Property improvement of mixed dairy and plant-based yogurt alternatives using formulation and lactic acid bacteria co-cultures

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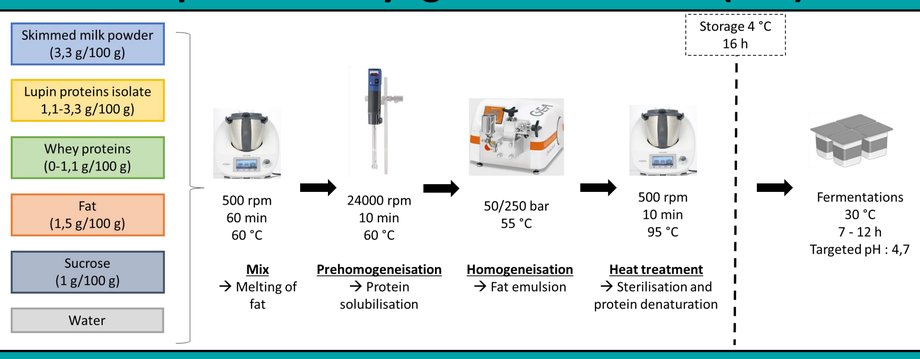
CONTEXT

In a chemically defined medium containing casein and lupin proteins as sole nitrogen sources, positive interactions were observed in co-cultures that associated a **proteolytic** strain, either *Enterococcus faecalis* (Efa) or *Lactococcus lactis* (Lla), and a **non-proteolytic** strain, *Lactiplantibacillus plantarum* (Lpl). The positive interactions were mediated by the peptides and amino acids provided by the proteolytic strains and were stronger with Efa (Canon *et al*. 2021).

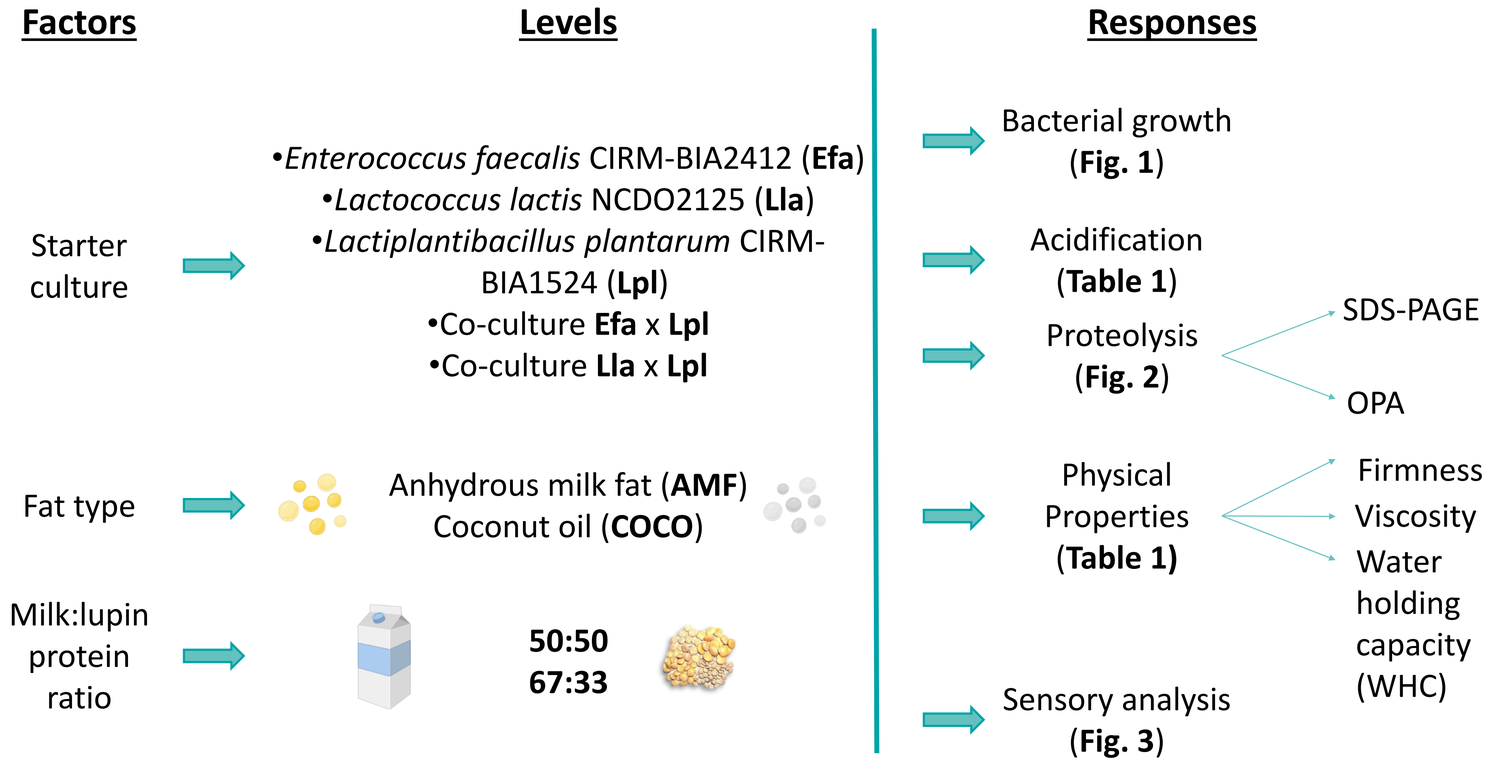
The aim of the study was to evaluate the impact of these **positive interactions** on the **organoleptic** and **textural properties** of mixed dairy and plant-based yogurt alternatives (YAs).

As lupin proteins are associated with unpleasant flavour, two different milk:lupin protein ratios were tested (67:33 and 50:50). In order to substitute animal-based fat by **plant-based** fat, anhydrous milk fat and coconut oil were also tested.

Preparation of yogurt alternatives (YAs)



EXPERIMENTAL DESIGN



RESULTS

BACTERIAL GROWTH

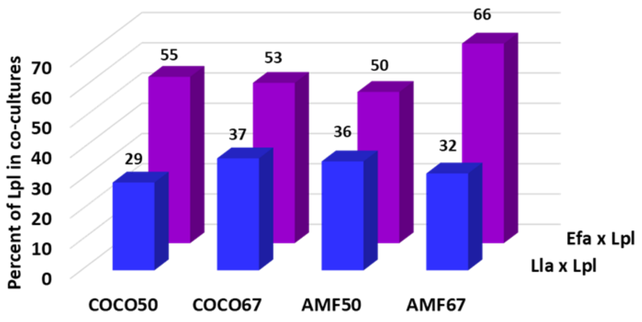


Fig 1. Percentages of the Lpl strain in co-cultures

- Total bacterial counts reached 10^9 cfu/mL in each YA
- Protein ratio and fat type had no impact on bacterial growth (**Fig. 1)** and acidification (**Table 1**)
- Lpl reached a significantly higher proportion when co-cultured with Efa compared to Lla (**Fig. 1**)

PHYSICAL PROPERTIES

Table 1. Physical properties of the 20 yogurt alternatives according to fat, protein ratio and cultures

	Fat type	Protein ratio (milk:lupin)	Efa	Efa x Lpl	Lla	Lla x Lpl	Lpl	p-values		
								Fat	Ratio	Cultures
Acidification rate (dpH/h)	-	-	0.34 ± 0.02 ^{b}	0.34 ± 0.01 ^{b}	0.68 ± 0.05 ^{c}	0.62 ± 0.06 ^{c}	0.28 ± 0.02 ^{a}	0.39	0.21	**< 2.2e-16**
Final pH	-	-	4.93 ± 0.05 ^{c}	4.74 ± 0.02 ^{b}	4.68 ± 0.03 ^{a}	4.67 ± 0.04 ^{a}	4.76 ± 0.07 ^{b}	0.72	0.93	**< 2.2e-16**
Apparent viscosity (η_{app}) at 50 s^{-1} Pa.s	COCO	50:50	0.73 ± 0.02 ^{a}	0.95 ± 0.09 ^{b}	1.44 ± 0.07 ^{d}	1.39 ± 0.09 ^{cd}	1.28 ± 0.07 ^{c}	**2 E-08**	**1E-07**	**< 2.2e-16**
		67:33	0.98 ± 0.02 ^{a}	1.15 ± 0.08 ^{b}	1.61 ± 0.06 ^{d}	1.59 ± 0.07 ^{cd}	1.42 ± 0.11 ^{c}			
	AMF	50:50	0.88 ± 0.06 ^{a}	1.17 ± 0.10 ^{b}	1.65 ± 0.10 ^{d}	1.6 ± 0.10 ^{cd}	1.5 ± 0.09 ^{c}			
		67:33	0.94 ± 0.05 ^{a}	1.24 ± 0.07 ^{b}	1.76 ± 0.07 ^{d}	1.71 ± 0.15 ^{cd}	1.58 ± 0.19 ^{c}			
Firmness N	COCO	50:50	0.17 ± 0.00 ^{a}	0.21 ± 0.02 ^{ab}	0.24 ± 0.01 ^{b}	0.22 ± 0.01 ^{ab}	0.21 ± 0.01 ^{ab}	**2 E-11**	**4 E-09**	**9.2E-04**
		67:33	0.29 ± 0.02 ^{a}	0.37 ± 0.02 ^{b}	0.45 ± 0.02 ^{c}	0.42 ± 0.02 ^{bc}	0.41 ± 0.06 ^{bc}			
	AMF	50:50	0.23 ± 0.01 ^{a}	0.29 ± 0.01 ^{ab}	0.29 ± 0.01 ^{b}	0.27 ± 0.00 ^{ab}	0.26 ± 0.01 ^{ab}			
		67:33	0.33 ± 0.01 ^{a}	0.44 ± 0.04 ^{b}	0.5 ± 0.04 ^{c}	0.49 ± 0.05 ^{bc}	0.48 ± 0.06 ^{bc}			
Water holding capacity (WHC, %)	COCO	50:50	81.5 ± 7.3 ^{c}	96.5 ± 2.3 ^{b}	98.9 ± 0.1 ^{a}	98.8 ± 0.5 ^{a}	98.7 ± 0.5 ^{a}	**3 E-04**	**8 E-05**	**7 E-15**
		67:33	87.8 ± 3.6 ^{b}	91.4 ± 0.9 ^{ab}	98.1 ± 0.1 ^{a}	98.1 ± 0.2 ^{a}	96.1 ± 1.8 ^{a}			
	AMF	50:50	75 ± 3.8 ^{c}	81.5 ± 6.1 ^{b}	98.1 ± 0.8 ^{a}	97.3 ± 1.7 ^{a}	97.4 ± 1.4 ^{a}			
		67:33	86 ± 1.7 ^{c}	86.5 ± 3.3 ^{bc}	98 ± 0.1 ^{a}	97.5 ± 0.6 ^{a}	94.8 ± 0.8 ^{ab}			

a,b,c,d: statistical differences between cultures in the same row
For each property, the color scale from red to green represents the highest and lowest value, respectively.

PROTEOLYSIS

- Proteolysis was impacted by the starter and the protein ratio but not by the fat type
- Efa showed a strong proteolytic activity and hydrolysed both milk and lupin proteins (**Fig. 2**)
- Protein hydrolysis was hardly observed with Lla
- Lpl decreased the levels of free NH_2 compouds

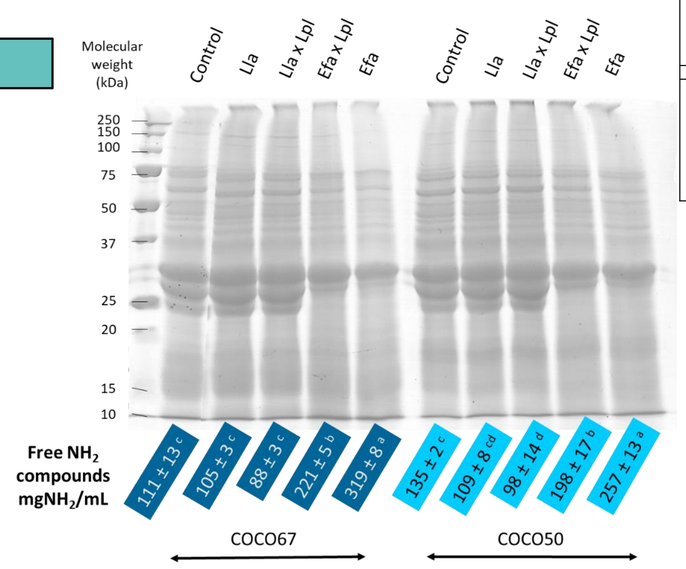


Fig 2. SDS-PAGE and quantification of free NH_2 groups
a,b,c : statistical differences between cultures

- Only the YAs fermented by Efa did not reach the targeted pH within 12 h incubation.
- Lla acidified faster, followed by Efa and Lpl. In both co-cultures, the acidification rates did not differ from that of Efa and Lla cultures (**Table 1**).
- Apparent viscosity at 50 s^{-1}, firmness and WHC were significantly higher for YAs: containing AMF, with a milk:lupin protein ratio of 67:33, and fermented with Lla or Lla x Lpl.
- The association Lpl x Efa resulted in more viscous and firmer YAs, as well as less wheying off, compared to the YAs fermented with Efa alone.

SENSORY ANALYSIS

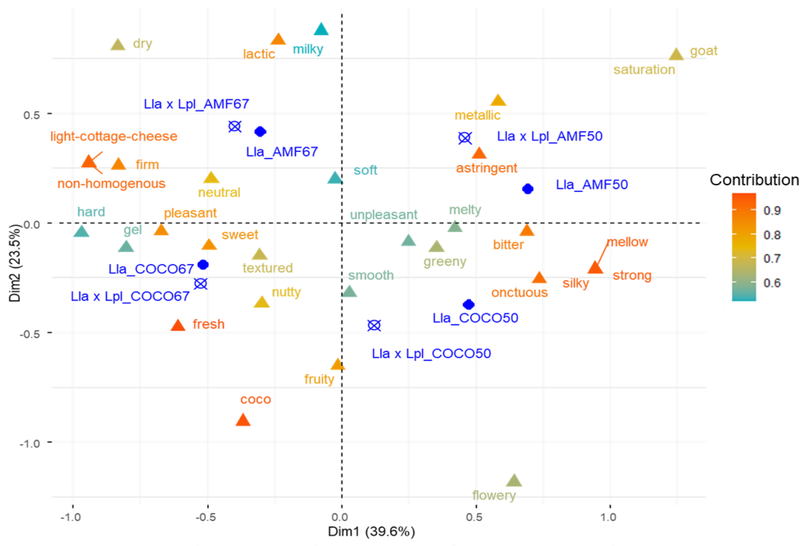


Fig. 3 Correspondence analysis map of sensory analysis (sorting task) data obtained for the 8 YAs fermented with Lla and Lla x Lpl

- YAs were differentiated according to the protein ratio (Dim1) and the fat type (Dim2) but not the starter (**Fig. 3**).
- The protein ratio of 67:33 was described as pleasant, textured (hard gel) and nonhomogenous as opposed to the ratio 50:50 described as unpleasant, bitter and with a mellow texture (**Fig. 3**).
- AMF was described as milky, lactic and "goaty" and COCO as fruity, fresh and nutty.

CONCLUSION

- 12 yogurt alternatives were prepared.
- With positive interactions (Efa x Lpl), both strains contributed to the final properties of the YAs. Without positive interactions (Lla x Lpl), only Lla contributed in the final properties of YAs.
- More textured Yas (firmer, less wheying off, more viscous) were obtained with anhydrous milk fat and a milk:lupin protein ratio of 67:33.
- Substitution of milk protein by lupin protein can be acceptable, in terms of flavour and texture, if the milk:lupin protein ratio is about 67:33.
- Coconut oil and anhydrous milk fat gave different physical properties that were not perceived during the sensory analysis. Substitution of animal-sourced fat by plant-based one is thus achievable.

Canon, F., Maillard M-B., Henry G., Thierry, A., & Gagnaire, V. (2021). Positive interactions between lactic acid bacteria promoted by nitrogen-based nutritional dependencies. AEM. doi: 10.1128/AEM.01055-21.

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