1	4.48 ± 0.04	8.96 ± 0.06	<1	4.27 ± 0.05
	Cream t0	7.65 ± 0.14	3.52 ± 0.36	4.70 ± 0.04	8.00 ± 0.04	<1	4.78 ± 0.06
	Cream tf	9.35 ± 0.03	<1	4.09 ± 0.04	9.41 ± 0.06	<1	4.34 ± 0.04
	Cream 7 days	9.42 ± 0.02	<1	3.94 ± 0.02	9.46 ± 0.02	<1	4.42 ± 0.04
	Cream 14 days	9.07 ± 0.07	<1	3.80 ± 0.01	9.22 ± 0.20	<1	3.97 ± 0.03
FFt$_0$I + KC11	Water t0	6.84 ± 0.06	<1	5.81 ± 0.11	7.06 ± 0.13	<1	5.92 ± 0.04
	Water tf	9.17 ± 0.06	<1	4.45 ± 0.01	9.12 ± 0.12	<1	4.28 ± 0.06
	Cream t0	7.73 ± 0.12	3.11 ± 0.21	4.78 ± 0.01	8.13 ± 0.07	<1	4.81 ± 0.03
	Cream tf	9.31 ± 0.02	<1	3.97 ± 0.02	9.48 ± 0.03	<1	4.21 ± 0.02
	Cream 7 days	9.19 ± 0.08	<1	3.71 ± 0.02	9.50 ± 0.06	<1	4.21 ± 0.03
	Cream 14 days	9.30 ± 0.03	<1	3.50 ± 0.03	9.13 ± 0.06	<1	3.84 ± 0.04

Tab. 2 Microbial counts (log CFU/g) of nut-based fermented products obtained with three different microbial consortia

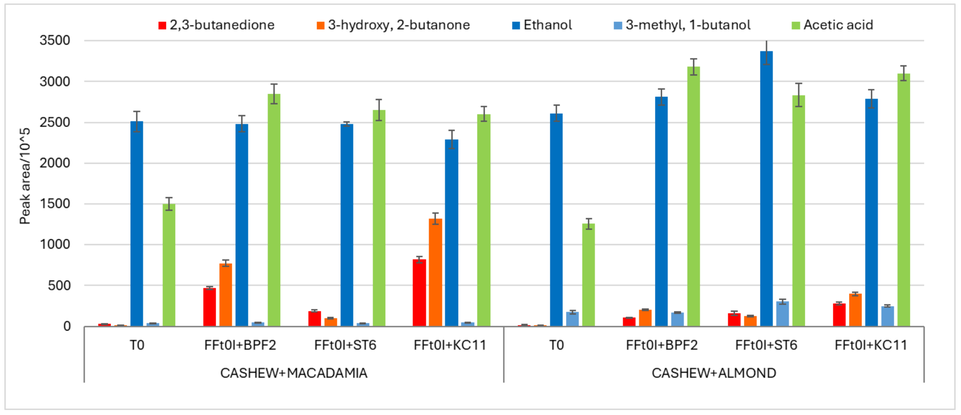

Fig.4 Main volatile compounds (SPME-GC-MS technique) detected in nut-based fermented products before (T0) and after fermentation with three different microbial consortia

- The use of microbial consortia increased acidification kinetics, reaching safe pH values (<4.4) within 24 h of fermentation. This was due to high proliferation of the LAB starter cultures up to 9 log CFU/g, already during soaking phase.
- Pre-treatment of nuts before soaking successfully controlled enterobacteria, that were always below the detection limit, except for some samples of the cream before fermentation
- Aroma profiles were characterized by the presence of ethanol and acetic acid but also compounds able to confer cheese-notes (acetoin and diacetyl) were accumulated, especially in the mixture cashew/macadamia, when strains belonging to the genus *Lactiplantibacillus* were used.

CONCLUSIONS

- This work provided valuable insights for developing innovative plant-based foods using microbial diversity from traditional fermented products.
- The optimization of the process (nut pre-treatment and two-phase fermentation) allowed to obtain a rapid acidification reaching pH values considered safe (challenge tests with food borne pathogens are in progress to confirm final product safety).
- The use of selected LAB consortia increased the accumulation of compounds that confer cheese-notes, favoring sensory acceptability.

REFERENCES: 1 Boukid, F.; Hassoun, A.; Zouari, A.; Tülbek, M.Ç.; Mefleh, M.; Aït-Kaddour, A.; Castellari, M.. Foods 2023, 12, 1005; 2 Tabanelli, G., Montanari, C., Gómez-Caravaca, A.M., Díaz-de-Cerio, E., Verardo, V., Zadeh, F.S. et al. (2024). Foods, 13, 3095.

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