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

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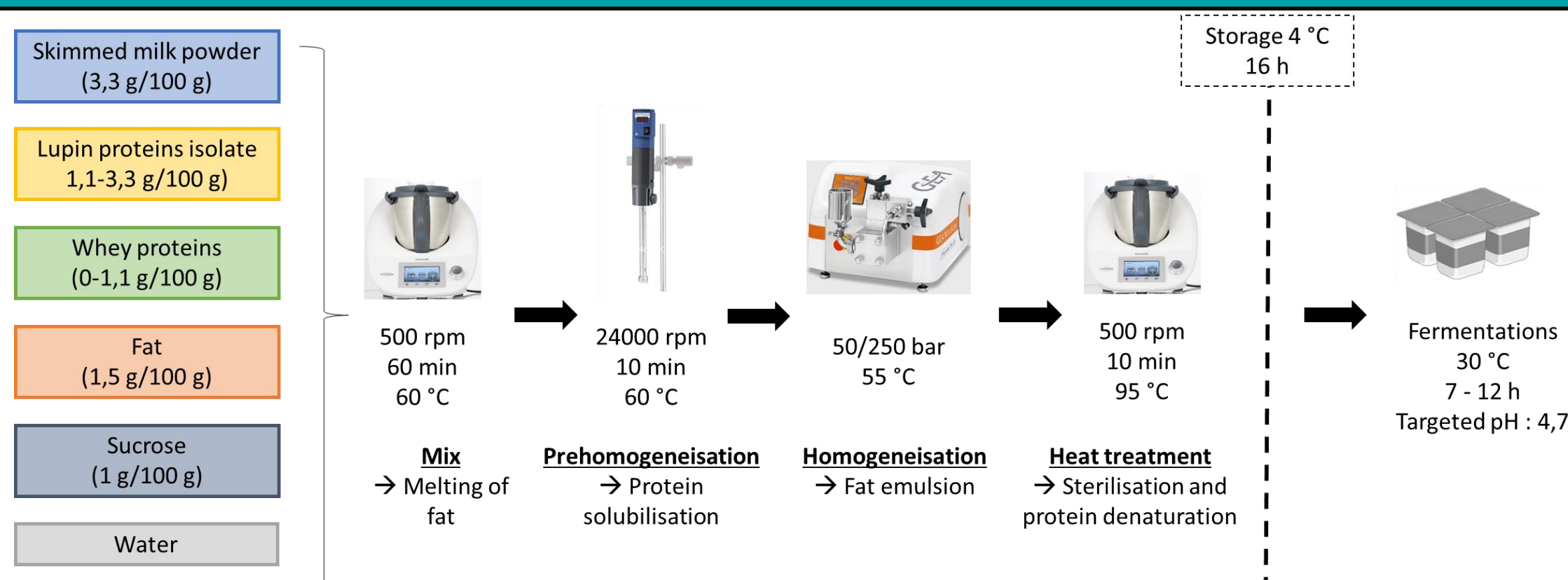
## CONTEXT

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

### Factors

### Levels

### Responses

Starter culture →  
 • *Enterococcus faecalis* CIRM-BIA2412 (Efa)  
 • *Lactococcus lactis* NCDO2125 (Lla)  
 • *Lactiplantibacillus plantarum* CIRM-BIA1524 (Lpl)  
 • Co-culture Efa x Lpl  
 • Co-culture Lla x Lpl

Fat type →  
 Anhydrous milk fat (AMF)  
 Coconut oil (COCO)

Milk:lupin protein ratio →  
 50:50  
 67:33

→ Bacterial growth (Fig. 1)  
 → Acidification (Table 1)  
 → Proteolysis (Fig. 2) → SDS-PAGE, OPA  
 → Physical Properties (Table 1) → Firmness, Viscosity, Water holding capacity (WHC)  
 → Sensory analysis (Fig. 3)

## RESULTS

### BACTERIAL GROWTH

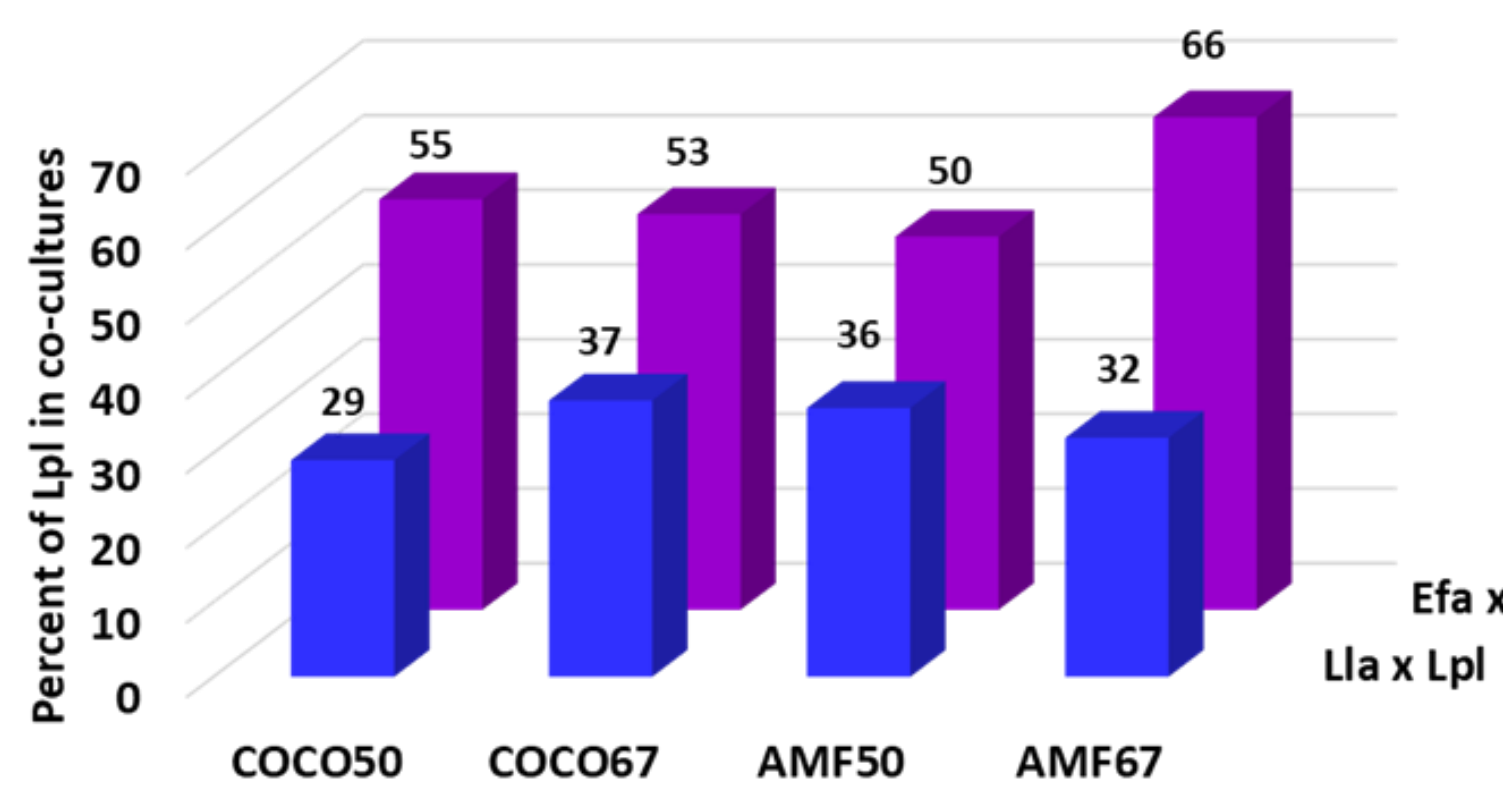


Fig. 1. Percentages of the Lpl strain in co-cultures

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

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.

- 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<sup>-1</sup>, 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.

### 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<sub>2</sub> compounds

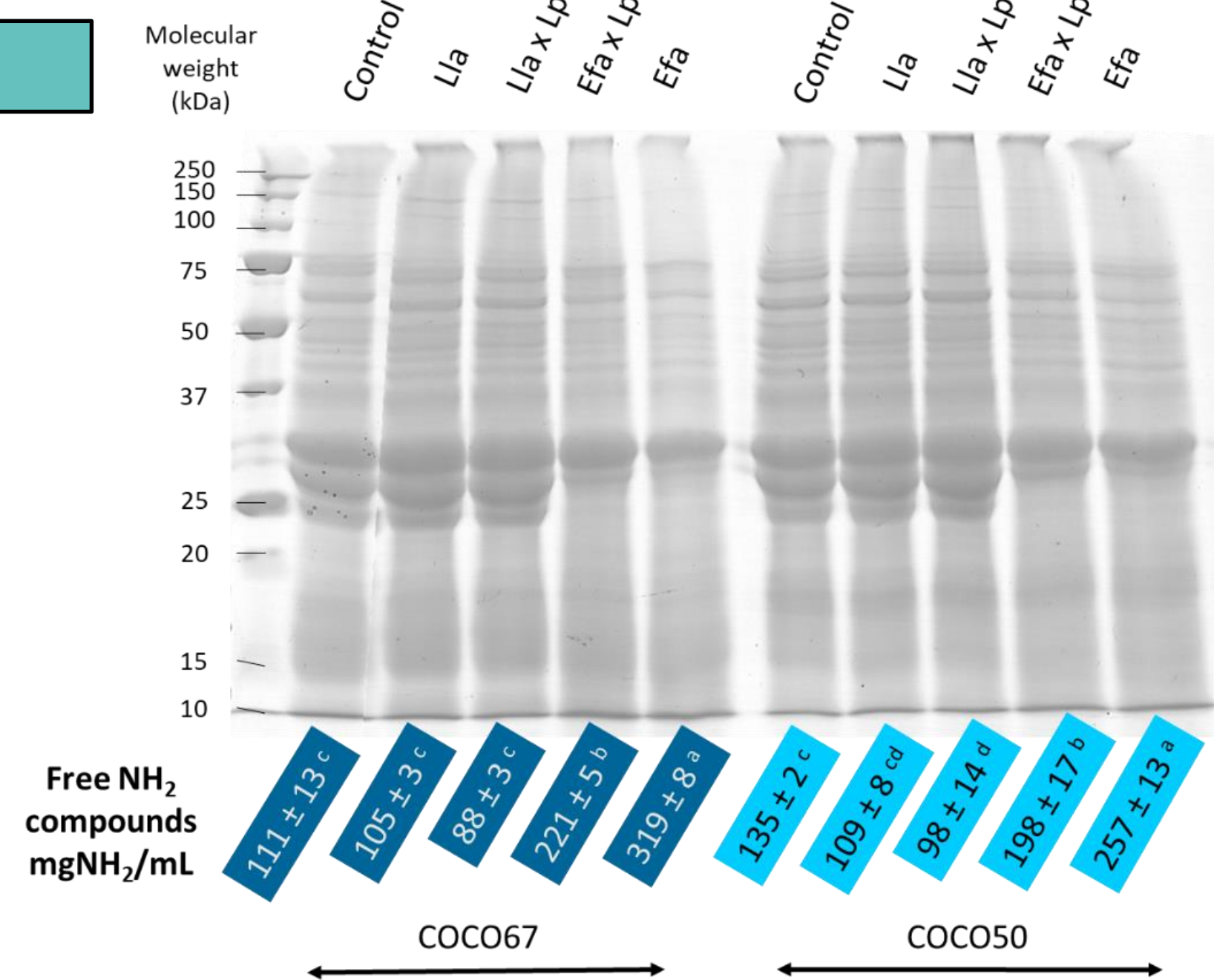


Fig. 2. SDS-PAGE and quantification of free NH<sub>2</sub> groups  
 a,b,c : statistical differences between cultures

### SENSORY ANALYSIS

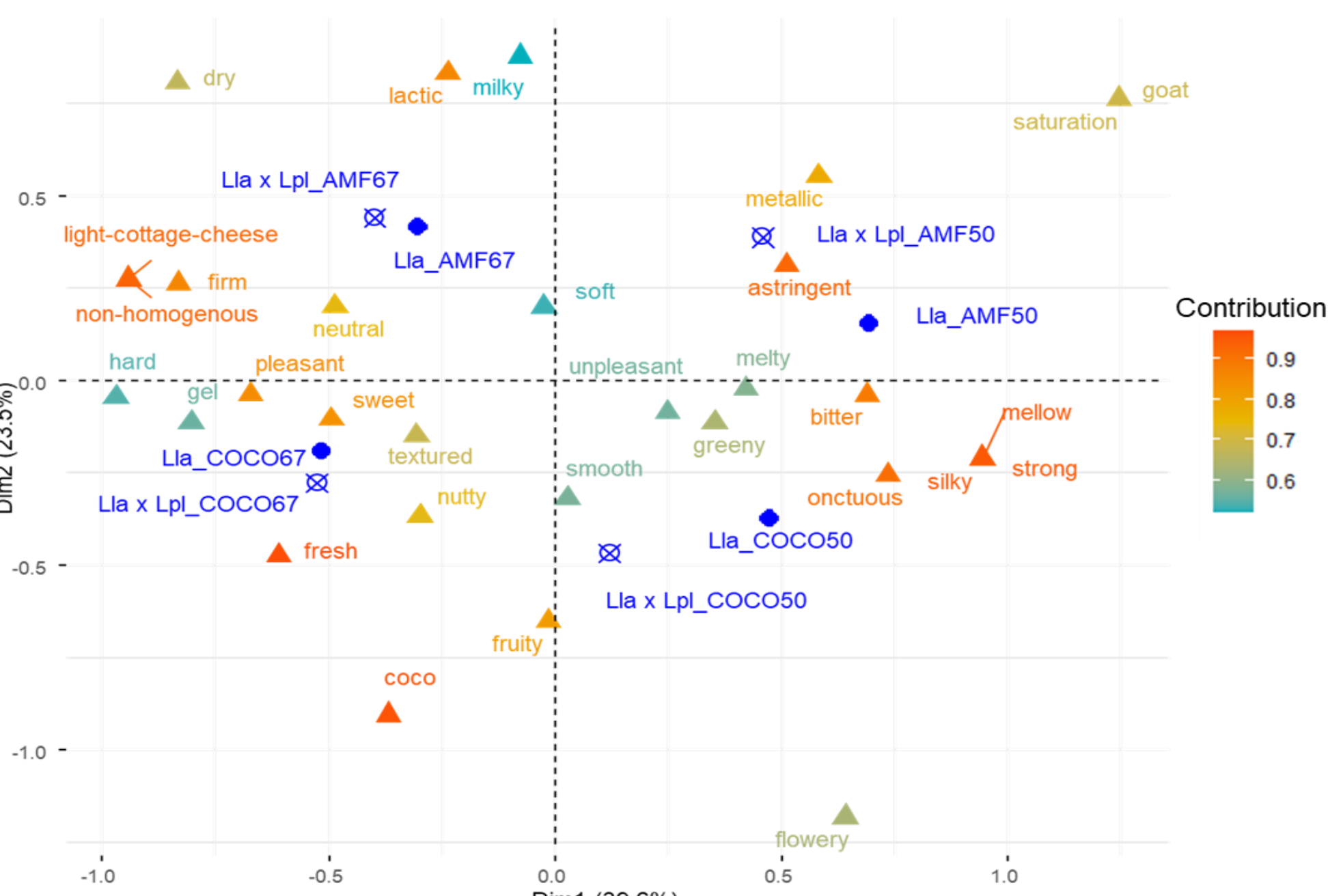


Fig. 3 Correspondence analysis map of sensory analysis (sorting task) data obtained for the 8 YAs fermented with Lla and Lla x Lpl

- 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.