DEVELOPMENT OF SHRIKHAND WITH CLA (CONJUGATED LINOLEIC ACID) USING CONSORTIUM OF PROBIOTIC AND DAIRY STARTER CULTURES

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Abstract: To assess Probiotic and Dairy starter cultures for their ability to produce CLA (Conjugated Linoleic Acid) utilising nuts and vegetable oils as source of (Linoleic acid) LA. Based on an in vitro study and product simulation appropriate consortia was selected and a functional food product (Shrikand) was developed utilising nuts as a source of Linoleic Acid. Consortia of *S thermophilus, L acidophilus* and *L rhamnosus* was used in this final product and highest CLA was recorded in Pista shrikhand (256.25mg/gm) followed by Almond (231.25mg/gm), cashew (228.12 mg/gm) and walnut (221.87 mg/gm) flavours. Pista as source of Linoleic acid was efficiently utilised by the consortia and along with highest CLA yield, organoleptic evaluation results also showed pista shrikhand to score higher and tasteful compared to the other three nut flavours.

***Keywords*: CLA, LA, Probiotic, Dairy starter culture**

I. INTRODUCTION

Conjugated linoleic acid (**CLA**) is a collective term used to describe a mixture of positional and geometric isomers (forms) of linoleic acid, an essential fatty acid. The fatty acids that cannot be synthesized by the body and therefore should be supplied in the diet are known as essential fatty acids (EFA).

CLA occurs naturally in human plasma phospholipids, majority of dairy products and meats of different origin. Of the two biologically important isomers, c9, t11 is the most prevalent one comprising around 80 to 90% of total CLA in ruminant products, and t10, c12 is present in lower amounts as 3-5% of total CLA. (Carina Paola Van et.al, 2012**)** Double bonds of CLA are found mainly at positions 9 and 11, or 10 and 12, while isomers with double bonds at other positions also have been reported.

The formation of the CLA in milk and meat of ruminants can be explained by the conversion of dietary linoleic acid through ruminal bacteria and mainly by conversion of vaccenic acid in the mammary gland. (Ruth E. Lawson 2001**)**

Human milk is devoid of CLA when ruminant products are removed from their diet. Some fermented dairy products contained higher levels of CLA than non-fermented milk. Shantha *et al*. (1995) observed an increase in CLA content from 4.4 mg CLA/g fat of unprocessed milk to 5.3 mg CLA/g fat of a yogurt product with 0.05% fat. Ha et al. (1989) reported a higher level of CLA in Cheese Whiz of 8.81 mg CLA/g fat than in unprocessed milk of 0.83 mg CLA/g fat. Dahi, an Indian equivalent of yogurt, also contained more CLA (26.5 mg CLA/g fat) than the raw material (5.5 mg CLA/g fat) (Tunh y lin 1999)

Research on the biological functions and health benefits of CLA dates back to the 1980s when scientists at the University of Wisconsin observed that an anti-carcinogenic compound (later identified as CLA) isolated from grilled ground beef inhibited chemically induced skin cancer in mice. Since then, numerous studies have investigated the effects of CLA on cancer, cardiovascular disease, body composition, and other conditions (e.g., insulin resistance, immune function, bone health). (Belury et.al 2002)

In the present study we investigated the ability of probiotic and dairy starter cultures in shrikhand to produce CLA by utilising Almonds, Cashews, Pistachios and Walnuts. Nuts have appreciable amount of linoleic acid present and hence have been used in the final product development to provide both flavour and health benefit.

II. MATERIALS AND METHODS

2.1 Market Survey of Dairy foods containing CLA by checking nutritional labels on products:

A market survey of different Dairy and oil based products was carried out in the suburbs of Mumbai, so as to investigate the mention of CLA in nutritional labels of the products.

2.2 Isolation of probiotic and dairy starter cultures:

1. Lactobacillus cultures *L acidophilus*, *L rhamnosus* and *S thermophilus* were isolated from commercial probiotic sources.
2. *Saccharomyces boulardii*isolated from pure lyophilized culture available commercially.
3. *Bacillus clausii* isolated from a commercially available spore suspension.

4. LAB isolated from fermented products as referred below: (Table 2.1)

4a.Sauerkraut: Mature cabbage heads were washed and shredded finely. 2.5% salt (w/w) was added mixed and filled till the brim in sterile glass bottle and fitted tightly with a lid. The bottles were incubated at (22-25ºC) for fermentation till 3 weeks.

4b. Dosa batter:Parboiled rice and black gram dhal in a proportion of 3:1 were soaked for 8h and ground. The batter was mixed and allowed to ferment overnight at RT.

Table2.1: Sample processing of Sauerkraut and Dosa batter

Fermented Product	Sample processing
Sauerkraut	A Loopful of brine solution obtained after 3 weeks of fermentation was streaked on MRS (De Man Rogosa and Sharpe) agar. Plates were incubated at RT/24hrs under microaerophilic conditions.
Dosa Batter	1 g batter was transferred into 10ml sterile saline and vortex. A loopful of suspension was streaked on MRS agar incubated at RT/24hrs under microaerophilic conditions.

2.3 Identification of isolates obtained on:

2.3.1 MRS agar:

Presumptive identification of isolates obtained on MRS agar were done by Gram staining and catalase test later followed by Biochemical identification.Homo hetero differential agar (HHD) and Gibson's medium was used to identify the nature of lactic acid fermenters.

2.3.2 SAB agar (Sabouraud's Agar): *S boulardii*Identification was done by Gram staining and morphology.

2.3.3 *Streptococcus thermophilus* isolation Agar:Presumptive identification of *S thermophilus*was done by Gram staining followed by Biochemical identification.

2.4 In Vitro screening of LAB cultures for their ability to produce CLA[8]:

The pure cultures were inoculatedas specified in Table 2.1 and incubated for the defined period to prepare seed cultures. For assay of CLA:10ml culture medium containing 1% tween 80 and 1% LA source (Table 2.2)were inoculated with 5% seed culture(24hrs old) and incubated for 24hrs(Table 2.3).Each of the culture medium wassubjected to lipid extraction to extract CLA.

Table 2.2 Linoleic acid containing substrates used in In vitro study and product development

Linoleic acid source	Source details	%LA	Reference
Cashews	UNBRANDED	8	USDA Nutrient Database
Almonds		12	
Pistachios		13	
Walnuts		38	
Sunflower oil	BRANDED	68	Oils rich in Linoleic acid, Dr. Tomislav Mestrovic 2015
Sesame oil		45	
Olive oil		10	
Peanut oil	UNBRANDED	32	

Table 2.3 Culture specific medium and Incubation conditions

Identified Cultures	Media	Incubation conditions
Lactobacillus cultures	MRS Broth	At 22-25ºC for 24hrs under microaerophilic conditions
Saccharomyces boulardii	Sabouraud's Broth	At 22-25ºC for 24hrs
Bacillus clausii	Nutrient broth	At 37ºC for 24hrs

2.5 Product Simulation study

CLA production was studied in de-skimmed cow milk and optimised with different nut sources containing Linoleic acid.

Seed culture was prepared as described previously.

Conditioning: 10ml of de-skimmed cow milk was inoculated with 24hrs old 2% seed culture and incubated at 22-25ºC for 24hrs.

Assay of CLA: 10ml de-skimmed milk as medium containing1% tween 80 and 1% LA source was inoculated with 5% seedculture from conditioning tube and incubated for 24hrs at 22-25ºC. Cultured tubes subjected to lipid extraction to extract CLA.

2.6 Product Development

Curd Setting with Individual cultures: All the cultures were cultured in selective media and a suspension prepared to a cell density of 3 x 10^8 cells/ml by comparing with McFarland standards. Curd setting was done by adding 2% culture suspension of one of the following consortia to buffalo milk.

1. *S thermophilus + L acidophilus + L rhamnosus*
2. *S thermophilus + L fermentum + S boulardi*

Curd setting was donefor different time periods and each curd sample was studied for acidity, organoleptic and physical properties.

Acidity:1 gm of set curd was weighed in a clean glass beaker, homogenised and 10ml Distilled water added. The homogenized sample was titrated with N/9 NaOH solution using phenolphthalein indicator till light pink colour. The Gm% lactic acid content was calculated.

Organoleptic properties (colour, texture, odour, taste, mouth feel) of developed Shrikhand was studied [2]. 25 Trained and untrained panellist were part of the sensory evaluation. A small amount of sample (50gm) was given for evaluating the taste, appearance, odour, flavour, mouthfeel(taste) to panellists in Mahanand and students at Bhavan's College

2.7Final Product Development at Mahanand Dairy Plant:

Based on curd setting studies final product was developedusing the mother culture consortia prepared in buffalo's milk. 3% starter culture consortia and 1%desired crushed nuts as source of Linoleic acid were added to the milkAfter desired curd acidity was attained (0.65%) the curd was hung in a muslin cloth for 8 – 10hrs to obtain chakka (solid mass) and whey. Chakka was further processed to shrikhand by adding sugar and other flavourings.The presence of CLA was checked at different phases of Shrikhand development;a) Phase of Starter culture (plain set curd without LA source), b) Phase of Mother Culture (curd set with LA source i.e. crushed nuts), c) Phase of Chakka (solid mass obtained by hanging mother culture in a muslin cloth for 8hrs), d) Phase of Whey (by-product obtained when chakka is hanged), e) Final product: Shrikand For all the above lipid extraction (as in 2.8) was done using 1g of a) to c) and e) or1ml whey- d).

2.8Estimation of CLA in product:

a) Lipid Extraction

To 3ml of cell free supernatant, 6ml isopropanol was added and vortexed for a minute. 5ml hexane was added and again vortexed for a minute,centrifuged at 2000rpm/5mins.The Hexane layer was collected and assayed spectrophotometrically at 233nm against a hexane layer extracted with uninoculated medium.

b)Fatty acid methyl esters (FAME) preparation method for GC analysis[European Pharmacopeia]

FAME preparation for detection of CLA were validated using standard CLA.

Take 1 ml of extracted lipid in 5ml of 14% BF3 in methanol in round bottom flask and boil for 10 minutes in a water bath. Add 4 ml of hexane to it and again boil for 10 minutes. Allow to cool and add 20 ml of saturated NaCl solution.Remove organic layer and dry over Na_2SO_4.Analyse by GC equipped with a flame ionization detector and a fused silica capillary column. (PerkinElmer Clarus 500gas chromatograph equipped with FID)The gas flow rates Carrier gas hydrogen: -1.4 mL min-1, Make-up gas nitrogen 30 mL min-1 flame gases, H2 and flame synthetic air: -30 and 300 mL per min.The sample injection rate (split): 1/100.The injector and detector temperatures: 235 °C .The column temperature programming: 65 °C for 4 min, followed by a ramp of 16 °C min-1 up to 185 °C, which was kept for 12 min. A second ramp of 20 ° C min-1 was run up to 235 °C for 14 min Injection volume: - 1 µL

III RESULTS AND DISCUSSION

In a market survey 25 Different dairy products and different oil samples of various brands were investigated to look for prevalence of CLA mentioned in their nutritional labels. Amongst all the products screened,only one product,AMUL - Lite Bread Spread mentioned 300mgCLA/100gm in its nutritional label. The present study investigated the possibility of safe lactic acid bacteria (LAB) and yeast for the production of CLA. In addition to the probiotic cultures, LAB isolated from Sauerkraut and Dosa batter were also investigated. The reason for choosing these sources was dual, firstly the cultures are safe and secondly CLA being a secondary metabolite, LA sources can be used in dressings and CLA content can be fortified in fermented products.

All the isolates were studied for their Gram nature, catalase test (in case of LAB) and biochemically identified by using conventional identification methods. (Table 3.1 and 3.2).

Table 3.1Results of Biochemical test of LAB isolated on MRS Agar from different sources:

Biochemical Test	Sources			
	L acidophilus	*L rhamnosus*	Sauerkraut	Dosa batter
Gram Staining	Gram positive rods	Gram positive rods in chains	Gram positive rods	Gram positive rods
Catalase test[a]	-	-	-	-
Gas from Glucose	-[c]	-[c]	+[d]	+[d]
Acid from				
a. Arabinose	-[e]	+[f]	+[f]	+[f]

b. Galactose	+[f]	+[f]	+[f]	+[f]
c. Lactose	+[f]	+[f]	+[f]	+[f]
d. Maltose	+[f]	+[f]	+[f]	+[f]
e. Mannitol	-[e]	+[f]	+[f]	-[e]
f. Raffinose	+[f]	+[f]	+[f]	+[f]
g. Sorbitol	-[e]	+[f]	+[f]	-[e]
Aesculin hydrolysis	+[g]	+[g]	+[g]	+[g]
Growth on HHD Agar Yellow: Homofermenter Green: Heterofermenter	Yellow colour colonies	Yellow colour colonies	Green colour colonies	Green colour colonies
Confirmation of isolate	*L acidophilus*	*L rhamnosus*	*L plantarum*	*L fermentum*

[a] Absence of effervescence,[b] Effervescence, [Ref: A comprehensive Dairy Microbiology (1993)]
[c] No gas bubbles, [d] Gas bubbles,
[e] Pink colour, [f] Colourless

Table 3.2Results ofbiochemical identification of *Streptococcus thermophilus*

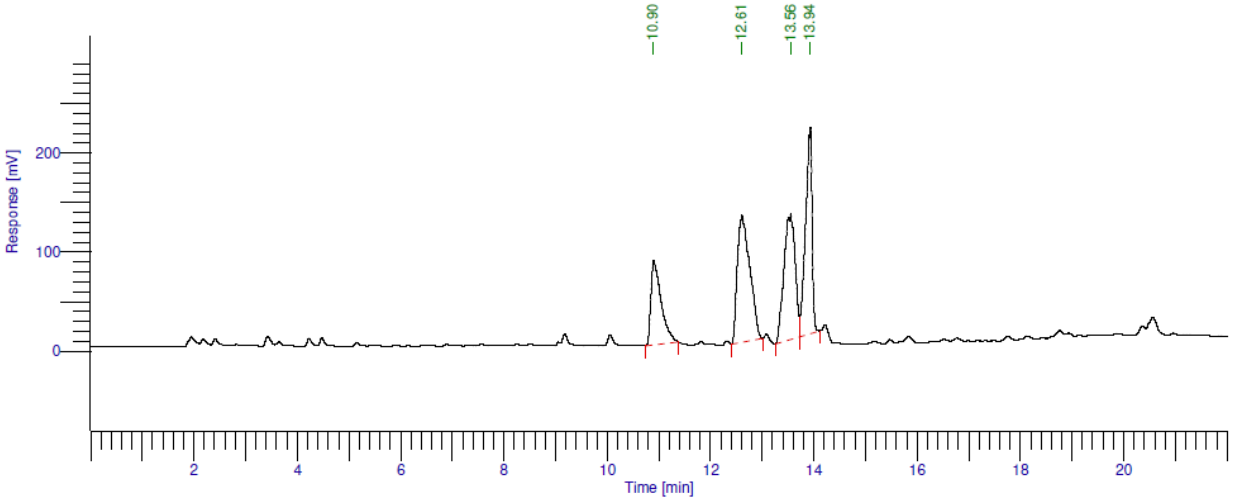
Biochemical test	Result
Gram staining	Gram positive cocci in chain
Growth in 10% bile	-[a]
Growth in 40% bile	-[a]
Optimum temperature	40-50 degree Celsius
Acid from	
a. Arabinose	+[b]
b. Lactose	+[b]
c. Mannitol	-[c]
d. Sucrose	+[b]
e. Maltose	-[c]
Aesculin hydrolysis	-[d]

[a] No turbidity, [Ref: A comprehensive Dairy Microbiology (1993)]
[b]Pink colour, [c]colourless,
[d]No blackening

*Saccharomyces boulardii*was only confirmed by Gram Staining to ascertain the purity of the culture isolated from the lyophilized culture.

The cultures identified and used in the current research were *L acidophilus, L rhamnosus, L fermentum, S boulardii, S thermophilus,* and *B clausii.*R sieber et al (2004) has described several strains of LAB for their ability to produce CLA in cultivation media or in milk. They include *Lactobacillus acidophilus, Lactobacillus brprevis, Lactobacillus casei, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus paracasei, Lactobacillus plantarum, Lactoboacillus rhamnosus, Lactococcus lactis ssp. cremoris and Lactococcus lactis ssp. lactis* strains. Linoleic acid ((C18:2) varies from 1.6% to 79% in various vegetable oils like coconut, sunflower, sesame, olive peanut etc.[Orsavova et al Int. J. Mol. Sci. 2015,]
CLA produced by all the cultures was extracted into hexane and quantified at 233nm. Spectrophotometric methods do not distinguish between CLA isomers thus a chromatographic analysis was necessary. GC-FID method was used for CLA determination in milk products after validating the derivatization of standard CLA using BF3 hexane method. The method resolved peaks of the four major conjugated linoleic moieties. [fig 3.1]

Figure 3.1 Standard CLA FAME prepared using BF3-Hexane (european pharmacopeia)



Using different vegetable oils in culture media, *S.thermophilus*and *S. boulardii* gave highest CLA with Sesame oil followed by *L. acidophilus* with Sunflower oil and *L.plantarum* with olive oil. *(*Table 3.3).Groundnut oil used in the study was a filtered oil while others were branded and refined, and for reasons not known could be the cause for least CLA production by all cultures.

Table 3.3 CLA production by different isolates using Vegetable oils as LA source

	Sunflower oil (mg/ml)*	Sesame oil (mg/ml)*	Groundnut oil (mg/ml)*	Olive (mg/ml)*
L acidophilus	**45.13 ± 1.2**	31.58 ± 2.1	2.87 ± 0.51	41.29 ± 1.5
L rhamnosus	5.75 ± 0.5	**37.84 ± 1.59**	1.15± 0.37	33.32 ± 1.04
L plantarum	36.45 ± 1.04	39.92 ± 1.2	0.81 ± 0.28	**43.01 ± 2.44**
L fermentum	31.58 ± 1.57	4.59 ± 0.5	0.82 ± 0.13	**34.02 ± 1.58**
S thermophilus	32.63 ± 1.58	32.63 ± 1.58	32.63 ± 1.58	32.63 ± 1.58
S boulardii	32.97 ± 1.58	**50.34 ± 0.6**	1.31 ± 0.14	30.9 ± 1.59
B clausii	**33.65 ± 0.61**	3.78 ± 0.37	2.38 ± 0.37	32.28 ± 1.02

* Results expressed as mean values of triplicate determination ± SD

Table 3.4 CLA production in mg/ml by all cultures with Cashew and Almond as LA sources

	Cashew(mg/ml)*			Almonds (mg/ml)*		
Period of fermentation	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
L acidophilus	**61.10 ± 2.17**	**74.3 ± 1.59**	**73.26 ± 2.16**	57.98 ± 3.18	73.26 ± 2.1	69.44 ± 2.1
L rhamnosus	62.14 ± 2.17	**91.64 ± 2.08**	**89.23 ± 1.58**	49.30 ± 2.17	64.23 ± 1.5	56.59 ± 3.1
L plantarum	**53.81 ± 4.9**	**68.37 ± 3.9**	44.78 ± 2.08	44.44 ± 3.18	63.54 ± 1.04	**47.91 ± 2.7**
L fermentum	**66.29 ± 2.6**	**72.21± 1.5**	49.99 ± 2.08	39.58 ± 2.75	50.69 ± 1.5	**53.81 ± 2.6**
S thermophilus	51.03 ± 2.7	65.96 ± 3.9	64.92 ± 0.6	**51.38 ± 1.58**	**72.91 ± 1.04**	**78.81 ± 1.2**
S boulardii	5.26 ± 0.37	6.33 ± 0.37	4.27 ± 0.86	**36.10 ± 1.59**	**48.95 ± 4.1**	**43.05 ± 2.6**
B clausii	2.45 ± 0.25	2.79 ± 0.28	0	4.75 ± 0.39	2.29 ± 0.37	0

* Results expressed as mean values of triplicate determination ± SD

Table 3.5 CLA production in mg/ml by all cultures with Pistachio and Walnut as LA sources

	Pistachio(mg/ml)*			Walnut (mg/ml)*		
Period of fermentation	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
L acidophilus	**58.36 ± 4.11**	62.14 ± 1.2	72.56 ± 1.5	37.84 ± 0.6	42.35 ± 1.59	53.46 ± 2.16

L rhamnosus	57.63 ± 1.58	63.19 ± 1.5	59.71 ± 0.6	**67.33 ± 2.18**	70.13 ± 1.58	76.73 ± 1.2
L plantarum	6.64 ± 0.63	32.96 ± 0.58	31.59 ± 0.6	3.12 ± 0.37	30.20 ± 1.04	34.70 ± 1.6
L fermentum	6.49 ± 0.37	35.41 ± 1.04	35.05 ± 1.6	5.34 ± 0.37	6.31 ± 0.24	31.94 ± 0.6
S thermophilus	35.75 ± 2.18	46.17 ± 1.5	53.81 ± 1.5	33.67 ± 0.6	37.15 ± 1.59	42.70 ± 2.7
S boulardii	5.92 ± 0.49	32.98 ± 2.16	35.75 ± 2.1	6.39 ± 0.21	5.58 ± 0.51	0.57 ± 0.14
B clausii	3.6 ± 0.36	3.61 ± 0.14	**1.39 ± 0.51**	**5.18 ± 0.49**	**4.26 ± 0.5**	0

* Results expressed as mean values of triplicate determination ± SD

Cashew was readily utilised by most of the organisms however, highest CLA production utilising cashew as LA source was recorded for *L fermentum* and *L rhamnosus*, *L acidophilus* showed promising results with almonds and pistachios while walnut was utilised effectively by *L rhamnosus*. *S boulardii*a probiotic yeast was able to produce CLA from LA present in almond and pistachios effectively as compared to other nut sources thus holds promising application of this organism as a CLA producing starter along with its probiotic benefits. (Table 3.4, 3.5)

The data obtained from in vitro study was used to make the functional product, thus studies were done in skim milk. Milk inherently contains some amount of CLA. Cow's milk was used and CLA was estimated to be around 3.6 mg/ml. In product simulation study nuts were added to milk and the fermentation process lead to increased CLA production in milk as compared to unfermented milk as control (Table 3.6). Hence based on these results Shrikhand was developed and suitable cultures were selected for the production of CLA.

The objective to use nuts was to incorporate these natural LA sources in functional food (shrikand) at the onset of growth and fermentationas done in the culture medium and observe for production of CLA. Three days of fermentation period was selected in this study because shrikand manufacture from first stage till finished product takes 2-3 days. Nuts were added while curd setting hence took advantage of its conversion during curd fermentation and later when it is hung for shrikhand making.Thus nuts otherwise being added as a garnish to shrikhand were studied for another dimension of health benefit. *L rhamnosus* and *L acidophilus* consistently showed high CLA in In vitro studies hence were chosen to be in the consortia used for curd setting.

Table 3.6 Product simulation study

Isolates	LA sources used in product simulation studies/ concentration of CLA in mg/ml			
	Cashews	Almonds	Pistachios	Walnut
L acidophilus	57	49	52	42
L rhamnosus	54	38	46	62
L plantarum	53.81	43.2	5.48	4.12
L fermentum	62	33.3	5.83	5.6
S thermophiles	46	47.4	39	30.04
S boulardii	25.2	27.35	19.3	22
B clausii	0	3.75	0	0
Control	3.6 mg/ml CLA			

CLA is an intermediate metabolite of lactic acid bacteria for converting polyunsaturated fatty acid to saturated fatty acid in order to reduce the toxicity of free fatty acids (Jun et al. 2005). The concentrations of CLA and CLNA isomers in ruminal food products are relatively small (4–6 mg/g) so large quantities of these food items should be consumed to obtain beneficial effects.

Current measures of usual or actual dietary intakes of CLA are very limited, most being only estimates. Average estimated CLA intake in U.S. adults is 0.2 g/day, whereas in some other countries such as Germany where the population consumes more energy from ruminant fat, CLA intake is much higher (e.g., about 0.4 g/day). In a small study of free-living adults in Canada, average intake of c9, t11 CLA (rumenic acid) was about 0.1 g/day (range of 0.02-0.17 g/day). (Sohail Mushtaq 2009)

There are variations in experimental models about effective doses of CLA, depending on animal model and the biological effect evaluated, the recommended dose for human daily intake also varies widely.

In general, by extrapolation of results found in animals, the recommended CLA daily intake is around 0.35 to 1 g/day. Some authors estimated a daily dose of 650 mg, but other studies considered that higher doses (3.0 to 4.2 g/day) are adequate to reduce body fat mass. Thus studies concerning to increase CLA content in foods receives great attention since bacterial inclusion improves CLA levels in some fermented dairy products or could generate CLA at intestinal level after a probiotic administration.[Carina Paola Van 2012]

Based on curd setting studies, end product development used a mother culture prepared in buffalo's milk, with 3% starter culture consortia and 1% crushed nuts as source of Linoleic acid. Buffalo's milk is recommended because milk fat in chakka should not be less than 8.5 percent by weight (dry weight). The acidity and curd setting time of mother culture for both the culture consortia used in this study was determined with and without nuts added to buffalo milk. The curd setting time was 5h with a desired

acidity of 0.5 to 0.6% as per Dairy norms[table 3.7]. When nuts were added the curd setting time altered and so also the acidity [table 3.8]

Table 3.7Parameters studied of set curd

Parameters	Concentration of starter	% Acidity	Setting time
Consortia 1 (*S thermophilus + L acidophilus + L rhamnosus*)	3%	0.59	5 h
Consortia 2 (*S thermophilus + L fermentum + S boulardii*)	3%	0.53	5 h

Table 3.8Parameters studied of Mother Culture

Linoleic acid source	Consortia 1 *S thermophilus + L acidophilus + L. rhamnosus*		Consortia 2 *S thermophilus + L fermentum + S.boulardii*	
	Setting time	Acidity %	Setting time	Acidity %
Almonds	4.8hrs	0.6	5 hrs	0.495
Cashew	5.2hrs	0.449	After 8 hrs	0.275
Pistachio	5hrs	0.55	After 6 hrs	0.405
Walnut	5hrs	0.58	After 7.5 hrs	0.311

Consortia 2 had longer curd setting time and acidity was variable with different nuts as well as not as per Dairy specifications. (Desirable acidity is maximum 0.65 % for curd and 1.4% for shrikand as per FSSAI specifications). Thus consortia 2 was not accepted in product development.

The final shrikhand was made with *S thermophilus + L acidophilus + L rhamnosus*and all the four nut flavours. The presence of CLA along with acidity (Gm% Lactic acid) was checked at different phases of product development (Table 3.9, Figure 3.2)Apparentlyfermented dairy products contain higher levels of CLA than non-fermented milk. Shantha et al. (1995) observed an increase in CLA content from 4.4 mg CLA/g fat of unprocessed milk to 5.3 mg CLA/g fat of a yoghurt product with 0.05% fat. Ha et al. (1989) reported a higher level of CLA in Cheese Whiz of 8.81 mg CLA/g fat than in unprocessed milk of 0.83 mg CLA/g fat. Dahi, an Indian equivalent of yogurt, also contained more CLA (26.5 mg CLA/g fat) than the raw material (5.5 mg CLA/g fat) (Tunh y lin 1999) In line with this study the product developed using nuts as source of LA reported a CLA content of 256.25mg/gm of Pista shrikhand followed by Almond (231.25mg/gm), cashew (228.12 mg/gm) and walnut (221.87 mg/gm) flavours.Each flavour of shrikhand was surrendered to organoleptic testing using trained and untrained panel members. The product was scored for colour, flavour, odour, appearance and taste in a hedonic testing. Hedonic ratings relate to pleasurable or unpleasable experiences and is used to measure consumer acceptability of food products. Among the four flavours, Pista flavour scored high on all tested parameters. [Table 3.10]

Table 3.9 CLA estimation at different satge of shrikhand development

Linoleic acid source	% acidity of final product	Almond	Cashew	Pistachio	Walnut
Mother culture	0.98	8.8 mg/gm	10.37 mg/gm	9.6 mg/gm	10.37 mg/gm
Whey	1.04	134.37 mg/ml	13.3 mg/ml	190.62 mg/ml	203.12 mg/ml
Chakka	0.78	162.5 mg/gm	150 mg/gm	196.8 mg/gm	218.75 mg/gm
Shrikand	1.13	231.25 mg/gm	228.12 mg/gm	256.25 mg/gm	221.87 mg/gm

Figure 3.2 CLA estimation at different satge of shrikhand development

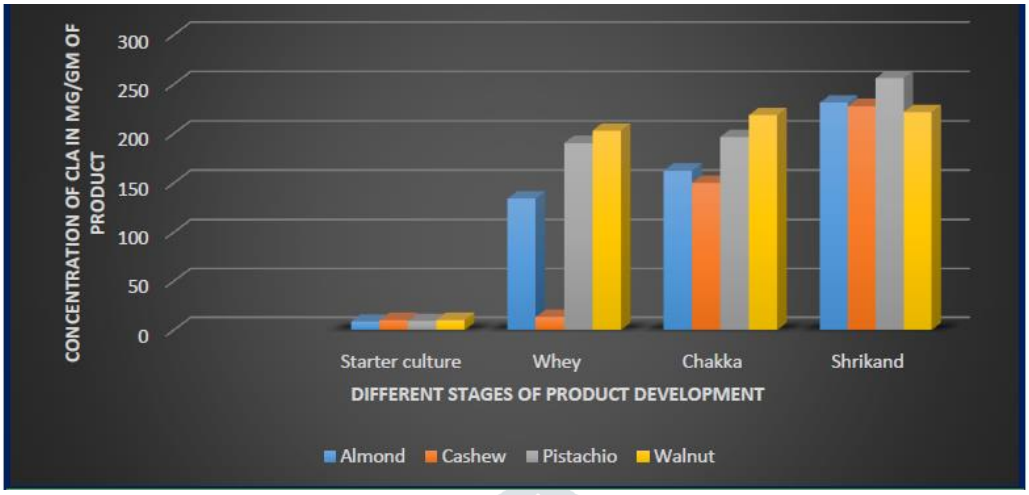


Table 3.10 Organoleptic test Results

Shrikhand	Colour*	Flavour*	Odour*	Appearance*	Taste*
Pista	4.62	4.62	4.12	5	5
Cashew	4.25	3	3.5	4.25	3.75
Almond	4.62	3.62	4.12	4	3.87
Walnut	4.12	4	3.37	4.12	4.25

*Ratings: 1=Worst, 2=Bad, 3=Average, 4=Good, 5=Excellent

CLA content was measured through the different phases till final product. An increase in CLA content was observed after fermentation and even in finished product hence proving that fermentation leads to increase in CLA and that fermented product Shrikand will offer CLA benefits.Till date no study on development of shrikand as functional food with appreciable CLA has been conducted, value added soy shrikand with increased protein content was however developed. (G. P. Kadam 2016)

In the present study substantial amount of CLA was estimated in whey thus whey can also be utilised to make whey powder that canoffer health supplement. Current nutritional recommendations for whole fat dairy products consumption is limited, hence the CLA and CLNA content of human diet is too low for obtaining health benefits. Therefore, a promising strategy to increase human intake of these bioactive lipids would be to include CLA and/or CLNA producer bacteria in fermented dairy products. In the last years, several studies have reported that some lactic acid bacteria and bifidobacterial strains are able to efficiently convert LA to CLA in milk, milk-based media, and dairy products [Fariborz Akbarzadeh 2012]
Developing shrikand with increased CLA utilising nuts and dried fruits as source of linoleic acid for microorganism is a promising economic venture.

Conclusion

In recent years, scientific investigators have moved from primary role of food as the source of energy and nutrients to action of biologically active food components on human health. In this way, a novel term -functional food- was introduced which refers to prevention and/or curing effects of food beyond its nutritional value. (Aziz Homayouni et.al, 2012)
The claimed health benefits of fermented functional foods are expressed either directly through the interaction of ingested live microorganisms, bacteria or yeast with the host (probiotic effect) or indirectly as a result of ingestion of microbial metabolites produced during the fermentation process (biogenic effect).
The biogenic properties of fermented functional foods result from the microbial production of bioactive metabolites such as certain vitamins, bioactive peptides, organic acids or fatty acids during fermentation. (Catherine Stanton et.al, 2005)
One such bioactive metabolite is CLA (Conjugated linoleic acid). The present study was designed to investigate the CLA content in an Indian fermented product (Shrikhand) utilising the ability of Probiotic and dairy starter culture to convert exogenously supplied natural Linoleic acid contained in nuts into CLA.

Use of lactic acid bacteria with high CLA-producing abilities could further increase the possibilities for an additional formation of the CLA content in common fermented dairy foods or cheese varieties without major changes in processing regimes. (R sieber et.al 2004)
Thus attempts have been made in development of western dairy products with increased CLA however, an Indian dairy product (shrikhand) with such increased CLA content can also enter in the category of functional food products.

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