Italian Journal of Food Science, 2021; 33 (SP1) 1–11

Bacterial conjugated linoleic acid bio-fortification of synbiotic yogurts using *Propionibacterium freudenreichii* as adjunct culture

Omid Zahed[a], Kianoush Khosravi-Darani[b]*, Amir Mohammad Mortazavian[b], Abdorreza Mohammadi[b]

[a]Student Research Committee, Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Science and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [b]Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Science and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Corresponding Author:** K. Khosravi Darani, (Prof. of Food Biotechnology) National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Science, P.O. Box: 19395-4741, Tehran, Iran. Email: k.khosravi@sbmu.ac.ir and kiankh@yahoo.com

Received: 20 September 2020; Accepted: 28 December 2020; Published: 6 January 2021

PAPER

Abstract

In this study, *Propionibacterium freudenreichii* was used for *in situ* production of conjugated linoleic acid (CLA) in yogurt. Firstly, effects of process variables, including strain type, percentage of milk fat, percentage of inoculum, quantity of sunflower oil, concentration of inulin, temperature of fermentation and time of storage at 4°C, on production of CLA by *Propionibacterium freudenreichii* were investigated using screening method of the Plackett–Burman design. Then optimisation of CLA production process was conducted using three major factors of milk fat percentage, inulin concentration and storage time at 4°C using central composite design. Analysis of variance established that the models were highly significant ($P \leq 0.05$). The model demonstrated that the production of CLA was affected by these three factors. Optimised CLA production by *Propionibacterium freudenreichii* ssp. *shermanii* in yogurts was achieved after 17 days of storage at 4°C in skim-milk containing 1.75% (w/w) fat and 2.25% (w/v) inulin as prebiotic. Reconfirmation test established that at the highlighted optimum conditions, the highest concentration of produced CLA was 6.4 mg g^{-1} lipid in yogurt, which is a 256% increase in total CLA production, compared with control samples. Results demonstrated that *Propionibacterium freudenreichii* ssp. *shermanii* not only leads to production of synbiotic yogurts containing inulin but also increases CLA production in yogurts.

Keywords: conjugated linoleic acid, probiotic, *Propionibacterium freudenreichii,* yogurt

Introduction

In addition to nutritional and sensory characteristics of food products, health beneficial aspects are other important criteria for consumers to choose food products. One of the best manners to receive essential nutrients with minimum side effects is enrichment of food products (Grunert, 2005). Functional foods play important roles in this area as tendency to consume functional foods has increased recently. Such characteristics are found in a new group of products called synbiotics, which contain probiotics and prebiotics simultaneously (Holzapfel and Schillinger, 2002). Various food products are established as probiotic carriers, of which fermented dairy products, such as yogurt and cheese, include the largest proportion in research and marketing (Pandey and Mishra, 2015). Propionic acid bacteria (PAB) are widely applied as beneficial probiotic bacteria in several food technologies

(Zárate *et al.*, 2011) because of their ability to produce important metabolites, for instance, propionic acid (Van Wyk *et al.*, 2018), folate (Rad *et al.*, 2016), vitamins B_2, B_7, B_{12} and K (Abou Ayana *et al.*, 2016; Zárate, 2012) and bacteriocins (Ahmadi *et al.*, 2015) are used in industrial and commercial scales (Farhadi *et al.*, 2012; Kouya *et al.*, 2008). Use of PAB in production of dairy products such as yogurt increases product viscosity through the production of exopolysaccharides and inhibits growth of undesirable microorganisms in the product through the production of propionic acid and bacteriocins. This increases shelf life of the product. In addition, growth of PAB does not interfere with the growth of lactic acid bacteria (LAB) in dairy products (Ekinci and Gurel, 2008; Gorret *et al.*, 2001).

Conjugated linoleic acid (CLA) is another valuable metabolite produced by PAB in culture media (Van Wyk *et al.*, 2018; Yang *et al.*, 2017). In fact, CLA is a fatty acid naturally found in milk fats and dairy products such as yogurt, butter and cheese (Van Wyk *et al.*, 2018). The compound belongs to a group of omega-6 fatty acids, and is a geometric isomer of linoleic acid (LA; Yang *et al.*, 2017). Beneficial properties of CLA include preventing increase of body fats (Corbo *et al.*, 2014), anti-carcinogenesis properties (colon, prostate, skin and breast cancers) (Masso-Welch *et al.*, 2004), antioxidant properties (Zárate, 2012), lowering of blood serum cholesterol (Hernandez, 2013), anti-inflammation properties (Olson *et al.*, 2017), anti-diabetic properties (Balci Yuce *et al.*, 2017) and regulation of the system. Daily intake of 3 g of CLA is recommended to prevent cancers; however, the CLA content of dairy products is only 0.5–9.9 mg g^{-1} of fats (Zárate, 2012). Commercially, most of CLA is produced through the chemical isomerisation of LA, in which harmful by-products are produced as well. In the chemical production method, various isomers of CLA are produced (Ogawa *et al.*, 2001). Studies have verified that c9t11-CLA, t9t11-CLA and t10c12-CLA isomers prevent diseases in the human body and include medical uses (Yang *et al.*, 2017). Dairy PAB has the potential to convert unsaturated fatty acids cis-9 cis-12 LA (c9c12-18:2) to cis9-trans-11 (c9t11-18:2), trans-10-cis-12 (t10c12-18:2) and trans-9-cis-11(t9c11-18:2) conjugate isomers (Hennessy *et al.*, 2012). Thus, it is possible to produce dairy products with high CLA levels by developing products fermented by PAB, which produces increased CLA levels by converting LA present in milk to CLA.

Environmental and growth factors greatly affect CLA production in dairy products (Yang *et al.*, 2017). Several studies have been conducted on the effects of process variables on microbial production of CLA, including probiotic strains (*Lactobacillus* sp., *Bifidobacterium* sp., *Propionibacterium* (*P.*) sp., *Leuconostoc* sp., *Lactococcus* sp., *Enterococcus* sp. and *Pediococcus* sp.) (Fukuda *et al.*, 2006; Kim, 2003; Ross *et al.*, 2010), inoculum size (Yang *et al.*, 2017), pH-value (Cousin *et al.*, 2016), incubation and fermentation temperatures (Khan *et al.*, 2011), added prebiotics (Ogawa *et al.*, 2001), LA-rich sources (Xu *et al.*, 2004), dissolved oxygen (Kim *et al.*, 2000) and storage time at 4°C (Akalin *et al.*, 2007). Therefore, optimisation of conditions is critical for the growth and production of CLA by PAB (Khodaiyan *et al.*, 2008).

The aim of this study was to investigate factors affecting CLA production in yogurts by *P. freudenreichii* ssp. *freudenreichii* and ssp. *shermanii* using the Plackett–Burman design (PBD). In addition, effect of variables (bacterial strains, milk fat concentration, inoculum percentage, prebiotic (inulin) concentration, sunflower oil quantity, fermentation temperature and storage time at 4°C) on production of CLA was investigated. To optimise the most important affecting factors, response surface methodology (RSM) design was used in yogurts containing *P. freudenreichii* ssp. *shermanii* for production of CLA.

Materials and Methods

Materials

Skim-milk powder and 40% (w/w) fat cream were kindly gifted by Pak Dairy, Tehran, Iran. Inulin powder with an average degree of polymerisation of ≥25 was provided by Ava Salamat Javid, Tehran, Iran. Sunflower oil (Margarine Foods, Tehran, Iran) was purchased from supermarkets. CLA standard was purchased from Sigma, St. Louis, MO, USA. All analytical reagents and chemicals were purchased from Merck, Darmstadt, Germany. All solvents used were of analytical or High Performance Liquid Chromatography (HPLC) grade.

Preparation of cultures

A commercial yogurt starter culture (YoFlex Express 1.0) containing *Streptococcus thermophilus* (ST) and *L. delbrueckii* ssp. *bulgaricus* (LB) was selected because of the mild acid-production activity of PAB used in this study. The YoFlex Express 1.0 was purchased from Chr. Hansen, Horsholm, Denmark, and used based on manufacturer's recommendations. Commercial starter culture (PS-4) containing *P. freudenreichii* ssp. *shermanii* was purchased from Chr. Hansen, Horsholm, Denmark. Cultures were obtained in freeze-dried (DVS) form and stored at –18°C. The PAB (PS-4) was weighed to prepare an initial count of 8 log colony-forming unit (CFU) mL^{-1}. Pre-cultures were prepared by dissolving each culture in 60 mL of sterilised skim-milk and activating them at 42°C for 20 min before use. The *P. freudenreichii* ssp. *freudenreichii* PTCC No. 1674 was provided by the Research and

Technology Department of Ministry of Sciences (Persian Type Culture Collection), Tehran, Iran. The strain was sub-cultured in sodium lactate medium (SLM) containing 1% (v/v) sodium lactate syrup, casein peptone 10 g L^{-1} and yeast extract 10 g L^{-1} at 30°C under micro-aerobic conditions (Grinstead and Barefoot, 1992)

Milk preparation

After preparing of reconstituted milk with 13% (w/v) of commercial skim-milk powder in distilled water (DW), the milk was pasteurised at 90°C for 30 min and cooled in an ice bath to temperature below 35°C to prevent possible heat shocks to probiotic bacteria. For preparing various percentages of milk fats, Pearson square method was used.

Fermentation

In this study, values of independent variables in yogurt samples were calculated based on the design of experiments (PBD and RSM) at each stage. After inoculation, yogurt samples were transferred into 100-mL polypropylene cups, and milks were incubated at 30–43°C (based on the design of experiments) using laboratory oven until a pH of 4.6 was reached. pH values of yogurt samples were determined with a pH meter 605 (Methrohm AG, Herisau, Switzerland). Then samples were quickly cooled using ice bath and stored at 4°C. Three yogurt samples were prepared to verify the model and compare productions of CLA by *P. freudenreichii* ssp. *shermanii*. The control yogurt, which contained traditional yogurt starter cultures (ST and LB) only, was not supplemented with *P. freudenreichii* ssp. *shermanii* (PS4) and prebiotics (inulin). Other samples (YC and PS4) included yogurt starter culture and *P. freudenreichii* ssp. *shermanii*, and in the third yogurt sample (YC, PS4 and inulin), *P. freudenreichii* ssp. *shermanii* was added in addition to traditional starter cultures and 2.27% (w/v) inulin. Fat content of milk in all three yogurt samples was 1.75% (w/w). Analyses were conducted after an overnight storage of yogurt samples and after 7, 16 and 21 days of storage at 4°C.

Count of viable bacteria

Cell count of the starter cultures (ST and LB) and probiotics (PAB) was conducted in duplicate after incubation time. Yogurt samples (1 mL) were added to 9 mL of 0.15% (w/v) sterile peptone water (Merck, Germany) and viable bacteria were counted as formed colonies using the pour plate method. LB and ST were plated in MRS agar and M17 agar (Merck, Germany) (Dave and Shah, 1996). The MRS agar was acidified to pH 5.4 using acetic acid. Sodium lactate agar was used for selective enumeration of PAB (Tharmaraj and Shah, 2003). The incubation temperature for *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus* and *P. freudenreichii* ssp. *shermanii*, respectively, were 45°C for 72 h, 37°C for 24 h and 30°C for 5–7 days under anaerobic conditions using gas generating pack A (Merck, Darmstadt, Germany), except for *S. thermophilus*.

Lipid extraction and CLA analysis

Extraction of CLA was conducted based on the method by Lin *et al*. (1999), in which yogurt was mixed with chloroform–methanol in a ratio of 2:1 (v/v), and was refrigerated and centrifuged for 6 min at 4,500 × g. The organic phase layer was collected and dehydrated with 0.3 g of sodium sulphate and stored in refrigerator for 24 h. The middle phase was separated from sodium sulphate using decantation and used in experiments. To remove the organic solvents (chloroform-methanol), rotary evaporator was used to dry off. Thereafter, in order to saponify fatty acids 1 mL solution of 1N sodium hydroxide in methanol was added into the solution and then it was incubated at 100°C for 15 min. Then hydrochloric acid solution in methanol was added to methylate present fatty acid, and the mixture was incubated at 60°C for 20 min using water bath. At this stage, 2 mL of distilled water was added and homogenised for 15 min using vortex mixer to release methyl esters from methanol, followed by formation of polar bonds between methanol and water. Then n-hexane was added and homogenised to transfer methyl esters from aqueous phase to organic phase. After removing aqueous phase, anhydrous sodium sulphate was mixed with organic phase and 1 µL of this mixture was injected into gas chromatographic columns (Capillary BP10; Philips Scientific Model 4410, UK) fitted with a flame ionisation detector. The column was 25 m in length and 0.22 mm in diameter with a thickness of 0.25 µm. The initial temperature of the column was 150°C with 1-min holding time, injection temperature was 250°C, final temperature was 230°C with 10-min duration and a temperature ramp of 5°C in 1 min. In this study, the total quantity of CLA (mg g^{-1} lipid) was reported as the sum of the production of two isomers (c9t11-18:2 and t10c12-18:2).

Experimental design

This study was conducted progressively at three levels step by step. As mentioned previously, different factors might affect bio-production of CLA in yogurt samples by PAB. The first optimisation step included identification of variables with significant effects on CLA production by PAB using PBD. After identification of effective and

significant variables in CLA production, effective factors identified at three levels were optimised using central composite design (CCD) under RSM designations. Moreover, the best conditions for independent variables in CLA production by PAB were provided and the quantities of CLA production in PAB yogurt samples were compared with those in control yogurt samples, which only contained starter cultures (YoFlex Express 1.0).

Plackett–Burman design

Effective factors and their levels were selected based on the literature review. The selected variables, including media compositions (e.g. strain type, milk fat percentage (MFP), inoculum percentage, sunflower oil quantity and inulin concentration) and environmental factors (e.g. incubation and fermentation temperatures and storage time at 4°C), are shown in Table 1. High levels (+) and low levels (–) represent two different levels of independent variables.

RSM design

The RSM is a set of statistical techniques for designing experiments, constructing models, assessing effects of factors and searching for the optimal conditions of the factors for optimal responses. In general, RSM is a great tool for optimising conditions when several factors are involved in production of a product (Cousin *et al*., 2016; Grinstead and Barefoot,1992; Khodaiyan *et al*., 2008). A combination of factors that produces a specific optimal response can be identified using design factor and RSM (Khodaiyan *et al.*, 2008). For additional accurate predictions on the optimum conditions of CLA bioproduction and to minimise the number of test sets, CCD under RSM was designed. In this study, all factors were used at three levels (Table 2). Experimental ranges of the three significant variables for CCD trials are shown in Table 2.

Statistical analysis

Statistical analysis of the results was conducted using MINITAB statistical software v.16 (Minitab, USA), and response surface plots were drawn. Data were statistically treated using analysis of variance (ANOVA). All data were presented as the mean value ± standard deviation (SD) of independent experiments on various days. In general, $P \leq 0.05$ was established statistically significant.

RESULTS AND DISCUSSION

Selection of the most important affecting factors using the Plackett–Burman design

The primary purpose of screening experiments is to select important major effects from less important ones.

Table 1. Process variables, selected levels and eight trials of the Plackett–Burmann design to study the impact of seven factors (and finding main variables) on microbial production of CLA in synbiotic yogurt.

Run	Independent variables							Response		
	A Strains	B Milk fat % (w/w)	C Inulin % (w/v)	D Sunflower oil (g L^{-1})	E Inoculum size (%)	F Temperature (°C)	G storage time (days)	cis-9,trans-11 CLA mg g^{-1} lipid	trans-10, cis-12 CLA mg g^{-1} lipid	Experimental total CLA mg g^{-1} lipid	Predicted total CLA mg g^{-1} lipid
1	PFF**	1	0	0.1	1	43	14	4.1 ± 0.11	0.2 ± 0.13	4.3 ± 0.14	4.3
2	PFF	3	0	0	2	30	14	4.4 ± 0.17	ND	4.4 ± 0.17	4.4
3	PFF	3	2	0	1	43	1	4.6 ± 0.09	0.8 ± 0.05	5.4 ± 0.07	5.3
4	PFS*	3	2	0.1	1	30	14	4.5 ±0.11	0.2 ±0.14	4.7 ± 0.14	4.7
5	PFF	1	2	0.1	2	30	1	4.6 ± 0.14	1.1 ± 0.12	5.7 ± 0.05	5.6
6	PFS	3	0	0.1	2	43	1	4.8 ± 0.10	ND§	4.8 ± 0.10	4.8
7	PFS	1	2	0	2	43	14	5 ± 0.09	ND	5.0 ± 0.09	4.9
8	PFS	1	0	0	1	30	1	5.5 ± 0.07	ND	5.5 ± 0.07	5.4

*PFS: *Propionibacterium freudenreichii* ssp. *shermanii* (PS4) (code-1).
**PFF: *Propionibacterium freudenreichii* ssp. *freudenreichii.*
§ND: The amount was less than detection limit.
CLA: conjugated linoleic acid.

Table 2. Main process variables, range and 17 trials of central composite design to study the impact of main and interaction effects on optimisation of microbial production of CLA in synbiotic yogurt.

Run	X1 – Milk fat % (w/w)	X2 – Inulin % (w/v)	X3 – Storage time (days)	cis-9,trans-11 CLA (mg g^{-1} lipid)	trans-10,cis-12 CLA (mg g^{-1} lipid)	Total CLA (mg g^{-1} lipid)
1	1.00	1	1	4.2	ND	4.2
2	3.50	1	1	4	0.1	4.1
3	1.00	3	1	4.1	ND*	4.1
4	3.50	3	1	3.9	0.1	4.0
5	1.00	1	21	5	0.3	5.3
6	3.50	1	21	4.9	0.2	5.1
7	1.00	3	21	4.9	0.9	5.8
8	3.50	3	21	4.7	0.5	5.2
9	1.00	2	11	5.2	0.4	5.6
10	3.50	2	11	4.9	0.4	5.3
11	2.25	1	11	4.8	0.6	5.4
12	2.25	3	11	5.1	0.4	5.5
13	2.25	2	1	4.4	ND	4.4
14	2.25	2	21	5.2	0.6	5.8
15	2.25	2	11	5.2	0.8	6.0
16	2.25	2	11	5.4	0.5	5.9
17	2.25	2	11	5.1	0.7	5.8

*ND: The amount was less than detection limit.
CLA: conjugated linoleic acid.

In this study, Student's *t*-test was conducted to demonstrate significance of each factor (*t*-value = coefficient/standard error (S_b)) (Khosravi-Darani and Zoghi, 2008). The tabulated *t*-value (degree of freedom = 6) at $P \leq 0.05$ was 1.94. Each variable linked to *t*-value higher than the tabulated *t*-value (1.94 for $P \leq 0.05$) was significant. Table 3 refers to statistical calculations of CLA production in yogurt samples by PAB. Results established that MFP, prebiotic (inulin) concentration and storage time at 4°C were significant due to their *t*-values being higher than 1.94. Based on Table 3, addition of 2% (w/v) inulin to yogurts increased the production of CLA. This increase might be due to the prebiotic role of inulin, which was an important factor in growth and maintenance of probiotics and caused longer survival of *P. freudenreichii* during the storage period at 4°C as well as greater production of CLA in yogurt. Mohanty *et al.* (2018) reported that prebiotics, especially inulin, were good candidates of functional foods. Salem *et al.* (2007) demonstrated that addition of 1% inulin to dairy cheese promoted growth and longer survival of existing strains. In another study done by Effat et al. (2019), it was reported that addition of 1–3% prebiotics, such as inulin, to milk increased survival and viability of the probiotic *Propionibacterium* strains. Table 3 shows that storage of yogurt containing *P. freudenreichii* at 4°C for 14 days increased the production of CLA. The PAB may adapt and survive at acidic pH of 2 (Van Wyk *et al.*, 2018). Owing to the fact that yogurt samples containing *P. freudenreichii* had pH higher than 2, *P. freudenreichii* was able to grow and produce CLA during the storage time. Akalin *et al.* (2007) reported increase in CLA production in yogurts during storage for 28 days. In addition, results in Table 3 indicate that yogurt samples containing 1% fat (w/w) with *P. freudenreichii* increased CLA production. Biohydrogenation pathway is also a mechanism for the formation of CLA in yogurts (Ha *et al.*, 1989). In order to convert LA to CLA in this pathway, LA isomerase plays an important role. Starter cultures, such as PAB, did not affect CLA formation without presence of LA. Increase in the proportion of milk fat and LA in yoghurt with *P. freudenreichii* increased production of CLA. Kishino *et al.* (2002) found that *Lactobacillus plantarum* AKU 1009a could produce high content of CLA (3.88 mg mL^{-1}) in nutrient media with 0.06% (w/v) LA. Khosravi-Darani *et al.* (2014) reported that CLA content in probiotic yogurts containing PAB increased by 40% from average 8.01 mg g^{-1} fat in non-treated yogurts to 11.03 mg g^{-1} fat in probiotic yogurts containing grape seed oil as a source of LA.

Optimisation of CLA production using response surface methodology

After selecting the most important affecting factors, central composite design and RSM method were used to

Table 3. Statistical data for analysis of variance of CLA production in yogurt by PAB.[a]

Factors	Coefficient	*t*-value
A (Strains)	–0.025	–0.35
B (Milk fat (%) w/w)	–0.150	–2.14
C (Inulin (%) w/v)	0.225	3.14
D (Sunflower oil, g/L)	–0.100	–1.42
E (Inoculum size, %)	0	0
F (Temperature, °C)	–0.1000	–1.42
G (Storage time, days)	0.375	5.28

[a]A_0 = 4.9 (mean of experimental CLA), standard error, S_b = 0.07, estimated error, S^2_e = 0.04, tabulated *t*-value (degree of freedom 6) at $P \leq 0.05$ is 1.94.
CLA: conjugated linoleic acid; PAB: propionic acid bacteria.

optimise the three factors (MFP, prebiotic concentration and storage time at 4°C). Design matrix for these factors in optimisation sets is described in Table 2. Results of RSM in the form of ANOVA are provided in Table 4. $P < 0.05$ demonstrates that the model terms are significant. The ANOVA results established that quadratic regression for the production of CLA by *P. freudenreichii* ssp. *shermanii* in yogurt models was significant. The lack-of-fit test was insignificant ($P = 0.314$) and only 1.8% of the total variations were not explained by the model (R^2 = 98.2%). The quadratic model was based on Eq. (1):

$$Y = 2.626 + 0.741X_1 + 1.001X_2 + 0.187X_3 - 0.155 (X_1)^2 - 0.242 (X_2)^2 - 0.005 (X_3)^2 - 0.04X_1X_2 - 0.006X_1X_3 + 0.01X_2X_3, \quad (1)$$

where Y, X_1, X_2 and X_3 were equivalent experimental response, MFP, inulin concentration and storage time at 4°C, respectively. Effects of various levels of variables on CLA production in yogurts by *P. freudenreichii* ssp. *shermanii* can be achieved using Eq. (1). Based on *t*-test and *P*-value, Table 4 shows that MFP, inulin concentration and storage time at 4°C significantly affected production of CLA, while the three affecting factors were not significant ($P \leq 0.05$).

Effects of inulin and milk fat percentage on CLA production

Figure 1 shows the effects of MFP, concentration of inulin and storage time at 4 °C in yogurt on production of CLA by *P. freudenriechii* in surface plots. In surface plot, response is plotted for two independent variables at a time, while other variables are fixed. Quantities of fat and free LA in milk and presence of inulin play important roles in survival of probiotic bacteria such as PAB as well as production of CLA in yogurts (Akalin *et al.*, 2007; Xu *et al.*, 2005). Figure 1a shows that increase in MFP up to 2.1% (w/w) increased production of CLA in yogurt by *P. freudenreichii* ssp. *shermanii*; however, production of CLA decreased at higher fat proportions. Results were similar to those established by Wang *et al.* (2007), who reported that the maximum production of CLA (78.8 μg mL^{-1}) was produced by *P. freudenreichii* ssp. *shermanii* in MRS media containing 12 mg mL^{-1} of sunflower oil as a source of LA. However, production of CLA decreased at higher concentrations of sunflower oil. Wang *et al.* (2007) demonstrated that at 9.6 mg mL^{-1} sunflower oil in SLM media, 73.9 μg mL^{-1} CLA was produced by *P. freudenriechii*. Again, the concentration of CLA decreased significantly when concentration of oil was higher than 9.6 mg mL^{-1}.

Table 4. Analysis of variance results for CLA production in yogurt by *P. freudenreichii* ssp. *shermanii*.

Source of variation	Degree of freedom	Sum of squares	Mean square	*P*
Regression	9	7.839	0.871	0.000
Linear	3	4.290	0.769	0.000
Square	3	3.404	1.256	0.000
Interaction	3	0.145	0.048	0.157
Lack of fit	5	0.123	0.024	0.314
Pure error	2	0.020	0.010	
Total	16	7.982		

Factors	Degree of freedom	Coefficient estimate	Standard error	*P*
Intercept	1	2.626	0.391	0.000
X_1	1	0.741	0.270	0.029
X_2	1	1.001	0.368	0.030
X_3	1	0.187	0.023	0.000
X_1^2	1	–0.155	0.055	0.027
X_2^2	1	–0.242	0.087	0.027
X_3^2	1	–0.005	0.000	0.000
X_1X_2	1	–0.040	0.040	0.356
X_1X_3	1	–0.006	0.004	0.182
X_2X_3	1	0.010	0.005	0.088

CLA: conjugated linoleic acid; PAB: propionic acid bacteria.

Nieman (1954) reported that free fatty acids disrupted permeability of cytoplasmic membranes in gram-positive bacteria and negatively affected the production of CLA. Wang *et al.* (2007) reported antibacterial activity of LA. Other studies have demonstrated that free fatty acids have negative and inhibitory effects on production of CLA by bacteria such as *Lactobacillus plantarum*, *P. freudenreichii* and *Lactobacillus* spp. (Alonso *et al.*, 2003; Lin, 2000; Lin *et al.*, 1999). As shown in Fig. 1a, production of CLA by *P. freudenreichii* ssp. *shermanii* in yogurts increased with increase in the concentration of inulin to nearly 2% (w/v). Increase in concentration

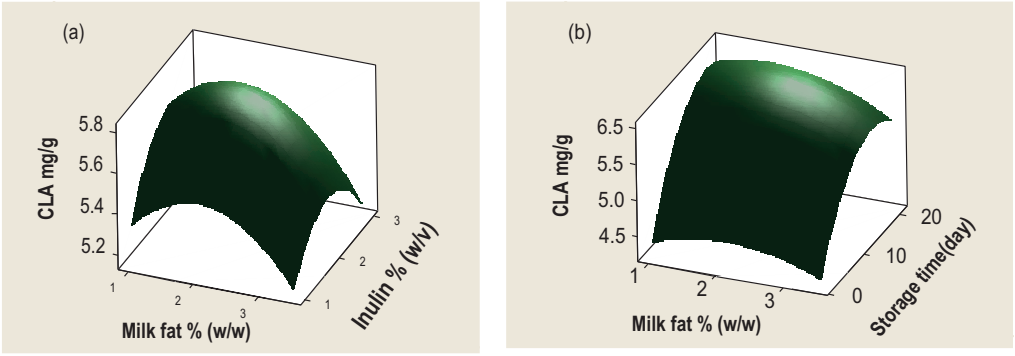

Figure 1. Surface plot of interactive effect on CLA production in yogurt by *P. freudenreichii* ssp. *shermanii*. (a) Effect of inulin and milk fat percentage; (b) effect of storage time of yogurt at 4°C and milk fat percentage.

of inulin by 2% (w/v) or more decreased production of CLA. The figure also shows that high concentrations of inulin had negative effects and decreased the production of CLA. Addition of high concentrations of inulin to yogurts favoured further survival of yogurt starter culture bacteria, resulting in greater decrease in yogurt pH. At lower pH, probiotic bacteria, such as PAB, have less ability to grow and function and hence CLA production decreases by these bacteria. Results of this study are similar to the results of a study done by Effat *et al.*(2019), who reported that increasing inulin concentration in yogurts from 3% to 5% decreased survival rate of probiotic bacteria.

In another study performed by Akalin *et al.* (2007), significant increase in CLA levels was reported when fructooligosaccharides (FOS) were added to yogurts and a 2.90-fold increase was observed in total CLA production in yogurts manufactured with 2% FOS using *Bifidobacterium animalis*.

Effects of yogurt storage time at 4°C and MFP on CLA production

Figure 1b shows that at a constant MFP, production of CLA in yogurts increased with increasing storage time at 4°C. Increase in the concentration of CLA continued until day 16 of storage of yogurt at 4°C, and then concentration of CLA decreased mildly. Studies have been conducted on the effects of yogurt storage time at 4°C on CLA production by different probiotics with various results. The results obtained by Boylston and Beitz (2002) indicated no significant change in yogurts' CLA content during storage for 7 days. In another study, Shantha *et al.* (1995) also showed stability in yogurts' c9t11-CLA isomer concentration at refrigerated storage for 42 days. In a study done by Akalin *et al.* (2007), relative decrease was reported in the concentration of c9t11-CLA isomer after 28 days. The major reason for decrease in yogurts' CLA concentration at storage time included oxidative

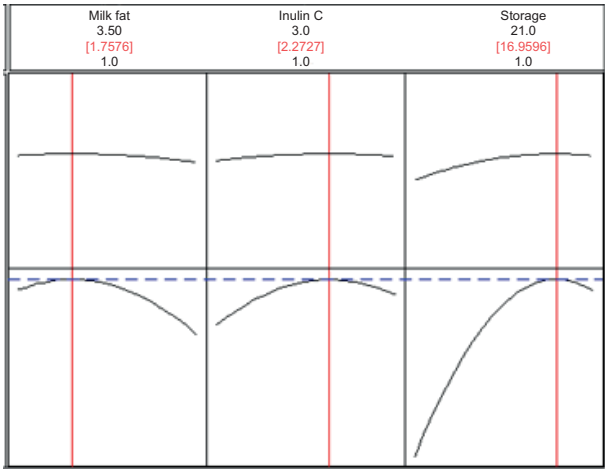

Figure 2. Optimisation plot of CLA production in yogurt by *P. freudenreichii* ssp. *shermanii*.

reactions that caused destruction of conjugated double bond system. Figure 2 points the best conditions for the production of CLA in yogurts by *P. freudenreichii* ssp. *shermanii*. The best values for the three variables of MFP (X_1), inulin concentration (X_2) and storage time at 4°C (X_3) included 1.75% (w/w), 2.27% (w/v) and 17 days, respectively; the highest CLA production by *P. freudenreichii* ssp. *shermanii* was seen in yogurts containing inulin (X_2).

Verification of the model

To verify the model, yogurt samples were prepared under optimal conditions of MFP (1.75% w/w), inulin concentration (2.27% w/v) and storage time at 4°C (~17 days) in three replicates, and the quantity of CLA in yogurts containing *P. freudenreichii* ssp. *shermanii* under optimal conditions was compared with two other yogurt samples from Section 2.4. The highest quantity of CLA included 6.4 ± 0.2 mg g^{-1} lipid. Model and regression didn't establish significant lack of fit between experiments and

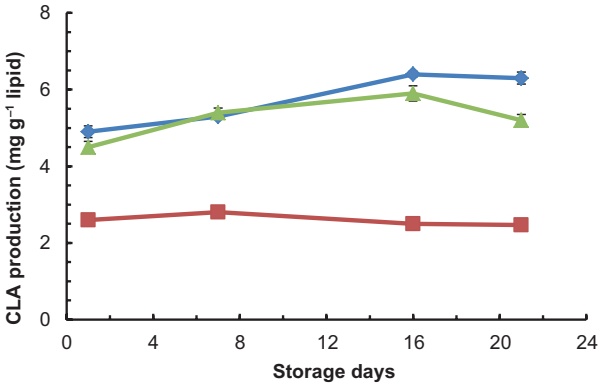

Figure 3. CLA production during storage of yogurt samples at 4°C. Yogurts: YC (■), YC + PS4 (▲), YC + PS4 + inulin (◆).

predicted values of CLA production by *P. freudenreichii* ssp. *shermanii* in yogurts (6.70 mg g^{-1} lipid; Fig. 3).

As seen in Fig. 3, the highest production rate of CLA occurred in yogurt samples containing *P. freudenreichii* ssp. *shermanii*, compared with control yogurt within the first 24 h of storage. This production rate of CLA was equal to 4.9 and 4.5 mg g^{-1} lipid, respectively, for the yogurt samples of *P. freudenreichii* ssp. *shermanii* and inulin and those without inulin, while this value of CLA was 2.6 mg g^{-1} lipid for control yogurts. Similar results were reported in a study done by Wang *et al.* (2007), which resulted in the highest production of CLA in three culture media of SLM, MRS and skim-milk at 24 h. Figure 3 shows that on early days of storage of yogurt samples (up to day 6), no significant differences were seen in the production of CLA by *P. freudenreichii* ssp. *shermanii* for yogurt samples (with or without inulin), with 189% and 191% increase in production of CLA, respectively, compared with control yogurt samples. On day 16 of storage, quantity of CLA in yogurts containing inulin reached to 6.4 mg g^{-1} lipid, increasing by 256%, compared with control samples. For yogurts without inulin, this increase was 239%. In yogurt samples containing *P. freudenreichii* and inulin, decreased concentration of CLA was observed after day 16, similar to the results of optimisation shown in Fig. 2. As previously stated, oxidative reactions that destroyed conjugated double bond systems were the major reasons for decrease in CLA concentration.

Microbiological viable count analysis

Bacterial count results of the three yogurt samples produced using the co-culture method during 3 weeks are compared with each other in Table 5. Viable counts of *S. thermophilus* in control yogurt samples without inulin during 21 days of storage decreased from 9.41 log CFU mL^{-1} on day 1 to 8.70 log CFU mL^{-1} on day 21. In this yogurt sample, a decrease in *L. delbrueckii* ssp. *bulgaricus* was seen from 8.19 log CFU mL^{-1} to 5.87 log CFU mL^{-1}, which was much higher for *L. delbrueckii* ssp. *bulgaricus* than for *S. thermophilus* in all samples. Low storage temperatures and over acidification have been reported for this decrease (Ekinci and Gurel, 2008).

Table 5. Viable cell count of starter cultures in fermented skim-milk during 21 days of storage at 4°C.[a]

Viable count	Storage time (days)	YC (log CFU mL^{-1})	YC + PS4 (log CFU mL^{-1})	YC + PS4 + Inolin (log CFU mL^{-1})
Streptococcus thermophilus	1	9.41 ± 0.02	9.43 ± 0.02	9.35 ± 0.04
	7	9.08 ± 0.03	9.42 ± 0.04	9.19 ± 0.07
	16	9.04 ± 0.06	9.31 ± 0.01	9.27 ± 0.02
	21	8.70 ± 0.05	8.76 ± 0.01	8.64 ± 0.1
Lactobacillus delbrueckii ssp. *bulgaricus*	1	8.19 ± 0.08	8.04 ± 0.04	8.32 ± 0.05
	7	7.12 ± 0.09	7.12 ± 0.09	8.02 ± 0.09
	16	6.43 ± 0.03	7.08 ± 0.06	7.07 ± 0.01
	21	5.87 ± 0.04	6.19 ± 0.02	6.08 ± 0.05
P. freudenreichii ssp. *shermanii*	1	—	9.18 ± 0.04	9.32 ± 0.5
	7	—	7.97 ± 0.01	9.05 ± 0.03
	16	—	6.16 ± 0.05	8.00 ± 0.01
	21	—	5.98 ± 0.08	6.33 ± 0.09

[a]Mean ± standard deviation (SD).
YC: yogurt starter culture containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* ssp. *bulgaricus*.
YC + PS4: yogurt starter culture containing *Streptococcus thermophiles, Lactobacillus delbrueckii* ssp. *bulgaricus* and *P. freudenreichii* ssp. *shermanii*.
YC + PS4 + inulin: yogurt starter culture containing *Streptococcus thermophiles, L. delbrueckii* ssp. *bulgaricus, P. freudenreichii* ssp. *shermanii* and 2.25% inulin added to yogurt sample.
CFU: colony-forming unit.

Similar results are reported from other studies (Ekinci and Gurel, 2008; Güler-Akin and Akin, 2007; Ranadheera *et al.*, 2012). Results presented in Table 5 indicate that a similar decrease was observed in the viable count of *S. thermophiles* and *L. delbrueckii* ssp. *bulgaricus* during 21 days of storage in yogurt containing *P. freudenreichii* ssp. *shermanii* (YC and PS4). This decrease was less pronounced for *L. delbrueckii* ssp. *bulgaricus.* Results demonstrated that addition of *P. freudenreichii* ssp. *shermanii* to yogurt samples did not affect negatively yogurt starter cultures (ST and LB). Comparison of yogurt samples with and without inulin demonstrated that presence of prebiotics, such as inulin, could significantly affect the count number of *P. freudenreichii* ssp. *shermanii*. As shown in Table 5 for yogurts containing inulin (YC, PS4 and inulin), number of *P. freudenreichii* ssp. *shermanii* after 16 days of storage was 8.00 log CFU mL^{-1}. In yogurts without inulin, this value was 6.16 log CFU mL^{-1}. Other studies (Capela *et al.*, 2006; Oliveira *et al.*, 2009) have reported positive effects of prebiotics, such as polydextrose, oligofructose and maltodextrin, on the survival of probiotics.

Conclusions

In this study, *P. freudenreichii* ssp. *shermanii* was used with traditional yogurt starter cultures (ST and LB) to enrich and produce CLA in yogurts. Results from PBD design demonstrated that three factors of MFP, inulin concentration and storage time at 4°C significantly affected CLA production in yogurts by *P. freudenreichii* ssp. *shermanii* using RSM design and optimising conditions of the three highlighted factors. The highest production of CLA (6.4 mg g^{-1} lipid) in yogurts was achieved with 1.75% (w/w) of fat, 2.25% (w/v) of inulin and 17 days of storage at 4°C, establishing a 256% increase in total CLA production compared with control yogurt samples. In conclusion, results of this study have revealed that *P. freudenreichii* ssp. *shermanii* exerts no negative effects on growth of yogurt starter cultures, and inulin could be beneficial for further survivals of *P. freudenreichii*.

Acknowledgements

This research was supported by student research committee of Shahid Beheshti University of Medical Sciences and National Nutrition and Food Technology Research Institute.

Conflict of interest

There was no conflict of interest to declare.

Compliance with ethical standards

The authors do not have any kind of interests. Research does not involve Human Participants and/or Animals. Informed consent is not applicable.

Funding

This study is related to the project NO.1397/75698 From Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran. We also appreciate the "Student Research Committee" and "Research & Technology Chancellor" in Shahid Beheshti University of Medical Sciences for their financial support of this study.

References

Abou Ayana I.A., El-Deeb A.M. and Ibrahim A.E. 2016. Research article using of dairy propionibacteria as bio-preservative in Kareish cheese. International Journal of Dairy Science 11: 114–123. https://doi.org/10.3923/ijds.2016.114.123

Ahmadi N., Khosravi-Darani K., Zarean-Shahraki S., Mortazavian M. and Mashayekh S. 2015. Fed-batch fermentation for propionic, acetic and lactic acid production. Oriental Journal of Chemistry 31: 581–590. https://doi.org/10.13005/ojc/310174

Akalın A., Tokuşoğlu Ö., Gönç S. and Aycan Ş. 2007. Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructooligosaccharide. Intnational Dairy Journal 17: 1089–1095. https://doi.org/10.1016/j.idairyj.2007.02.005

Alonso L., Cuesta E. and Gilliland S. 2003. Production of free conjugated linoleic acid by Lactobacillus acidophilus and Lactobacillus casei of human intestinal origin. International Journal of Dairy Science 86: 1941–1946. https://doi.org/10.3168/jds.S0022-0302(03)73781-3

Balci Yuce H., Akbulut N., Ocakli S., Kayir O. and Elmastas M. 2017. The effect of commercial conjugated linoleic acid products on experimental periodontitis and diabetes mellitus in Wistar rats. Acta Odontologica Scandinavica 75: 21–29. https://doi.org/10.1080/00016357.2016.1244355

Boylston T. and Beitz D. 2002. Conjugated linoleic acid and fatty acid composition of yogurt produced from milk of cows fed soy oil and conjugated linoleic acid. Journal of food science 67: 1973–1978. https://doi.org/10.1111/j.1365-2621.2002.tb08755.x

Capela P., Hay T. and Shah N.P. 2006. Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. International Food Research Journal. 39: 203–211. https://doi.org/10.1016/j.foodres.2005.07.007

Corbo M.R., Bevilacqua A., Petruzzi L., Casanova F.P. and Sinigaglia M. 2014. Functional beverages: the emerging side of functional foods: commercial trends, research, and health implications. Comprehensive Reviews in Food Science and Food Safety 13: 1192–1206. https://doi.org/10.1111/1541-4337.12109

Cousin F.J., Jouan-Lanhouet S., Théret N., Brenner C., Jouan E., Le Moigne-Muller G., Dimanche-Boitrel M.-T. and Jan G. 2016. The probiotic propionibacterium freudenreichii as a new adjuvant for TRAIL-based therapy in colorectal cancer. Oncotarget 7: 7161. https://doi.org/10.18632/oncotarget.6881

Dave R. and Shah N. 1996. Evaluation of media for selective enumeration of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, and bifidobacteria. International Journal of Dairy Science 79: 1529–1536. https://doi.org/10.3168/jds.S0022-0302(96)76513-X

Effat B.A., Ibrahim M.K., Tawfik N.F., Mehanna N.S. and Soliman N.R. 2019. Effect of different prebiotics on survival and viability of some dairy propionibacteria. International Journal of Advanced Research in Biological Sciences 6: 99–104. http://dx.doi.org/10.22192/ijarbs.2019.06.08.014

Ekinci F. and Gurel M. 2008. Effect of using propionic acid bacteria as an adjunct culture in yogurt production. International Journal of Dairy Science 91: 892–899. https://doi.org/10.3168/jds.2007-0244

Farhadi S., Khosravi-Darani K., Mashayekh M., Mortazavian A., Mohammadi A. and Shahraz F. 2012. Effect of incubation temperature and inoculation ratio of starter culture on propionic acid production in dairy beverage fermented with propionibacterium. Iranian Journal of Nutritional. Sciences 7: 41–50.

Fukuda S., Suzuki Y., Murai M., Asanuma N. and Hino T. 2006. Isolation of a novel strain of Butyrivibriofibrisolvens that isomerizes linoleic acid to conjugated linoleic acid without hydrogenation, and its utilization as a probiotic for animals. Journal of Applied Microbiology 100: 787–794. https://doi.org/10.1111/j.1365-2672.2006.02864.x

Gorret N., Maubois J., Ghoul M. and Engasser J. 2001. Exopolysaccharide production by Propionibacterium acidipropionici on milk microfiltrate. Journal of Applied Microbiology 90: 779–787. https://doi.org/10.1046/j.1365-2672.2001.01306.x

Grinstead D. and Barefoot S. 1992. Jenseniin G, a heat-stable bacteriocin produced by propionibacterium jensenii P126. Applied and Environmental Microbiology 58: 215–220. https://doi.org/10.1128/AEM.58.1.215-220.1992

Grunert K.G. 2005. Food quality and safety: consumer perception and demand. European Review of Agricultural Economics 32: 369–391. https://doi.org/10.1093/eurrag/jbi011

Güler-Akın M.B. and Akın M.S. 2007. Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goat's milk. Food Chemistry 100: 788–793. https://doi.org/10.1016/j.foodchem.2005.10.038

Ha Y.L., Grimm N.K. and Pariza M.W. 1989. Newly recognized anticarcinogenic fatty acids: identification and quantification in natural and processed cheeses. Journal of Agricultural and Food Chemistry 37: 75–81. https://doi.org/10.1021/jf00085a018

Hennessy A.A., Barrett E., Ross R.P., Fitzgerald G.F., Devery R. and Stanton C. 2012. The production of conjugated α-linolenic, γ-linolenic and stearidonic acids by strains of bifidobacteria and propionibacteria. Lipids 47: 313–327. https://doi.org/10.1007/s11745-011-3636-z

Hernandez E. 2013. Enrichment of baked goods with omega-3 fatty acids. In: "Food enrichment with omega-3 fatty acids". C. Jacobsen, N. S. Nielsen, A. Frisenfeldt Horn and A-D. Moltke Sorensen (Ed), Elsevier, pp. 319–335. Woodhead Publishing, Cambridge. https://doi.org/10.1533/9780857098863.3.319

Holzapfel W.H. and Schillinger U. 2002. Introduction to pre-and probiotics. International Food Research Journal.. 35: 109–116. https://doi.org/10.1016/S0963-9969(01)00171-5

Khan M.M., Mir N.A. and Khan M.M. 2011. Production of vitamin B12 by improved strains of propionibacterium freudenreichii. Biotechnology & Bioengineering 1: 19–24. http://dx.doi.org/10.1016/j.ejbt.2016.07.004

Khodaiyan F., Razavi S.H. and Mousavi S.M. 2008. Optimization of canthaxanthin production by Dietzianatronolimnaea HS-1 from cheese whey using statistical experimental methods. Biochemical Engineering Journal 40: 415–422. https://doi.org/10.1016/j.bej.2008.01.016

Khosravi-Darani K. and Zoghi A. 2008. Comparison of pretreatment strategies of sugarcane baggase: experimental design for citric acid production. Bioresource Technology 99: 6986–6993. https://doi.org/10.1016/j.biortech.2008.01.024

Kim Y.J. 2003. Partial inhibition of biohydrogenation of linoleic acid can increase the conjugated linoleic acid production of butyrivibrio fibrisolvens A38. Journal of Agricultural and Food Chemistry 51: 4258–4262. https://doi.org/10.1021/jf034057r

Kim Y.J., Liu R.H., Bond D.R. and Russell J.B. 2000. Effect of linoleic acid concentration on conjugated linoleic acid production by butyrivibrio fibrisolvens A38. Applied and Environmental Microbiology 66: 5226–5230. https://doi.org/10.1128/AEM.66.12.5226-5230.2000

Kishino S., Ogawa J., Omura Y., Matsumura K. and Shimizu S. 2002. Conjugated linoleic acid production from linoleic acid by lactic acid bacteria. Journal of the American Oil Chemists' Society 79: 159-163. https://doi.org/10.1007/s11746-002-0451-4

Kouya T., Tobita K., Horiuchi M., Nakayama E., Deguchi H., Tanaka T. and Taniguchi M. 2008. Production of extracellular bifidogenic growth stimulator (BGS) from propionibacterium shermanii using a bioreactor system with a microfiltration module and an on-line controller for lactic acid concentration. Journal of Bioscience and Bioengineering 105: 184–191. https://doi.org/10.1263/jbb.105.184

Lin T.Y. 2000. Conjugated linoleic acid concentration as affected by lactic cultures and additives. Food Chemistry 69: 27–31. https://doi.org/10.1016/S0308-8146(99)00218-6

Lin T.Y., Lin C.-W. and Lee C.-H. 1999. Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. Food Chemistry 67: 1–5. https://doi.org/10.1016/S0308-8146(99)00077-1

Masso-Welch P.A., Zangani D., Ip C., Vaughan M.M., Shoemaker S.F., McGee S.O. and Ip M.M. 2004. Isomers of conjugated linoleic acid differ in their effects on angiogenesis and survival of mouse mammary adipose vasculature. Journal of Nutrition 134: 299–307. https://doi.org/10.1093/jn/134.2.299

Mohanty D., Misra S., Mohapatra S. and Sahu PS. 2018. Prebiotics and synbiotics: Recent concepts in nutrition. Food bioscience 26: 152-160. https://doi.org/10.1016/j.fbio.2018.10.008

Nieman C. 1954. Influence of trace amounts of fatty acids on the growth of microorganisms. Bacteriological Reviews 18: 147.

Ogawa J., Matsumura K., Kishino S., Omura Y. and Shimizu S. 2001. Conjugated linoleic acid accumulation via 10-hydroxy-12-octadecaenoic acid during microaerobic transformation of linoleic acid by Lactobacillus acidophilus. Applied and Environmental Microbiology 67: 1246–1252. https://doi.org/10.1128/AEM.67.3.1246-1252.2001

Oliveira R.P., Florence A.C., Silva R.C., Perego P., Converti A., Gioielli L.A. and Oliveira M.N. 2009. Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk. International Journal of Food Microbiology 128: 467–472. https://doi.org/10.1016/j.ijfoodmicro.2008.10.012

Olson J.M., Haas A.W., Lor J., McKee H.S. and Cook M.E. 2017. A comparison of the anti-inflammatory effects of cis-9, trans-11 conjugated linoleic acid to celecoxib in the collagen-induced arthritis model. Lipids. 52: 151–159. https://doi.org/10.1007/s11745-016-4228-8

Pandey S.M. and Mishra H. 2015. Optimization of the prebiotic & probiotic concentration and incubation temperature for the preparation of synbiotic soy yoghurt using response surface methodology. Food Science 62: 458–467. https://doi.org/10.1016/j.lwt.2014.12.003

Rad A.H., Khosroushahi A.Y., Khalili M. and Jafarzadeh S. 2016. Folate bio-fortification of yoghurt and fermented milk: a review. Dairy Science & Technology 96: 427–441. https://doi.org/10.1007/s13594-016-0286-1

Ranadheera C.S., Evans C., Adams M. and Baines S. 2012. Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. Food Chemistry 135: 1411–1418. https://doi.org/10.1016/j.foodchem.2012.06.025

Ross R., Mills S., Hill C., Fitzgerald G. and Stanton C. 2010. Specific metabolite production by gut microbiota as a basis for probiotic function. International Dairy Journal 20: 269–276. https://doi.org/10.1016/j.idairyj.2009.12.003

Salem MM., El-Gawad A., Hassan FA. and Effat BA. 2007. Use of synbiotics for production of functional low fat Labneh. Polish Journal of Food and Nutrition Sciences 57: 151-159.

Shantha NC., RAM LN., O'leary J., HICKS CL. and DECKER EA. 1995. Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. Journal of Food Science 60: 695-697. https://doi.org/10.1111/j.1365-2621.1995.tb06208.x

Tharmaraj N. and Shah N. 2003. Selective enumeration of Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus, and propionibacteria. Journal of Dairy Science 86: 2288–2296. https://doi.org/10.3168/jds.S0022-0302(03)73821-1

Van Wyk J., Morkel R.A. and Dolley L. 2018. Metabolites of propionibacterium: techno-and biofunctional ingredients. Alternative and Replacement Foods. Elsevier, pp. 205–260. https://doi.org/10.1016/B978-0-12-811446-9.00008-3

Wang L.-M., Lv J.-P., Chu Z.-Q., Cui Y.-Y. and Ren X.-H. 2007. Production of conjugated linoleic acid by propionibacterium freudenreichii. Food Chemistry 103: 313–318. https://doi.org/10.1016/j.foodchem.2006.07.065

Xu S., Boylston T.D. and Glatz B.A. 2004. Effect of lipid source on probiotic bacteria and conjugated linoleic acid formation in milk model systems. Journal of the American Oil Chemists' Society 81: 589–595. https://doi.org/10.1007/s11746-006-0946-z

Xu S., Boylston T.D. and Glatz B.A. 2005. Conjugated linoleic acid content and organoleptic attributes of fermented milk products produced with probiotic bacteria. Journal of Agricultural Food Chemistry 53: 53(23): 9064–9072. https://doi.org/10.1021/jf051030u

Yang B., Gao H., Stanton C., Ross R.P., Zhang H., Chen Y.Q., Chen H. and Chen W. 2017. Bacterial conjugated linoleic acid production and their applications. Progress in Lipid Research 68: 26–36. https://doi.org/10.1016/j.plipres.2017.09.002

Zárate G. 2012. Dairy propionibacteria: less conventional probiotics to improve the human and animal health. In: "Probiotic in Animals". Rigobelo E (Ed), pp 153–202. InTech, Rijeka. https://doi.org/10.5772/50320

Zárate G., Babot J., Argañaraz-Martinez E., Lorenzo-Pisarello M. and Perez Chaia A. 2011. Dairy propionibacteria: technological importance and probiotic potential for application on human and animal nutrition. In: Filip Rosana, editor. Paper presented at the Multidisciplinary Approaches on Food Science and Nutrition for the XXI Century Research Signpost. Trivandrum-695-023, Kerala, India: Transworld Research Network India, pp. 75–213.