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Fortification of Indian curd with chia oil Nanoliposome as source of α-linolenic acid

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Abstract
Chia oil is a rich source of α-linolenic acid (ALA). ALA is well known for its several therapeutic potentials; however it is prone to oxidative degradation. Therefore, this study was conducted to encapsulate chia oil in nanoliposome. Chia oil nanoliposome was successfully encapsulated in nanoliposomes with encapsulation efficiency of 88.31%. This chia oil nanoliposome was incorporated in curd and the physiochemical properties of fortified curd were evaluated. No significant difference in pH, acidity, colour and flow behaviour of control curd and fortified curd were observed. The syneresis of control curd and fortified curd were significantly different. The saturated fatty acid of fortified curd was decreased however unsaturated fatty acid content increased. The ALA content of fortified curd was increased from 2.17% to 5.95%. Thus the fortified curd can be used for the supplementation of ALA in different age group of consumers.

Keywords: Chia oil, α-linolenic acid, fortification, curd

1. Introduction
α-linolenic acid (ALA) is an essential ω-3 polyunsaturated fatty acids obtained from various plant sources. Numerous investigations have showed the health benefits of omega-3 fatty acids (Zimet & Livney, 2009) [28]. Among all vegetable oil sources, chia seed oil contains around 68% ALA, and flax seed contains about 57%, which also have antioxidants, minerals and vitamins (Campos *et al*., 2014) [21]. Chia seed oil also contains ω-6 linolenic acid, myricetin, quercetin, kaempferol and caffeic acid (Mohd *et al*., 2012). However, it is prone to oxidative degradation which leads to the production of secondary metabolites which is responsible for off-flavors and odors. Controlling this degradation is a challenge for unsaturated fatty acids.
Milk and milk products are commonly consumed by different age groups due to its nutritive value (Upadhyay *et al*., 2014) [24]. Indian curd is similar product to yogurt popular in Indian subcontinent (Rathi *et al*., 2015). Curd is prepared by fermenting milk with help of misophilic lactic acid bacteria such as *Lactococcus lactis* ssp. *Lactis*, *Lactococcus lactis* ssp. *Diacetylactis* and *Lactococcus lactis* ssp. *Cremoris*. Curd possesses various therapeutic potentials such as anticancer, it enhances appetite and vitality, testament of disorders such as diarrhoea, dyspepsia and other intestinal disorders, and good for lactose intolerant people (Agarwal and Bhasin 2002) [1]. In India, about 9% of milk production is used for the production of curd.
Recently, nanoliposome based encapsulation technique used for encapsulation of various food bioactives (Munin & Edwards, 2011) [18]. The vesicular particles are able to encapsulate water-soluble, lipid-soluble and amphiphilic materials. In our earlier publication nanoliposomal encapsulation of chia oil has been studied with 88.31% efficiency (Choudhary *et al*., 2021) [4, 5]. The objectives of the current investigation are to fortify Indian curd with chia oil nanoliposomes and study the physiochemical properties of the fortified curd.

2. Materials and Methods
2.1 Materials
Cold extracted chia oil (CO), and curd sample were purchased from local market of Thanjavur local market based in Tamil Nadu. Soy lecithin and Tween 80 were procured from Himedia, India. All chemicals used in this work were of AR grade.

2.2 Preparation and estimation of encapsulation efficiency of CO nanoliposome
CO nanoliposomes were prepared using the method described by Choudhary *et al*., (2021a) [4, 5].

Briefly, CO was dissolved in lipid phase of phosphatidylcholine and Tween 80. To obtain thin film of lipid phase, ethanol was evaporated using a gentle stream of N_2 gas then the film was rehydrated with 20 mL of phosphate buffer saline (0.01 M, pH 7.2). Probe sonication (Labman PRO 650) using 6 mm probe with 70% amplitude at 20 kHz for 30 min (3 min sonication, 2 min interval for 6 times) was done to reduce size and lamellarity. After preparation of liposome, the unentrapped CO was separated using dialysis technique. The nanoliposome was stored in amber screw capped vials, in an inert atmosphere at 4 °C in dark for further application. The encapsulation efficiency was recorded by UV-Vis spectrophotometer (multimode microplate reader, SpectraMax iD3, Molecular Devices, US) (Viriyaroj, *et al*., 2009).

2.3 Fortification of curd with nanoliposomes

Indian curd was fortified by the addition of 15 ml of CO nanoliposomes into 100 g of Indian curd (3% fat; 4% protein), and kept in glass container at 4 ºC for further analysis (Fig. 1). Control sample is curd without nanoliposomes.

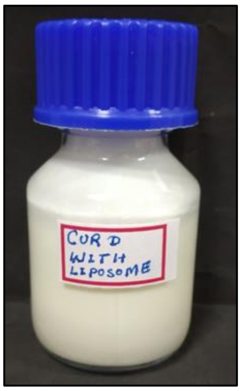

Fig 1: Chia oil nanoliposome fortified curd

2.4 Physiochemical properties of fortified curd sample

2.4.1 Titratable acidy and pH

AOAC official method 942.15 (AOAC, 2000) was used for the determination the acidity. Titratable acidity was measured in terms of g of lactic acid/100 g product. For the determination of titratable acidity 10 g of curd sample was mixed with 10 mL of hot distilled water and titrated with 0.1 N NaOH using 0.5% phenolphthalein indicator. pH of control curd and fortified curd were recorded by digital pH meter (PC 700, Eutech Instrument, Singapore).

$$\%\, Lactic\ acid\ (w/v) = \frac{9 \times V_1 \times N}{V_2} \qquad (1)$$

Where,
V_1 is the titre value of NaOH (mL),
N is the normality of NaOH and
V_2 is the volume (mL) of milk used for titration.

2.4.2 Syneresis

Syneresis is shrinkage of gel due to expulsion of whey/liquid that reduces the capability of gel to hold the available liquid phase. It occurs in curd when the casein particles rearrange themselves in such way that they start losing the whey without any without application of any eternal force (Serra *et al*. 2009). To evaluate syneresis, 20 g of curd was centrifuged at 500 rpm for 5 minutes. The separated liquid was collected and measured using measuring cylinder. Syneresis percentage was calculated using equation given below (Ghorbanzade *et al*., 2016) [6].

$$Syneresis\ (\%) = \frac{Total\ weight\ of\ lipquid\ separated\ (g)}{Total\ weight\ of\ curd\ (g)} \times 100 \qquad (2)$$

2.4.3 Colour value

Colour (L*, a*, b*) of nanoliposome incorporated curd was measured using Hunter colour lab (Model: D25 Optical sensor; Hunter Associate Laboratory, Reston, VA) and results were presented in L* (Lightness), a*(redness) and b* (yellowness) values.

2.4.4 Flow behaviours

Flow behaviour of control and fortified curd samples were examined by procedure used by Choudhary *et al*. (2021b) [4, 5] for curd sample during fermentation. Briefly, 25 mL of sample was taken in a cuvette. A rheometer (Modular Compact Rheometer Anton Paar, MCR 52, Austria) with concentric cylinder probe (ST24-20 2V/2V-30) assembly was used to measure the viscosity of curd sample. During the measurement the temperature of the samples was maintained at 25 °C. Fifty shear-stress/shear-rate data points were obtained from the analysis, at one point per 2 s, during the shearing of the sample from 0.01 s^{-1} up to 100 s^{-1} shear rate within the experiment time of 100 s. The whole process of sample preparation was repeated twice, while all viscosity investigations were performed on duplicate samples.

2.5 GC-MS analysis of fatty acid profile of curd samples

Fat from CO nanoliposome and both the curd samples were extracted using chloroform-methanol (2:1, v/v). The extracted sample was converted in fatty acid methyl ester (FAME) (AOCS, 2007). Fatty acid profile was analyzed using gas chromatography mass spectroscopy (GC-MS) technique (436-GC Bruker, Livingston, UK). GC-MS analysis was done with a 2 µL split mode (10:1) sample volume using capillary column (5% Diphenyl / 95% Dimethyl polysiloxane, 30m x 0.25mm ID x 0.25µm). The inlet temperature was maintained at 280 ºC. The analysis oven temperature was programmed as follows: 50 ºC for 1 min, 25 ºC/min to 175 ºC, and 4 ºC/min to 230 ºC, remaining at 230 ºC for 5 min, with total run time of 26 min for each cycle (Rasti *et al*., 2012) [20].

2.6 Statistical Analysis

Duncan's multiple range tests with p value<0.05 was performed with IBM SPSS V20 (IBM, USA) to verify the significant difference of the data. All experiments were conducted in triplicate and the data are expressed as means ± SD of three independent experimental runs.

3. Result and Discussions

3.1. Chia oil nanoliposomes

CO nanoliposome, as stated in our previous publication, showed 88.31±0.64% encapsulation efficiency, with spherical shape geometry and average particle size of 49.25 nm with moderate repulsion among them and good polydispersity. The nanoliposomes also showed sustained release of chia oil at simulated gastric condition and intestinal condition (Choudhary *et al*., 2021) [4, 5].

3.2. Physicochemical properties of CO fortified curd

3.2.1. Acidity, pH, and colour

The acidity of control curd and fortified curd were found to be

0.77±0.01% and 0.73±0.02%, respectively; whereas pH of control and fortified curd were 4.38±0.07 and 4.65±0.09, respectively (Table 1). There was no significant difference between acidity and pH of control and nanoliposome fortified curd. The study conducted by Goyal *et al.*, (2016) [7] for fortification of curd with encapsulated flaxseed oil was also reported no significant change in pH and acidity. Veena *et al.* (2014) also reported no significant change in acidity and pH of flaxseed oil emulsion fortified curd. The increase in pH was also reported for the flaxseed powder fortified yogurt sample by Marand *et al.*, (2020) [30]. Colour of curd is related visual appearance which is very import for judging the quality of curd. There was no significant difference between colour values of control curd and fortified curd. Liutkevicius *et al.* (2010) [13] also reported no significant difference in colour of control and polyunsaturated omega-3 fatty acids fortified curd.

Table 1: pH and acidity of control and chia oil nanoliposome fortified curd

Characteristics	Product	
	Control curd	**Fortified curd**
pH	4.38±0.07^a	4.65±0.09^a
Titratable acidity (% lactic acid)	0.77±0.01^a	0.73±0.02^a
Syneresis (%)	14.77±0.25^a	20.03±0.93^b
Color		
L*	91.07±0.07^a	90.90±.07^a
a*	-1.85±0.41^a	-1.50±0.07^a
b*	13.31±0.28^a	13.26±0.01^a

*Different letters in a row indicate significant difference at $p \leq 0.05$

3.2.2. Flow behaviour
Flow behaviour is an important quality parameter which influence the acceptability of curd. The flow behaviour of control curd sample was similar to that of the fortified curd as presented in Figure 2. The maximum apparent viscosity of control and fortified curd were 57.5 and 50.9 Pa.S at 2.14 shear rate. Slight decrease in initial viscosity may be due to reduction in solid content after addition of nanoliposomes. Control and fortified curd showed shear thinning behaviour. With increasing shear rate the apparent viscosity of control and fortified curd increased, which became constant after reaching certain shear rate. This is owing to the breakdown of curd's gel structure with increases and achieve equilibrium after particular shear rate. This behaviour was also reported by Choudhary *et al.* (2021b) [4, 5] for control curd and curd prepared with ultrasound treated milk.

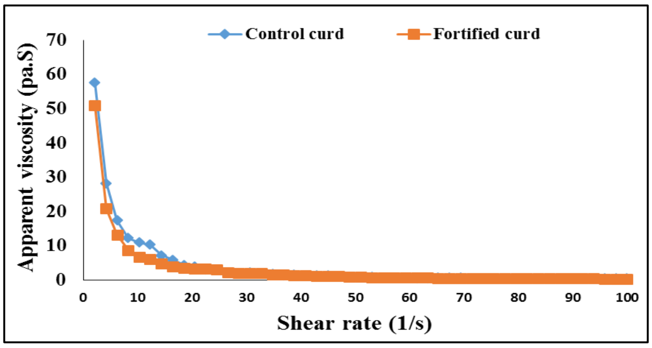

Fig 2: Flow behaviour of control Indian curd and chia oil nanoliposome fortified curd

3.2.3. Syneresis
Syneresis is an important physical parameter to evaluate the quality of curd. The change in syneresis after fortification was evaluated. The syneresis of control and fortified curd were A significant change in syneresis was observed between control and fortified curd (Table 1), probably owing to the additional liquid medium through nanoliposome suspension. Goyal *et al.* (2016) [7] also reported significant change in syneresis of encapsulated flaxseed oil fortified curd (Goyal *et al.*, 2016) [7]. The resulted reported by Ghorbanzade *et al.*, (2016) [6] for omega-3 fatty acid fortified also observed significant changes in syneresis. Syneresis is also influenced by various factors such total solid, fat content, protein content and nature of protein present in the sample (Farnsworth *et al.*, 2006) [29].

3.3 Fatty acid profile
The fatty profile presented in Table 2 showed that saturated fatty acid content of control and fortified curd were 94.52% and 85.92%, respectively; whereas monounsaturated fatty acid was 1.37% and 4.16%, respectively. The increase in unsaturated fatty acids probably was contributed by CO nanoliposomes. In fortified curd decanoic acid, lauric acid, myristic acid, palmitic acid and stearic acid decreased, however unsaturated fatty acids such as oleic acid, linoleic acid and α-linolenic acid increased. Marand *et al.* has also reported a decrease in saturated fatty acid and increase in unsaturated fatty acid content in flaxseed powder enriched yogurt (Marand *et al.*, 2020) [30]. The increase in α-linolenic content for encapsulated flaxseed oil fortified curd was also reported by Goyal *et al.*, (2016) [7].

Table 2: Fatty acid profile of control and chia oil nanoliposome fortified curd

Fatty acids	Control	Fortified curd
Decanoic acid	4.05^a	3.30^b
Lauric acid	5.26^a	4.40
Myristic acid	15.24^a	14.20^a
Palmitic acid	42.20^a	40.90^a
Palmitoleic acid	0.27^a	2.71^b
Stearic acid	10.08^a	3.48^b
Oleic acid	19.34^a	21.8^a
Linoleic acid	1.90^a	3.58^b
Linolenic acid	2.17^a	5.95^b

*Different letters in a row indicate significant difference at $p \leq 0.05$

4. Conclusions
Chia oil was successfully encapsulated in nanoliposome with encapsulation efficiency of 88.31±0.64%. The nanoliposome fortified curd showed no significant change in pH, acidity, colour and flow behaviour from the control curd. However, the syneresis of fortified curd were significantly increased. Fatty acid profile of fortified curd was improved significantly along with α-linolenic acid content that enhanced from 2.17% to 5.95%. This formulation can be used for the supplementation of α-linolenic acid.

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6. References
1. Agarwal KN, Bhasin SK. Feasibility studies to control

acute diarrhoea in children by feeding fermented milk preparations Actimel and Indian Dahi. European Journal of Clinical Nutrition 2002;56(4):S56-S59.
2. Bnyan R, Khan I, Ehtezazi T, Saleem I, Gordon S, O'Neill F, *et al*. Surfactant effects on lipid-based vesicles properties. Journal of pharmaceutical sciences 2018;107(5):1237-1246.
3. Chen X, Zou LQ, Niu J, Liu W, Peng SF, Liu CM. The stability, sustained release and cellular antioxidant activity of curcumin nanoliposomes. Molecules 2015;20(8):14293-14311.
4. Choudhary P, Rawson A. Impact of power ultrasound on the quality attributes of curd and its fermentation/gelation kinetics. Journal of Food Process Engineering 2021, e13698.
5. Choudhary P, Dutta S, Moses JA, Anandharamakrishnan C. Nanoliposomal encapsulation of chia oil for sustained delivery of α- linolenic acid. International Journal of Food Science & Technology 2021.
6. Ghorbanzade T, Jafari SM, Akhavan S, Hadavi R. Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt. Food chemistry 2017;216:146-152.
7. Goyal A, Sharma V, Sihag MK, Singh AK, Arora S, Sabikhi L. Fortification of dahi (Indian yoghurt) with omega-3 fatty acids using microencapsulated flaxseed oil microcapsules. Journal of food science and technology 2016;53(5):2422-2433.
8. Hao Y, Zhao F, Li N, Yang Y. Studies on a high encapsulation of colchicine by a niosome system. International journal of pharmaceutics 2002;244(1-2):73-80.
9. Jin HH, Lu Q, Jiang JG. Curcumin liposomes prepared with milk fat globule membrane phospholipids and soybean lecithin. Journal of dairy science 2016;99(3):1780-1790.
10. Karthik P, Anandharamakrishnan C. Enhancing omega-3 fatty acids nanoemulsion stability and *in-vitro* digestibility through emulsifiers. Journal of Food Engineering 2016;187:92-105.
11. Latifi S, Tamayol A, Habibey R, Sabzevari R, Kahn C, Geny D, *et al*. Natural lecithin promotes neural network complexity and activity. Scientific reports 2016;6(1):1-9.
12. Lavanya MN, Kathiravan T, Moses JA, Anand harama krishnan C. Influence of spray-drying conditions on microencapsulation of fish oil and chia oil. Drying Technology 2019.
13. Liutkevičius A, Speičienė V, Alenčikienė G, Mieželienė A, Zaborskienė GJFC, Kazernavičiūtė R. Impact of polyunsaturated omega-3 fatty acids on the quality of curd and curd products. Food Chemistry and Technology (Maisto Chemija ir Technologija) 2010;44(1):31-40.
14. Malakar J, Sen SO, Nayak AK, Sen KK. Formulation, optimization and evaluation of transferosomal gel for transdermal insulin delivery. Saudi pharmaceutical journal 2012;20(4):355-363.
15. Meng H, Xu Y. Pirfenidone-loaded liposomes for lung targeting: preparation and *in vitro*/*in vivo* evaluation. Drug design, development and therapy 2015;9:3369.
16. Mishra D, Garg M, Dubey V, Jain S, Jain NK. Elastic liposomes mediated transdermal deliveryof an anti-hypertensive agent: propranolol hydrochloride. Journal of pharmaceutical sciences 2007;96(1):145-155.
17. Mozafari MR, Khosravi-Darani K, Borazan GG, Cui J, Pardakhty A, Yurdugul S. Encapsulation of food ingredients using nanoliposome technology. International Journal of Food Properties 2008;11(4):833-844.
18. Munin A, Edwards-Lévy F. Encapsulation of natural polyphenolic compounds; a review. Pharmaceutics 2011;3(4):793-829.
19. Patel R, Singh SK, Singh S, Sheth NR, Gendle R. Development and characterization of curcumin loaded transfersome for transdermal delivery. Journal of pharmaceutical sciences and research 2009;1(4):71.
20. Rasti B, Jinap S, Mozafari MR, Yazid AM. Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods. Food chemistry 2012;135(4):2761-2770.
21. Segura-Campos MR, Ciau-Solís N, Rosado-Rubio G, Chel-Guerrero L, Betancur-Ancona D. Physicochemical characterization of chia (Salvia hispanica) seed oil from Yucatán, México. Agricultural Sciences 2014.
22. Serra M, Trujillo AJ, Guamis B, Ferragut V. Evaluation of physical properties during storage of set and stirred yogurts made from ultra-high pressure homogenization-treated milk. Food hydrocolloids 2009;23(1):82-91.
23. Teng J, Hu X, Wang M, Tao N. Fabrication of chia (*Salvia hispanica* L.) seed oil nanoemulsions using different emulsifiers. Journal of Food Processing and Preservation 2018;42(1):e13416.
24. Upadhyay N, Goyal A, Kumar A, Ghai DL, Singh R. Preservation of milk and milk products for analytical purposes. Food reviews international 2014;30(3):203-224.
25. Veena N. Development and evaluation of milk fortified with omega-3 fatty acids, phytosterols and soluble dietary fiber. PhD thesis submitted to National Dairy Research Institute, Karnal, India 2014.
26. Viriyaroj A, Ngawhirunpat T, Sukma M, Akkaramongkolporn P, Ruktanonchai U, Opanasopit P. Physicochemical properties and antioxidant activity of gamma-oryzanol-loaded liposome formulations for topical use. Pharmaceutical development and technology 2009;14(6):665-671.
27. Wang Q, Lv S, Lu J, Jiang S, Lin L. Characterization, stability, and *in vitro* release evaluation of carboxymethyl chitosan coated liposomes containing fish oil. Journal of food science 2015;80(7):C1460-C1467.
28. Zimet P, Livney YD. Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for ω-3 polyunsaturated fatty acids. Food Hydrocolloids 2009;23(4):1120-1126.
29. Farnsworth JP, Li J, Hendricks GM, Guo MR. Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. Small Ruminant Research 2006;65(1-2):113-121.
30. Marand MA, Amjadi S, Marand MA, Roufegarinejad L, Jafari SM. Fortification of yogurt with flaxseed powder and evaluation of its fatty acid profile, physicochemical, antioxidant, and sensory properties. Powder Technology 2020;359:76-84.