In situ CLA and CLNA production: a potential strategy to elaborate food products enriched in bioactive fatty acids

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Introduction

Conjugated linoleic acid (CLA) and, more recently, conjugated linolenic acid (CLNA) isomers have shown potential to be applied as new functional ingredients, given its bioactive potential to exert health benefits to human host[1,2]. CLA and CLNA are naturally present in meat and milk fat of ruminants, being CLNA isomers also found in vegetable oils[3,4]. Due to limitations in terms of CLA and CLNA concentration and availability in their natural sources, strategies that could enable the increment of CLA/CLNA daily intake have been studied, among which is the *in situ* microbial production. The biohydrogenation of linoleic acid (LA) and linolenic acid (LNA) from ruminants' diet by ruminal bacteria, leads to the formation of intermediate products such as CLA and CLNA[5]. However, strains of lactobacilli, bifidobacteria and propionibacteria have also revealed capacity to produce these compounds[3,6], yet require further assessment of such potential.

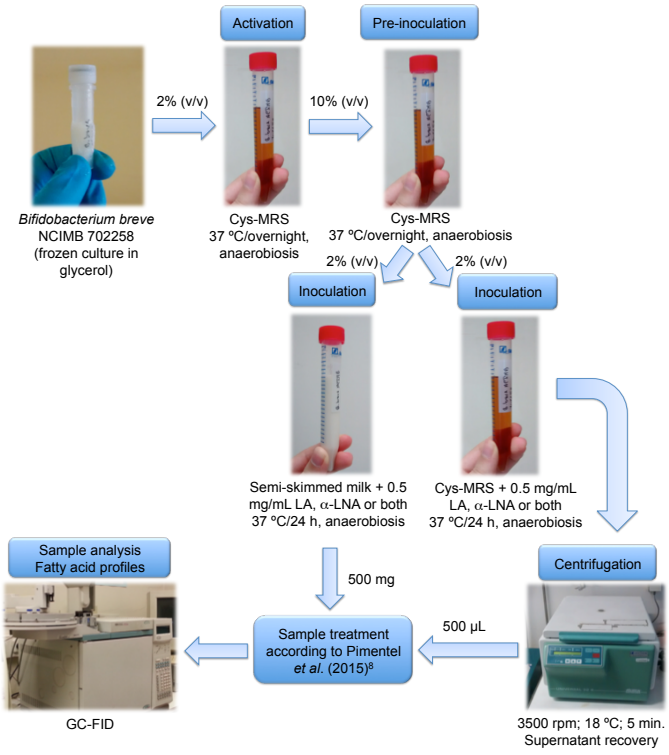
Objectives

The general aim of this work was to test the *in situ* production of CLA and CLNA isomers in semi-skimmed milk (1.6% fat) using *Bifidobacterium breve* NCIMB 702258, a strain that has previously shown CLA/CLNA production capacity[7], in order to verify the suitability of this strategy for CLA and CLNA enrichment of dairy food products.

To fulfill such major goal the following specific objectives were drawn, namely:

- To test the strain growth capacity when grown in MRS medium containing LA, α-LNA or both (at 0.5 mg/mL each) for 24 h at 37 ºC and analyze the fatty acid concentration through gas chromatography.
- To test *Bifidobacterium breve* producing capacity in semi-skimmed milk, under the same conditions applied in MRS medium.

Methods



Results

- **In MRS medium** LA and α-LNA conversion rates were found to be between 14.83% and 67.33%, and CLNA production was higher than CLA counterpart independently of substrates being added separately or together (Table 1).
- **In semi-skimmed milk**, CLA/CLNA isomers production was positive substrate conversion rates were 1.2-3.8 fold lower than those obtained in MRS medium (rates achieved were between 14.55% and 28.09%, Table 1). α-LNA conversion was higher than LA when the substrates were added separately, but not when both LA and α-LNA were co-assayed; in fact, in this second case LA conversion rate was two-fold higher than in MRS medium alone (Table 1; Figure 1).

Table 1. LA and LNA conversion rates by *B. breve* NCIMB 702258 after incubation in MRS medium and semi-skimmed milk (1.6% fat) with LA, α-LNA and both.

	MRS medium		Semi-skimmed milk	
Substrate	LA conversion (%)	α-LNA conversion (%)	LA conversion (%)	α-LNA conversion (%)
LA	30.36 ± 0.97	-	24.19 ± 1.78	-
α-LNA	-	67.33 ± 1.83	-	28.09 ± 2.31
LA+α-LNA	14.83 ± 0.56	55.40 ± 1.44	27.60 ± 1.42	14.55 ± 0.98

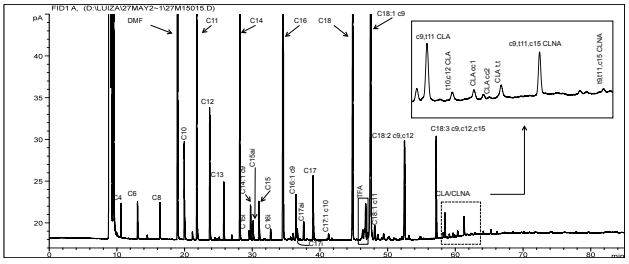


Figure 1. Fatty acid profile assessed by gas chromatography of the fermented milk inoculated with *B. breve* NCIMB 702258 and both LA and α-LNA.

Conclusions

In conclusion, *in situ* production of microbial CLA and CLNA isomers is a strategy with potential to be applied in the future elaboration of CLA- and CLNA-enriched dairy food products.

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Acknowledgements

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