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Lactobacillus plantarum as source of conjugated linoleic acid: Effect of pH, incubation Temperature and inulin incorporation

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Abstract

The effect of pH and temperature, and inulin use, on the growth and the fatty acid profile of *Lactobacillus plantarum* strain were evaluated. The best results were obtained at 6.5 pH broth, producing 3.2 g/L of biomass and about 20% of conjugated linoleic acid (CLA) in the cell lipids. Similar growth was observed with 37 and 45ºC, but a low CLA content (10.6%) was achieved at 45°C. In the case of inulin incorporation, a low biomass concentration (1 g/L) and low production of CLA (12.4%) were observed. These results suggest a pH and temperature dependence on CLA production by the microorganism.

Keywords: *Lactobacillus plantarum; CLA; oil supplementation; temperature; pH; inulin*

Introduction

Bacteria such as *Lactobacillus* are used in the food industry to produce fermentable vegetable; also, they are recognized as probiotics (Maragkoudakis et al. 2006). Additionally some of these microorganisms are capable of producing conjugated linoleic acid (CLA) (Ogawa et al. 2005). CLA is an isomer of linoleic acid, with two double bonds, which are in *cis* or *trans* configuration. Despite this, CLA is considered a good fat and is used in the prevention and control of cardiovascular disease, high cholesterol and cancer treatment, among others (Bhattacharya et al. 2006).

According to Ogawa et al. (2005) report at least nine strains of *Bifidobacterium*, five of *Lactobacillus*, one of *Propionobacterium* and one of *Megasphaera* have been used for CLA production, by means of different reaction methods and substrate kinds, obtaining CLA mixtures. When Kishino et al. (2002) studied the conversion of LA to CLA using lactic bacteria, a *Lactobacillus plantarum* (AKU 1009a) strain produce the greater amount of CLA (40 mg/mL) after 108 hours of culture. Dong and Qi (2006) evaluated CLA production feasibility using *Lactobacillus acidophilus* 11854 strains; an increment of CLA content was appreciated up to pH and temperature values of 6.4 and 37ºC respectively, then the fatty acid concentration decay. CLA was formed after the cultures reached the stationary phase (Alonso et al. 2003). Important variables in microbial growth are the pH and the temperature of culture, affecting among other things, the specific growth rate and formation of secondary products too (Guerzoni et al. 2001; Mataragas et al. 2003).

Other variable in growth and fatty acid composition is the culture media composition, specially the carbon source. It is recognized that oligosaccharide are able to produce several effects on bacteria as a protective molecule in the preservation (freeze dried or dried) and prebiotic effect (Wrolstad 2012). Also, can produce a effect on secondary products formation as has been reported by Akalin et al. (2006), whom evaluated the effect of fructooligosaccharides (FOS) incorporation in yoghurt when started culture and probiotics microorganism, observing an increment of CLA content.
The aim of this study was to determine the effect of pH and temperature on growth and fatty acid profile of *Lactobacillus plantarum,* when this strain is grown in a supplemented medium with oils rich in linoleic acid. In addition the effect of a prebiotic compound, such as inulin, incorporation was evaluated too.

Materials and Methods

Microbial strain and culture medium

The microorganism *Lactobacillus plantarum* NRRL - B4496 was donated by ARS-USDA. The culture medium used was Man-Rogosa-Sharpe (MRS) media, composed by casein peptone (10 g / L), meat extract (10 g / L), yeast extract (5 g / L), glucose (20 g / L), Tween 80 (1 mL / L), sodium acetate tri-hydrate (5 g / L), ammonium citrate (2 g / L), di-sodium phosphate (2 g / L), sulfate hepta-hydrate magnesium (0.2 g / L), manganese sulfate mono-hydrate (0.5 g / L) plus some micro-elements.

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Culture medium was supplemented with 2.67 mL / L of grape seed oil (59.3% linoleic acid).

Also, glucose of MRS media (as main carbon source) was replaced total or partially by an oligosaccharide as Inulin (Granotec, Chile).

Microbial growth and consumption of carbon source.

Lactobacillus culture was carried out in an aerobic environment by batch, using 150 rpm of agitation. Three pHs were tested: 5.5, 6.5 and 7.5. A phosphate buffer (200 mM) was used to keep the pH of culture media. Also, three incubation temperatures were evaluated 30, 37 and 45ºC. Microbial growth was determined by spectrophotometry, using a calibration curve developed with the same strain, and verified by gravimetry. Additionally, the glucose consumption using a specific enzymatic kit was determined.

Fatty acid profile

Fatty acid profile was determined after reaching a steady state for each experiment. Biomass was harvested, centrifuged and separated from the liquid medium. In the case of biomass lipids, they were trans-methylated from the cell, incorporating a stage of cell mill using micro-glass balls prior to the saponification step with methanolic sodium hydroxide solution, and a step of methylation of fatty acids using a boron trifluoride methanol solution. Methylated fatty acids were recovered with n-hexane and injected into the gas chromatograph.

Fatty acid profile determination was performed using a Perkin Elmer Clarus 600 gas chromatograph with FID detector and a Restek Rtx-2330 column; injector and detector were maintained at 220 °C and 250 °C respectively using temperature gradient in the furnace. Nitrogen was the carrier gas. FAME MIX 37 (Supelco), CLA c9t11 and t10c12 (Sigma Aldrich), and methyl ester of linoleic acid (Supelco) were used as external standards.

Results and Discussion

Figure 1 show the effect of pH on *Lactobacillus plantarum* growth and on the glucose uptake when the culture was supplemented with grape seed oil. As it is possible to observe the maximum cell concentration (3.2 g/L) was the same using either a buffer pH 5.5 or 6.5. After 20 hours of fermentation, *L.plantarum* reached a stationary phase of growth at pH 6.5, whereas at pH 5.5 this phase was achieve after 25 hours. In the case of glucose decrease, a similar behavior was observed in both cases, with a total consumption after 20-23 hours. With regard to the specific growth rate an increase from 0.089 h^{-1} to 0.141 h^{-1} was observed when pH increased from 5.5 to 6.5. When pH of 7.5 was applied growth was not observed.

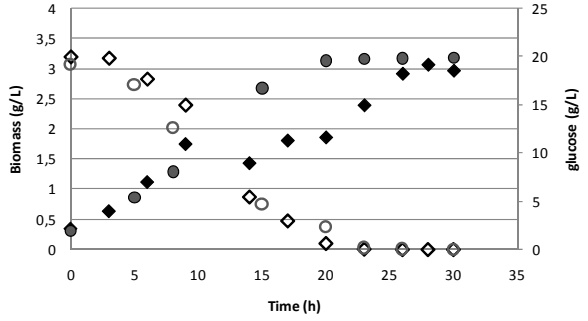

Figure 1: Effect of pH culture on cell growth of *L.plantarum*. Culture conditions: MRS medium supplemented with grape seed oil. 2.67 mL / L; 37ºC; aerobic culture, agitation 150 rpm; pH: 6.5 (○), 5.5 (◊)

As it is possible to observe, *Lactobacillus plantarum* is able to adapt to different pHs. Other strains of *Lactobacillus*, such as *L.curvatus* have a similar behavior (Mataragas et al. 2003), observed that at pHs between 5 and 6.5 produced different growth rates but a similar maximum cell concentration; as well as was determined by LeBlanc et al. (2004) for *Lactobacillus fermentum*.

Table 1 : Effect of pH in fatty acid profile of *L. plantarum* and in the depleted culture media. Incubation conditions:37ºC, broth supplemented with grape seed oil.

Fatty acid	pH 5.5		pH 6.5	
	intracellular	extracellular	intracellular	extracellular
Miristic	2.5%	-	4.2%	4.6%
Palmitic	12.2%	11.3%	13.1%	7.5%
Palmitoleic	-	-	4.1%	3.9%
Estearic	6.9%	13.7%	3.6%	2.9%
Oleic	11.1%	26.7%	31.8%	53.3%
Vaccenic	18.6%	-	6.4%	-
Linoleic	14.7%	27.9%	10.8%	13.9%
CLA	20.7%	-	21.5%	-
others	13.3%	20.5%	4.5%	14.0%

Regarding the effect of pH on fatty acid profile, Table 1 shows its effect on the main extra- and intracellular fatty acids, when *Lactobacillus* growth was performed using grape seed oil. As it is possible to appreciate, vaccenic and CLA fatty acids were observed only inside the microorganism. Similar CLA content was observed when pH 5.5 or 6.5 were used, obtaining about 20% of this fatty acid. In the case of LA a higher content is reported in depleted culture media. It is important to mentioned that LA hydrogenation, by the enzymatic system of the microorganism, have as final product the stearic acid, passing through vaccenic acid generation; also, some ruminal microorganism are able to produce CLA from oleic and vaccenic acids by Δ9-desaturase enzyme (Banni et al. 2001). Due to this fact, these results suggest a mechanism active by oil richest presence and satisfactory growth pH.

These results are agree those reported by Ando et al. (2003), whom indicated that CLA was most efficiently produced with a pH 6.0 sodium citrate buffer, using washed cells of *L. plantarum* JCM 1551. Authors as Dong and Qi (2006) report that LA conversion to CLA using *L. acidophilus* and alfalfa seed oil as LA source is higher at 6.4 pH (50% of conversion). The LA transformation at other pH conditions is low (5-30% of conversion), suggesting that enzymatic system to convert LA to CLA is pH-dependent, producing an inactivation or a structural change of LA isomerase. A Similar conclusion was obtained by Li et al. (2013) when *Lactobacillus acidophilus F0221* and free linoleic acid were used to produce CLA; a good amount of CLA was produced when the initial pH was in the range of 6.0 to 7.0, probably because this range is the most suitable for enzyme linoleic acid isomerase activity. Temperature is an important variable on microbial growth and molecule production. It is recognized that temperature can induce the variation in lipid composition of microorganism as way to maintain the cell membrane functionality (Guerzoni et al. 2001). Some authors (Guerzoni et al. 2001) report that thermal stress (a high temperature, 50ºC, in comparison to 42ºC) in *Lactobacillus helveticus* produces an increase in the most of fatty acids evaluated with the exception of vernolic acid, which is improved at the low temperature (42ºC).
When evaluating the effect for culture at pH 6.5, and under various conditions of temperature, it is possible to see in Figure 2, that 30 °C of incubation temperature produces a specific growth rate of 0.16 h^{-1}, and a low final cell concentration (1.9 g/L). In the case of 37 and 45 ° C, it is appreciated that final cell concentration reached is similar (3.1 g / L), but with different specific growth rates (0.18 h^{-1} for 37ºC and 0.16 h^{-1} for 45ºC). In both cases the steady state is reached about 20 hours of culture, at which time glucose uptake is complete. In this case the higher cell substrate yield is achieved with

37 ° C (0.148 g_{cell} / $g_{glucose}$). Mataragas et al. (2003) report that best growth of *L.curvatus* was produced when 25 and 30ºC was used; however the authors does not evaluated the effect of higher temperature. In the case reported by Ando et al. (2003), when washed cells of *L.plantarum* JCM 1551 and ricinoleic acid were used to produce CLA, the authors indicated that the best results was observed with 42ºC.

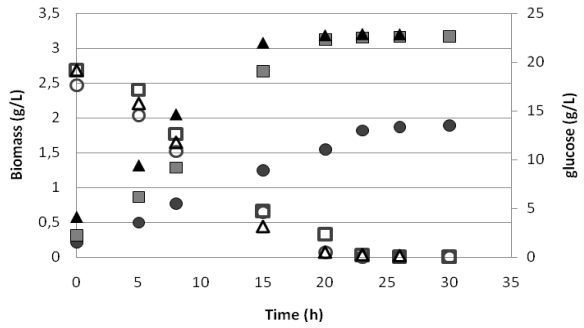

Figure 2: Effect of incubation temperature on cell growth of *L.plantarum*. Culture conditions: MRS medium supplemented with grape seed oil. 2.67 mL / L; pH 6.5; aerobic culture, agitation 150 rpm; temperature: 30ºC (○), 37ºC (□); 45ºC(Δ).

The intra and extracellular fatty acid profiles obtained using grape seed oil and a pH medium of 6.5, shows that the best result of CLA production is obtained with 37 ° C (Table 2). It is also possible to observe that this fatty acid as well as vaccenic acid occurs only in the intracellular profile. Interestingly, the culture medium have grape seed oil, consisting mainly palmitic, oleic, linoleic and linolenic fatty acids, but also have tween 80, having oleic acid; the latter, according to Lin (2006) is able to improve the production of some CLA species too. Dong and Qi (2006) report that *L.acidophilus* produces the best LA conversion to CLA at 37ºC (about 45%), with a strong decrease when the culture was done at 50ºC (only with a 15% of conversion), suggesting that 37ºC is the most suitable temperature for *L.acidophilus* growth and LA isomerase production. On the other hand Li et al. (2013) indicated that *L.acidophilus* F022 incubated in anaerobic conditions, shows the best growth at 37ºC, but CLA production was similar between 25 and 45ºC. The results obtained in this work, as well as those obtained by other authors suggest that the production of CLA depends on multiple factors including temperature and strain used. A consequence of temperature variation is the change in the cell membrane fluidity. Bacterial cell membrane is mainly composed by lipids (40-70%), and the fatty acid profile determined the viscosity of the membrane (Brillard and Broussolle, 2012).

Then, the changes in the composition of the membrane lipids are response to the need to maintain the homeostasis of the system. In addition, some enzymes, responsible of CLA production, such as LA isomerase are anchored to the cell membrane (Kim et al. 2000). Subsequently, an increment in incubation temperature can improve the LA isomerization; but an excessive temperature can promote the inactivation of the enzymes. Also, the increment of temperature can affect the dispersion state of linoleic acid in the culture media (Li et al. 2013).

Prebiotic compounds are non-digestible ingredients that beneficially affect the organism by stimulating the growth and activity of one or more bacteria strains in the colon, thus improving the health (Wrolstad, 2012). Inulin is a group of polysaccharides composed mainly by fructose, and it is a recognized as prebiotic compound. As it is possible to observe in Figure 3, when inulin was used in the culture media of *Lactobacillus plantarum*, a similar behavior was obtained with only glucose (20 $g_{glucose}$/L) and the mixture of glucose plus inulin (10 $g_{glucose}$/L and 10 g_{inulin}/L), reaching up to 3.4 g/L of biomass; however if only inulin is used as main carbon source, the maximum biomass concentration raised after 29 hours of incubation is 1 g/L. This result is according those reported by Goderska et al. (2008), whom report that glucose produce the best growth of *Lactobacillus acidophilus* bacteria in comparison to other sugars and prebiotic as saccharose, lactose, fructose and commercial oligosaccharides. A similar behavior was reported for *Bifidobacterium bifidum*. The result confirms that glucose is easiest metabolizable saccharide.

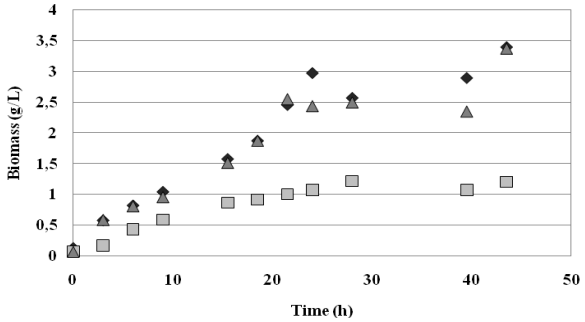

Figure 3: Effect of inulin incorporation in the culture medium of *L. plantarum*, when this is supplemented with 2.6 mL / L of grape seed oil. Incubation conditions: 37°C; pH free (6.5 initial); carbon source: glucose 20 g/L (◊); inulin 20 g/L (□); glucose 10 g/L plus inulin 10 g/L (Δ).

Table 3 shows that in the cultures done with glucose there is compounds with DP 7 or higher, which can be provided by yeast extract or peptone present in culture MRS broth. It is also observed that DP2 compounds are obtained with the incubation time. The glucose consumption is done after 13 hours, whereas when only inulin was used no significant differences were observed until 19 hours. If the mixture of glucose and inulin was used, significant consumption of glucose and the appearance of compounds DP2 were showed again.

The hydrolysis of inulin and the production of fructooligosaccharide can be observed. Kaplan and Hutkins (2003), reports that *L.*

Table 2: Effect of temperature on the fatty acid profile of *L. plantarum* in a medium at pH 6.5 and supplemented with 2.6 mL / L of grape seed oil.

Fatty acid	30°C		37°C		45°C	
	Intracellular	Extracellular	Intracellular	Extracellular	Intracellular	Extracellular
Miristic	2.93%	3.11%	4.22%	4.55%	4.73%	-
Palmític	12.85%	14.85%	13.11%	7.46%	21.76%	7.32%
Palmitoleic	2.91%	2.26%	4.08%	3.88%	1.99%	-
Estearic	3.61%	12.10%	3.61%	2.89%	6.26%	3.10%
Oleic	14.45%	38.09%	31.79%	53.33%	22.18%	29.18%
Vacénic	15.01%	3.59%	6.41%	-	1.88%	-
Linoleic	20.76%	13.76%	10.82%	13.89%	22.23%	43.08%
CLA	6.05%	-	21.50%	-	10.63%	-

paracasei was able to consume FOS, with 2 and 4 fructose units. The microorganism transports the oligosaccharide inside to the cell and then hydrolyzed it. This transportation is quickly for smaller molecules, and some monosaccharide such as glucose and fructose are highly metabolized promoting the inhibition of FOS consumption.

Table 3: Relative carbohydrate consumption in the culture of *L. plantarum* when the fermentation was done at 37ºC, using grape seed oil as linoleic acid source**.**

	Glucose			Inulin			Glucose: Inulin		
Time (h)	**DP7**	**DP2**	**DP1**	**DP7**	**DP2**	**DP1**	**DP7**	**DP2**	**DP1**
0	1	1	1	1	0	0	1	1	1
6	0.97	1.36	0.71	0.99	0	0	1	1.43	0.37
13	0.96	1.93	0.17	0.96	1	0	1	1.34	0.35
19	1	1.98	0.18	0.90	0.69	0	1.0	1.45	0.32
24	0.96	1.93	0.17	0.69	0	0	1	1.48	0.34
37	1	1.93	0.19	0.65	0	0	0.99	1.46	0.30

Regarding the fatty acid composition, Table 4 shows that only in the case of cultures with glucose plus inulin is observed the presence of fatty acid of interest. It is noteworthy that the fatty acid profile differs slightly from those obtained in studying the experiences of operational conditions, however the first experiments were performed using phosphate buffer, while current experiences are developed without pH control, which also generates differences in the level of cell growth.

Table 4: Effect of inulin incorporation on the fatty acid profile of *L. plantarum* in a MRS broth supplemented with 2.6 mL $_{\text{grape seed oil}}$ /L.

Fatty acid	**Inulin**		**Glucose:Inulin (1:1)**	
	Intracellular	**Extracellular**	**Intracellular**	**Extracellular**
Miristic	0.4%	1.11%	9.77%	0.5%
Palmitic	5.54%	9.82%	0.64%	9.51%
Palmitoleic	-	-	0.35%	-
Estearic	2.88%	9.80%	3.94%	7.16%
Oleic	22.65%	19.97%	8.15%	23.12%
Vacénic	-	-	-	-
Linoleic	66.81%	54.22%	59.14%	59.09%
CLA	-	-	12.44%	-

Akalin et al. (2006) evaluated the enrichment with fructooligosaccharides (FOS) when yoghurt was produced with started culture (*Streptococcus thermophilus* and *L. delbrueckii ssp. Bulgaricus*) and different probiotics (*Lactobacillus acidophilus* and *Bifidobacterium animalis*) were added. CLA increased from 2.45 and 0.06 mg/g fat to 5.68 and 0.19 mg/g fat of c9t11 and t10c12 CLA isomers respectively, at the first day of storage, when yogurt was elaborated in the presence of FOS and *B. animalis*, in comparison to 2.07 mg/g fat of total CLA for one conventionally prepared. Rodrigues et al. (2012) report the effect of probiotic bacteria (*L. casei* or *B. lactis*) and the prebiotic type (Fructooligosaccharides: FOS and inulin) in free fatty acids (FFA) profile of cheese, observing that prebiotic inclusion produce an increment of FFA content and specially of CLA; however the results using only FOS was better those reported using FOS plus inulin. On other research, oligosaccharides such as maltodextrin, polydextrose and oligofructose produce different effects on *L. acidophilus, L. bulgaricus, L. rahmnosus* and *B. lactis* growth when these bacterias were used for the fermentation of skim milk in co-culture with *S. thermophilus*. In general, polidextrose and oligofructose produce better increase of biomass respect to control samples. The CLA content depends of the prebiotic and the probiotic applied in the skim milk fermentation, but they observed increments of more than 20% when prebiotics were incorporated (Oliveira et al. 2009).

Conclusions

The pH and the temperature during the incubation of *Lactobacillus plantarum,* in a MRS broth supplemented with grape seed oil as source of linoleic acid, produce important effects on growth and the fatty acid profile of this microorganism recognized as probiotic. In the case of pH effect, it is observed a microorganism adaptation to environmental conditions, since at different pHs a similar biomass concentration was obtained at the end of culture; however, the difference in pH of the medium affects the activity of enzymes involved in the conversion of LA to CLA. The temperature also has an effect on growth and fatty acid profile. Its effect is related to the fluidity of the cell membrane and linoleate isomerase activity. On the other hand, inulin does not produces the expected effect probably because a high glucose consumption.

The results obtained in this study and those reported by other authors suggest that these effects are strongly dependent on the microorganism strain (*Lactobacillus*) studied

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References

Akalin AS; Tokusoglu O; Gönc S, Aycan S. (2007). Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructooligosaccharide. *Int Dairy J*, 17:1089-1095

Alonso L, Cuesta EP, Gilliland SE (2003). Production of free conjugated linoleic acid by *Lactobacillus acidophilus* and *Lactobacillus casei* of human intestinal origin. *J Dairy Sci*, 86: 1941-1946

Ando A, Ogawa J, Kishino S, Shimizu S. (2003). CLA production from ricinoleic acid by lactic acid bacteria. *J Am Oil Chem Soc*, 80: 889-894

Banni S, Angioni **E**, Murru E, Carta G, Melis MP, Bauman D, Dong Y, Ip C. (2001). Vaccenic Acid Feeding Increases Tissue Levels of Conjugated Linoleic Acid and Suppresses Development of Premalignant Lesions in Rat Mammary Gland. *Nutr Cancer*, 41:91-97

Bhattacharya A, Banu J, Rahman M, Causey J, Fernandes G. (2006). Biological effects of conjugated linoleic acids in health and disease. *J Nutritional Biochem*. 17:789-810

Brillard J,Broussolle V. (2012). Mechanism involved in low-temperature adaptation in Bacillus cereus. In: Stress Response in Microbiology. Requena Editor. Caister Academic Press, Norfolk UK., p 125.

Dong M, Qi S. (2006). Conjugated linoleic acid production by fermentation. *Int J Food Eng*, 2: article 3

Goderska K, Nowak J, Czarnecki Z. (2008). Comparison of the growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* Species in media supplemented with selected saccharides including prebiotics. *Acta Sci Pol, Technol Aliment*, 7:5-20

Guerzoni E, Lanciotti R, Cocconcelli S. (2001). Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and termal stresses in *Lactobacillus helveticus*. *Microbiol*, 147:2255-2264

Kaplan H; Hutkins R. (2003). Metabolism of fructooligosaccharides by Lactobacillus paracasei 1195. Appl Environ Microbiol. 69:2217-2222

Kim Y J, Liu R H, Bond D R, Russell J B .(2000). Effect of linoleic acid concentration on conjugated linoleic acid production by Butyrivibrio fibrisolvens A38. Appl Environ Microbiol, 66: 5226-5230

Kishino S, Ogawa J, Ando A, Omura Y, Shimizu S. (2002). Ricinoleic acid and castor oil as substrates for conjugated linoleic acid production by washed cells of Lactobacillus plantarum. Biosci Biotechnol Biochem, 66: 2283–2286

LeBlanc JG, Garro MS, Savoy de Giori G. (2004). Effect of pH on Lactobacillus fermentum growth, raffinose removal, α-galactosidase activity and fermentation products. Appl Microbiol Biotechnol, 65: 119-123

Li J,Zhang L, Han X, Yi H, Guo C, Zhang Y, Du M, Luo X, Zhang Y, ShanY. (2013). Effect of incubation conditions and possible intestinal nutrients on cis-9,trans-11 conjugated linoleic acid production by Lactobacillus acidophilus F0221. Int Dairy J, 29:.93-98

Lin TY. (2006).Conjugated linoleic acid production by cells and enzyme extract of Lactobacillus delbrueckii ssp. Bulgaricus with additions of different fatty acids. Food Chem. 94: 437-441

Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulo G, Pot B, Tsakalido E. (2006). Probiotic potential of Lactobacillus strains isolated from dairy products. Int Dairy J, 16:189–199

Matagaras M, Metaxopoulos J, Galiotou M, Drosinos EH. (2003). Influence of pH and temperature on growth and bacteriocin production by Leuconostoc mesenteroides L124 and Lactobacillus curvatus L442. Meat Sci, 64:265-271

Ogawa J, Kishino S, Ando A, Sugimoto S; Mihara K, Shimuzu S. (2005). Production of conjugated fatty acids by lactic acid bacteria. J BiosciBioeng, 100, 355-364

Oliveira R; Florence A; Silva R; Perego P; Converti A; Gioielli L; Oliveira M. (2009). Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acid profiles in nonfat symbiotic fermented milk. Int J Food Microbiol, 128:467-472

Rodrigues D, Rocha-Santos T, Gomes A, Goodfellow B, Freitas A. (2012). Lypolisis in probiotic and synbiotic cheese: The influence of probiotic bacteria, prebiotic compounds and ripening time on free fatty acid profile. Food Chem, 131:1414-1421

Wrolstad R. (2012). Nutritional roles of carbohydrates. In: Food Carbohydrate Chemistry. R. Wrolstad Editor. Wiley-Blackwell, UK. p147-164