In situ milk enrichment with microbial CLA/CLNA using vegetable oils as substrate source

Ana L. Fontes[1,2], Lígia L. Pimentel[1], M. Rosário Domingues[3,4], Luis M. Rodríguez-Alcalá[1] and Ana M. Gomes[1]

[1]Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal
[2]Laboratório Associado para a Química Verde – LAQV-REQUIMTE, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago 3810-193 Aveiro, Portugal
[3]Centro de Espectrometria de Massa, LAQV-REQUIMTE, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago 3810-193 Aveiro, Portugal
[4]CESAM, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago 3810-193 Aveiro, Portugal

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Introduction

Conjugated linoleic (CLA) and linolenic acid (CLNA) isomers have been attracting much scientific attention due to their potential bioactive properties[1,2]. These compounds are naturally produced during the biohydrogenation process of dietary linoleic (LA) and alpha-linolenic acid (α-LNA), respectively, by ruminal bacteria[3]. However, several probiotic strains have also revealed the ability to produce those bioactive fatty acids[4,5], and our research group has previously identified *Bifidobacterium breve* DSM 20091 with such capacity[6]. To produce those conjugated fatty acids directly in a dairy matrix may be a good strategy to increment CLA/CLNA daily intake, since there are limitations in their natural sources (ruminants' milk and meat and vegetable oils)[7,8]. To be used for human consumption, a safe substrate source is necessary, and for that purpose, there are edible vegetable oils commercially available which are rich in LA, such as soybean oil (SBO), or α-LNA, such as flaxseed oil (FSO)[9,10]. Therefore, the aim of this work was to verify if it is indeed possible to enrich milk in CLA and CLNA through *in situ* microbial production using vegetable oils as substrate sources.

Methods

Flaxseed oil

Soybean oil

↓ Hydrolysis by *Candida rugosa* lipase (45 ºC, 200 rpm, 24 h)

Oil-in-water emulsions with 2% (w/v) Tween 80

↓ 0.25-10 mg/mL LA and/or α-LNA

Bifidobacterium breve DSM 20091

1% (v/v) →

Pasteurized cows' milk + 0.05% (w/v) cysteine

→

37 ºC, 24 h, anaerobiosis

↓

Fermented cows' milk

←

Fatty acid analysis via GC-FID

Results

Higher yields were detected when *B. breve* DSM 20091 was inoculated with flaxseed oil (FSO) alone, where CLNA isomers were those mainly produced. Surprisingly, only traces of CLA were found, either with soybean oil (SBO) alone or in combination (Figure 1). FSO was further assayed at higher concentrations, in order to find out if *B. breve* DSM 20091 would be able to enrich milk in higher amounts of conjugated fatty acids.

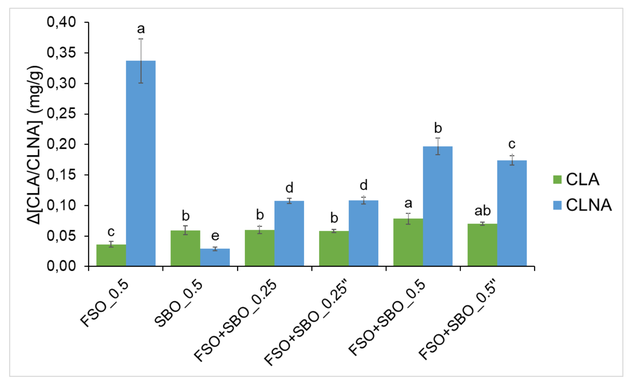

Fig.1 Variation in CLA/CLNA amounts (mg/g) after milk fermentation with *B. breve* DSM 20091 at 0.25 or 0.50 mg/mL of LA (from SBO) and/or α-LNA (from FSO), adjusted or not ('') when oils are combined.

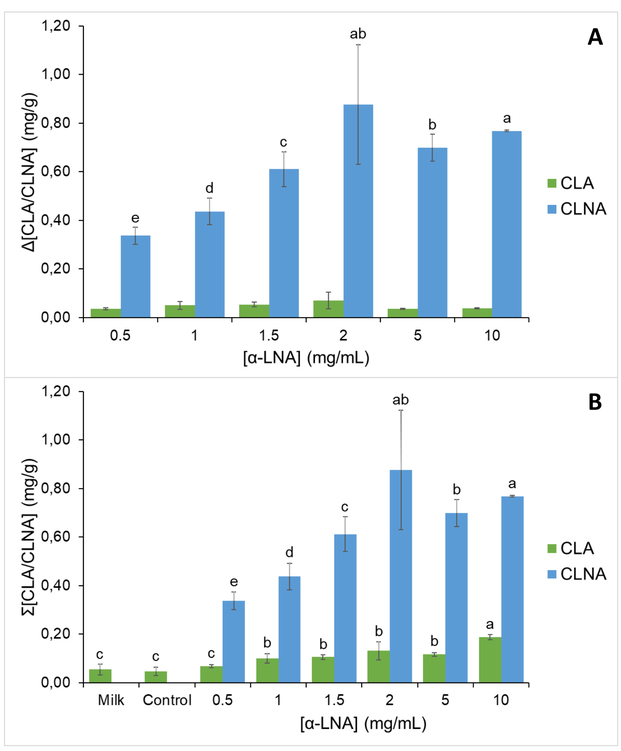

Fig.2 Variation in [A] and total [B] CLA/CLNA amounts (mg/g) after milk fermentation with *B. breve* DSM 20091 at different α-LNA (from FSO) concentrations (mg/mL).

Maximum yield of conjugated fatty acids was reached at 2 mg/mL α-LNA, being mostly produced CLNA isomers, once more. In fact, CLA production was similar between substrate concentrations (Figure 2-A).
Analysis of the milk natural fatty acid composition profile revealed absence of CLNA isomers; when *B. breve* DSM 20091 was added it was able to enrich milk with ~0.9 mg/g CLNA at 2 mg/mL α-LNA. At such concentration, CLA content was also increased, but not sufficiently to enrich milk in that conjugated fatty acid (Figure 2-B), as already figured out by the production yields (Figure 2-A).

Conclusions

Through the *in situ* production approach, it was possible to enrich milk in CLNA isomers, but not in CLA, by inoculating milk, previously added with flaxseed oil, with *B. breve* DSM 20091.

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