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Development and characterization of stable flaxseed oil emulsion prepared using soy protein isolate and modified starch as a potent source of omega-3 fatty acid fortificant in dairy & food products

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Abstract

A large population in country like India are vegetarian. Lack of certain essential components in vegetarian foods like omega-3 fatty acids have been shown in many research studies. Omega-3 fatty acids have been associated with reduced risk of several health diseases. Flaxseed oil is one of the richest vegetarian sources of omega-3 fatty acids. However, due to high susceptibility to oxidation, the use of flaxseed oil is limited in food and dairy products. This problem can be overcome by encapsulating flaxseed oil where a protective coat is formed on oil droplets. In the present study, effect of soy protein isolate (SPI) and modified starch (NC 46) as coating material for flaxseed oil emulsions and microcapsules were evaluated. Emulsion of flaxseed oil was prepared at varied level of oil i.e. 25, 30 and 35% of the total solids (TS) while the overall TS was maintained at 20, 25 and 30%. Emulsions were prepared by homogenization using Ultra-Turrax at 18000 RPM for 5 minutes. Among all the emulsion samples, the emulsion with 30% oil load and 30% TS was found most stable in terms of low creaming index (2.673%), narrow particle size distribution, high oxidative stability and high zeta potential (38.5mV). The development has potential application in successful Omega-3 fatty acids fortification in dairy and food products.

Keywords: Characterization of stable flaxseed oil emulsion, soy protein isolate, modified starch, dairy & food

1. Introduction

Omega-3 (ω -3) fatty acids and alpha linolenic acids (ALA) are considered to be functional ingredients owing to several physiological health benefits. Some of the health benefits of omega-3 fatty acids include prevention of cardiovascular disease, hypertension, hypocholesterolemic effect, anti-inflammatory properties [12, 4]. The estimated global demand for omega-3 fatty acid ingredients was USD 1595 million in 2010 and is expected to increase by about 2.5 folds, i.e. more than USD 4000 million in 2018 [5]. The rich sources of omega-3 fatty acids include fish oil, flaxseed oil, algal oil, canola oil etc. Although fish is the greatest contributor of ω -3 (EPA and DHA) but the vegetarian diets do not include enough fish products to meet dietary recommendations of ω -3 fatty acids. Flaxseed oil can be used to rescue such vegetarian populations as it is the richest vegetarian source of ω -3 fatty acids.

Flaxseed (*Linum usitatissimum*) oil, also known as linseed oil, is the rich source of ω -3 fatty acids, having 50-60% α -linolenic acid (ω -3, C18:3). Flaxseed oil contains both omega-3 and omega-6 fatty acids, which are needed for healthy operation of many bodily functions. Flaxseed oil comprises of essential fatty acid; alpha-linolenic acid (ALA) which our body converts into Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA). However, due to its high polyunsaturation (>73%), flaxseed oil is extremely susceptible to oxidation at high temperature, which leads to the production of toxic hydro-peroxides and off flavour during processing and handling of the products. Hence, flaxseed oil cannot be used as cooking oil. Stabilization of the flaxseed oil for its food applications is a challenging job. This can be achieved by stabilizing the flaxseed oil in emulsion form.

Emulsion droplet stabilization is often achieved through the addition of amphiphilic molecules such as emulsifiers, which decrease the interfacial tension between the phases and increase the steric hindrances and/or the electrostatic repulsion between the droplets [11].

Oil-in-water emulsions easily disperse into food systems such as beverages, dairy products, salad dressing, bakery products in which the as such added oil is separated out during processing or storage. Emulsions are thermodynamically unfavourable systems that tend to break down over time due to variety of physico-chemical mechanisms [29]. However, the kinetic stability of the emulsions can be improved by incorporation of certain emulsifiers and/or stabilizers. The soy protein isolates (SPI) can be used as coat material due to their superior emulsifying and stabilizing properties. It was reported that higher stability of oil-in-water emulsions prepared with SPI when compared to starch and Gum Arabic based emulsions [1, 25]. Further, the combination of proteins with carbohydrates as a carrier material favours better protection, oxidative stability and drying properties of emulsions [3]. In addition to proteins, modified starches also form uniform film around the core material thus stabilizing the emulsion. Octenyl Succinylated starches are amphiphilic in nature because of hydrophobicity introduced by Octenyl succinic anhydride (OSA) group in the hydrophilic starch backbone. These starches can thus be suitably used as efficient emulsifiers [40]. Both SPI and modified starches are quite stable, easily available, and affordable with effective functional properties. It has potential application for emulsion preparation of essential fatty acids.

Various oil-in-water emulsions were studied by authors using soybean, fish, corn, orange peel oil, rapeseed oil emulsion [12, 22, 37, 39] but studies are limited on preparation of flaxseed oil emulsion [22, 16]. Further, reports on combination of soy protein isolate and modified starches for flaxseed oil emulsion are scanty. With this background, the present study was planned to develop stable flaxseed oil emulsion using SPI and modified starch, which could be used as a potential fortificant of omega-3 fatty acids in various food products. The effect of oil load and total solid concentration on various emulsion characteristics such as zeta potential, droplet size, rheological properties was studied.

2. Material and methods

2.1. Material

Cold pressed flaxseed oil was received from AAK Kamani Pvt. Ltd. Andheri, Mumbai (Maharashtra). Soy protein isolate (SPI) with 90.5% protein was procured from Shridurga Sales Corporation, Bangalore, India. The specification of SPI as mentioned in the certificate of analysis given by manufacturer is given in Table 1. Modified starch (OSA modified tapioca maltodextrin) was obtained from Ingredion, Ingredion India Private Ltd, Thane Maharashtra. Other chemicals were of AR grade purchased from Sigma and Himedia, India

2.2. Preparation of emulsions

For preparation of flaxseed oil emulsion, flaxseed oil, soy protein isolates and modified starch (NC 46) were mixed in calculated amount by using high speed blender (Phillips, India) for approximately 5 minutes and then the prepared solution was homogenised using high shear mixture (IKA ULTRA-TURRAX T-18, Germany) in order to obtain stable emulsion and stored at lower temperature (4-7°C). It is a high shear mixer with speed range: 3000 - 25000 rpm and volume range: 1mL to 1500 ml. Concentration of SPI in emulsion was kept constant at 5% of total solids based on preliminary trials. Finally, The% TS of emulsion was maintained at 20, 25, and 30% using modified starch with various concentration of oil at 25, 30, 35% level of total solids (Table 2).

2.3. Physico-chemical characterization of emulsion

2.3.1. Creaming stability

Immediately after preparation, 15 mL of each emulsion was poured into a centrifuge tube (internal diameter = 11 mm, height = 94 mm), sealed with a plastic cap and centrifuged at 2000 rpm for 10 min. The emulsion stability was measured by the change in height of the bottom serum phase (H) with storage time. The creaming index (CI) was determined according to Eq. (1). The analysis was carried out in triplicate.

$$\text{Creaming index} = \frac{H}{H_0} \times 100 \quad (1)$$

Where,

H represents the separated phase of emulsion

H₀ represents the initial height of the emulsion

2.3.2. Physical stability

Samples of each emulsion were stored in 50 mL glass bottles (internal diameter = 45 mm, height = 70 mm) stored at refrigerated temperature (4-7°C) for 28 days and the emulsions were visually observed for any separation of oil. To facilitate visualization of the phase separation, Sudan III (red coloured dye) was added to the flaxseed oil before preparing the emulsion.

2.3.4. Particle size distribution and zeta (ζ) - potential

The electric charge and size of flaxseed oil emulsion droplets were measured using Zetasizer Nano Series ZS90 (Malvern Instruments Ltd., UK). About 1 mL of the emulsion was added to 99 mL of distilled water at 25 °C to measure the particle size. The emulsions were analysed 1 day after their preparation. Emulsion droplet size is expressed as Z-average diameter (nm) and ζ-potential in mV. The particle size distribution curves are expressed as percent intensity Vs diameter (nm).

2.3.5. Rheological measurements

Steady shear measurements were performed using a dynamic rheometer (Anton Paar Rheometer, MCR-52, Austria, Europe). The probe with 75 mm dia, 1° cone angle having cone-and-plate geometry (CP 75/1°) was used for viscosity measurements. Emulsion viscosity was measured at 25±0.1 °C, over a range of shear rate from 5-100/s. Viscosity was measured every 7th day till 28 days of storage at low temperature (4-7 °C).

2.3.6. Oxidative stability

The selected emulsion sample was evaluated for oxidative stability every 7th day till 28 days of storage at low temperature (4-7 °C).

2.3.6.1. Peroxide value

To determine the peroxide value, oil was extracted from emulsion by the method of with slight modifications [14]. Twenty grams of sample was mixed with 200 mL cold mixture of chloroform: methanol (2:1) in a separating funnel. After shaking gently for 3 min, mixture was allowed to stand for 10 min. A lower chloroform layer was removed separately. Upper layer was washed with 100 mL of chloroform: methanol (2:1) mixture and again lower chloroform layer was removed and mixed with previous one followed by mixing with 40 mL distilled water. After phase separation, lower chloroform layer was collected, passed through anhydrous sodium sulphate and dried using flash

evaporator (Metrex Scientific Instruments, India) under vacuum at 40 °C. Peroxide value of extracted oil was evaluated at every week during storage of 28 days by the standard iodometric method^[2]. Peroxide value was calculated according to the following formula (4).

$$\text{Peroxide value (milli eq/Kg oil)} = \frac{V \times N \times 100}{\text{Wt. of sample}} \quad (4)$$

2.3.6.2. Free fatty acids (FFA) content

Thirty millilitres of emulsion sample was taken in a 500 mL glass stoppered conical flask. To this 100 mL of freshly prepared extraction mixture (isopropanol, petroleum ether and 4 N sulphuric acid in the ratio of 40:10:1, respectively) was added followed by 50 mL petroleum ether. The conical flask was stoppered and shaken vigorously for 25-30 s and then it was transferred to separating funnel allowed to settle for 10-15 min or until the two layers got clearly separated. Solvent was evaporated in waterbath at 50°C. Separated fat was taken for titration by noting the weight, six drops of 1% methanolic phenolphthalein were added to it and titrated against 0.005 N methanolic potassium hydroxide (KOH) solution. A blank using 5 mL of water instead of milk was used to obtain the blank titre value. The FFA content in micro equivalents per mL emulsion was obtained using the following formula (5):

$$\text{FFA (}\mu\text{Eq/ mL)} = \frac{N}{5P} \times 1000 \times (V_2 - V_1) \quad (5)$$

Where,

N=Normality of KOH solution

P=Proportion of upper layer in separating funnel

V₂=Volume of standard KOH solution used for the milk sample

V₁=Volume of standard KOH solution used for the blank

2.4. Statistical analysis

All the data were analysed and expressed as mean of three replicates and standard deviations were calculated. All statistical analyses were performed using SPSS software and statistical significance was set at $p < 0.05$. The least significant difference (LSD) test was used to find out significant differences between sample means. Two way analysis of variance (ANOVA) was used to determine differences among treatment means using the Post Hoc Test (Duncan) to study the effect of total solids and oil load on emulsion properties..

3. Results and discussion

3.1. Physical stability and creaming index

Amount of coat material, type of coat material and method of homogenisation play an important role for stabilization of emulsion. During homogenisation the dispersed core material is covered by an encapsulating agent and protects the core from coalescence. Lack of coat material between two droplets may cause irreversible bridging between two adjacent droplets, however the more coat material may negatively affect properties of emulsion.

Prepared emulsion were analysed for creaming stability and physical stability. The results are described in Table 3. The creaming index values for emulsion samples varied from 2.88±0.38 to 9.80±0.35%. Emulsion with 30% TS and 25% oil load having maximum creaming stability followed by 30% TS and 30% oil load. While emulsion with 35% oil load and 20% TS having least stability. The creaming index values were significantly ($p < 0.05$) different for changing TS and oil load. The creaming index decreased significantly ($p < 0.05$)

with increasing TS, while it increased significantly ($p < 0.05$) with increasing oil load. This can be due to the lack of sufficient coat material (encapsulating agent), which causes sharing of core material (active material) and leads to irreversible bridging flocculation^[11]. Further, more coating material may increase surface load and adversely influences properties of emulsion, therefore very high concentrations of coat material were avoided^[28].

Based upon visual observation, E2, E3, E6 and E9 emulsion samples were found physically stable up to 28th day on storage at 4°C. This could be attributed to higher total solids content in these samples. Similar findings were reported where 7% WPC 80 with 12% oil load was stable up to 28th day of storage^[16]. Researcher reported a small separation (16.8%) after 24 hours of homogenisation in flaxseed oil emulsion encapsulated by Maltodextrin and WPC with ratio 25:75^[6].

3.2. Droplet size

Droplet size governs the emulsion based food properties like texture, appearance, shelf life etc. The mean droplet diameter (Z average size) varied significantly ($p < 0.05$) with the concentration of core and coat material (Table 4). It was observed that with the increase in oil load, the particle size increases significantly ($p < 0.05$), while with increasing TS, the particle size decreased considerably. The emulsion having oil load of 25% and total solid 30% had lowest mean droplet diameter value of 701.80±103.00 nm and the one with 35% oil load and 30% total solid had highest mean droplet diameter value of 2074.66±100.00 nm. Researchers prepared flaxseed oil emulsion using cross-linked sodium caseinate and reported mean droplet diameter of 1.6 μm (1600 nm) at zero day^[27]. The mean droplet size of flaxseed oil emulsions prepared in the present study are quite lower as compared to refined vegetable oil emulsions prepared with soy protein isolate in some reported study which had average particle droplet size of 4170 nm^[1]. Similar results were found by researcher who prepared emulsion with 5% WPC-80^[16]. In a reported study rapeseed oil: WPC emulsions with particle size ranging from 122.4 to 342 nm and 458 to 2,669 nm was found^[12]. Emulsion showing particle size distribution with a single peak and in a narrow range would be the most homogenous and physically stable. In this case, the emulsion prepared with 30% TS and 30% oil load showed such particle size distribution.

3.3. Zeta (ζ) potential

The charge on droplet can influence the rheological properties of an emulsion. Emulsions with high zeta-potential (negative or positive) are electrically stabilized while emulsions with low zeta-potential may be coagulate or flocculate. The ζ potential represents the charge of the droplets with adsorbed protein and/or biopolymer, positive charge is associated with any ions that move along with the droplet in the electric field^[38]. Table 4 summarizes the ζ -potential of the emulsion droplets as a function of concentration of the encapsulating agent. Emulsion having 30% oil load and 30% TS having highest ζ potential -36.83±2.18 mV. Thus can be interpreted that the emulsion having highest physical stability among all prepared samples. It was reported that ζ-potential on emulsion droplets, and the range varied from -28.6 to -33.5 mV^[16]. Similar findings have been reported by authors who studied the different oil-in-water emulsions and observed negative zeta potential^[7, 32, 35]. Higher ζ-potential with increase in total solids in emulsions could be explained by the maximum

utilization of coat material for the coverage of oil droplets. Similar results were reported by researchers for the soybean oil and flaxseed protein emulsions [42]. They reported that ζ -potential of the emulsions varied from -30.7 to -49.5 mV, with significant increase with flaxseed protein concentration. It was reported that higher negative zeta potential values prevent aggregation of emulsion droplets and increases stability through electrostatic repulsion [1]. Thus it can be concluded from the results that ζ -potential of the flaxseed oil emulsion produced by using 30 oil load of 30% TS was the most appropriate for its stability.

3.4. Rheological characteristics

Rheological properties of an emulsion useful during the processing conditions (like pumping, mixing, flowing in pipes) or in designing a delivery system for a particular food application. Apparent viscosity (cP) under the shear rate ($5-100$ s⁻¹) for emulsions having different concentration of SPI and modified starch (NC 46) during storage of 28 days was analysed. In present study, the emulsion shows decrease in viscosity with increasing shear rate, reflecting the non-Newtonian Pseudoplastic (shear thinning) nature. Viscosity increased with increase in total solid but decreased with increase in oil content at same TS level. Viscosity also increased during the storage period. This phenomenon may be attributed to the fact that flocculation between the droplet of emulsion occurred. Results reported by researchers suggest that the rheology of concentrated emulsions (viscosity) is fairly dependent on droplet concentration, size and shear rate, as well as the electrostatic repulsion between droplets [7]. Figure 1.0, and 2.0 shows that at lower shear rate ($10-20$ s⁻¹), high viscosity was observed irrespective of the emulsion composition. Similarly, high viscosities at low-shear rates have been reported for emulsions stabilized by soy protein isolate [1]. Shear-thinning may occur for a variety of reasons in food emulsions (e.g., the spatial distribution of the particles may be altered by the shear field, or temporary flocs may be deformed and disrupted. Researchers also reported shear thinning behaviour at lower shear rate (<100 s⁻¹) for rapeseed oil and soybean oil emulsions respectively [12, 42]. However, it was found that corn oil-in-water emulsions (50 g oil/ 100 g) stabilized by WPC presented a Newtonian behaviour [26]. Shear thinning behaviour can be observed due to irreversible deformation and breakdown of flocs under the shear stress [28]. The flow curves data for all the emulsions fitted well to the power law model equation. Researchers studied the flaxseed oil emulsions stabilized by whey protein isolates (total solids 33%) and reported that all O/W emulsions showed very low pseudoplasticity with increase in concentration of whey proteins, suggesting the increase in viscosity and droplets concentration [22]. Shear thinning behaviour was also observed by researchers who worked on whey protein isolate stabilized oil-in-water emulsions [37]. Pseudoplastic behaviour is the most common type of non-ideal behaviour exhibited by food emulsions.

3.5. Oxidative stability

Encapsulated oil possess more oxidative stability than the bulk oil. The progress in oxidation can be measured by production of primary oxidative products like lipid hydroperoxide and free fatty acids. Thus the peroxide value and Free fatty acid contents were measured for the developed emulsion for 28 days of storage at 4-7 °C. From Table 5.0,

there was no significant difference ($p < 0.05$) in peroxide value of flaxseed oil (Control) and emulsion was observed (2.62 mEq peroxide/Kg oil). While it gradually increased up to 6.20 and 5.06 mEq peroxide/kg oil in control and selected emulsion respectively. While the FFA contents increased from 0.80 to 1.53 μ Eq/ mL in control and in emulsion 0.80 to 1.37 μ Eq/ mL with storage at 4-7 °C (Figure 3.0). Similar findings were reported regarding increase in Peroxide value in emulsion and free flaxseed oil ~ 20.98 and $\sim 44.56\%$ respectively when prepared with 5% WPC [16]. The present results are in agreement with workers who reported improved oxidative stability of flaxseed oil (in powder form) encapsulated by different proteins [17, 19, 33]. Peroxide value of flaxseed oil in emulsions (prepared with 7.5–12.5% WPC) was within the limit of up to 15 mEq peroxide/kg oil under the Codex Alimentarius Commission (1999) standard for cold-pressed and virgin oils [9]. The high stability of emulsions containing SPI as it has antioxidative properties and their ability to bind some pro-oxidant impurities (such as transient metals) due to presence of Histidine, Glutamic acid, Aspartic acid [29]; thus protecting oil against oxidation. It was reported that improved oxidative stability of flaxseed oil emulsions encapsulated by sodium caseinate cross-linked by Transglutaminase during 30 days of storage [27]. Researchers reported a significant increase in PV from 0 to 1.777 mEq peroxides/kg oil of flaxseed oil emulsion homogenized at 80 MPa [22]. It was reported that flaxseed oil encapsulated with Maltodextrin: HiCap (modified starch) and Maltodextrin: Gum Arabic presented peroxide values of 22.6 and 24.8 mEq peroxides/kg oil, respectively after 1 week of storage [5].

Table 1: Technical specifications of commercial soy protein isolate

Sensory organ index		
Item	Standard	Result
Appearance	Light yellow powder	Light yellow powder
Odour	Neutral to nutty	Neutral to nutty
Flavour	Pleasant nutty	Pleasant nutty
Physical and chemical index		
Crude protein	90.00% min	90.50
pH value	7.00 \pm 0.5	6.75
Moisture %	7.0 max	5.65
Ash(dry basis)%	6.0 max	5.10
Functional index		
Dispersability	Within 25 sec.	Within 20 sec.
Microbial index		
TPC	20000 max	1000
E. coli	Negative	Negative
Conclusion	Eligible	

Table 2: Oil load and total solid content for preparation of emulsion samples

Sr. No.	Oil Load (% of T.S.)	Total Solids (%)	Sample Name
			NC 46
1	25	20	A1
		25	A2
		30	A3
2	30	20	A4
		25	A5
		30	A6
3	35	20	A7
		25	A8
		30	A9

Table 3: Oil load and total solid content for preparation of emulsion samples

Oil Load (% of T.S.)	Total Solids (%)	Sample (NC 46 emulsions)	Creaming index (%)	Physical stability
25	20	A1	0.647±0.034 ^e	Stable
25	25	A2	0.334±0.001 ^h	Stable
25	30	A3	0.159±0.036 ⁱ	Stable
30	20	A4	6.889±0.385 ^b	Stable
30	25	A5	3.991±0.031 ^d	Stable
30	30	A6	2.673±0.010 ^f	Stable
35	20	A7	7.811±0.328 ^a	Stable
35	25	A8	5.333±0.000 ^c	Stable
35	30	A9	3.333±0.000 ^e	Stable

Results are expressed as Mean±SD, n=3; Means with different small letters superscript (a,b,c) within the column differ significantly ($p<0.05$) among the samples

Table 4: Zeta potential and average droplet size of flaxseed oil emulsion at refrigerated storage (4-7 °C)

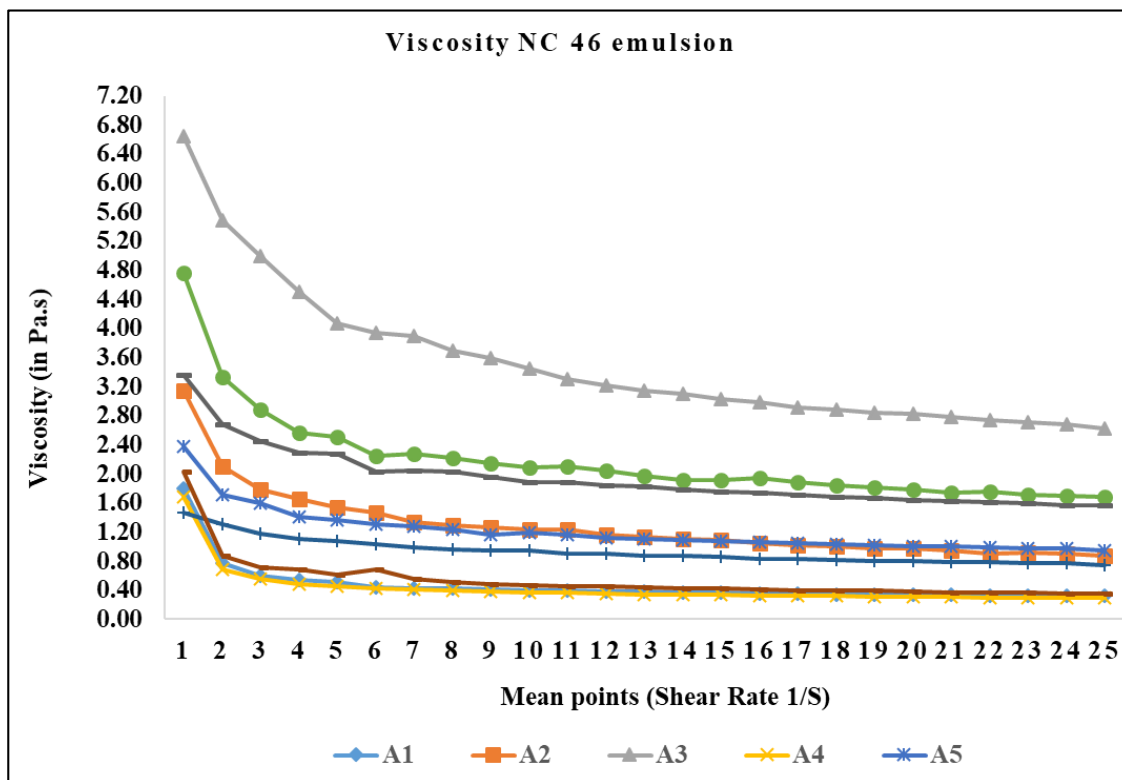
Oil Load (% of T.S.)	Total Solids (%)	Sample (NC 46 emulsions)	Zeta potential(mV)	Average droplet size (nm) (for NC 46 emulsions)
25	20	A1	-28.17±1.36 ^f	721.00±48.77 ^a
25	25	A2	-34.50±5.67 ^e	1360.13±407.35 ^{ab}
25	30	A3	-35.70±2.90 ^e	888.40±221.31 ^{cde}
30	20	A4	-37.93±3.61 ^c	1151.17±346.27 ^{de}
30	25	A5	-31.57±0.97 ^d	892.43±256.95 ^{ab}
30	30	A6	-38.50±2.26 ^d	1073.83±175.21 ^e
35	20	A7	-35.43±1.05 ^b	1119.47±267.11 ^{cd}
35	25	A8	-32.87±1.40 ^b	1219.00±302.91 ^{bc}
35	30	A9	-32.70±2.54 ^a	782.80±128.60 ^{bc}

Results are expressed as Mean±SD, n=3; Means with different small letters superscript (a,b,c) within the column differ significantly ($p<0.05$) among the samples

Table 5: Effect of storage (at 4-7 °C) on peroxide value (meq peroxide/kg oil) of flaxseed oil and emulsions

Sample	Storage period				
	Day 0	Day 7	Day 14	Day 21	Day 28
Oil (Control)	2.61±0.06 ^{eA}	3.30±0.04 ^{dA}	4.01±0.07 ^{cA}	5.05±0.04 ^{bA}	6.20±0.03 ^{aA}
N-Creamer 46 emulsion	2.60±0.03 ^{eA}	3.07±0.08 ^{dB}	3.35±0.03 ^{cB}	3.70±0.02 ^{bB}	4.02±0.06 ^{aB}

Results are expressed as Mean±SD, n=3; Means with different small letters superscript (a,b,c) within the rows and capital letters (A,B,C,..) within the column differ significantly ($p<0.05$) among the samples

**Fig 1:** Apparent viscosity (m Pa.s) of NC 46 emulsion samples with variable shear rate (5-100/S)

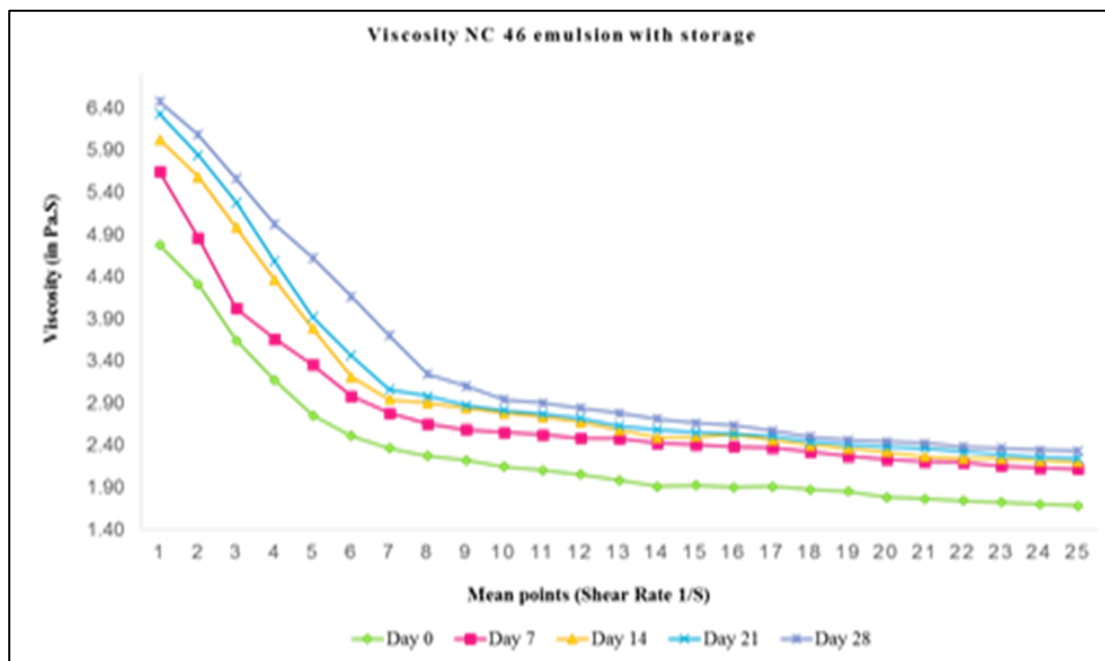


Fig 2: Apparent viscosity (m Pa-s) of selected (A6) NC 46 emulsion sample with variable shear rate during storage period of 28 days (5-100/S)

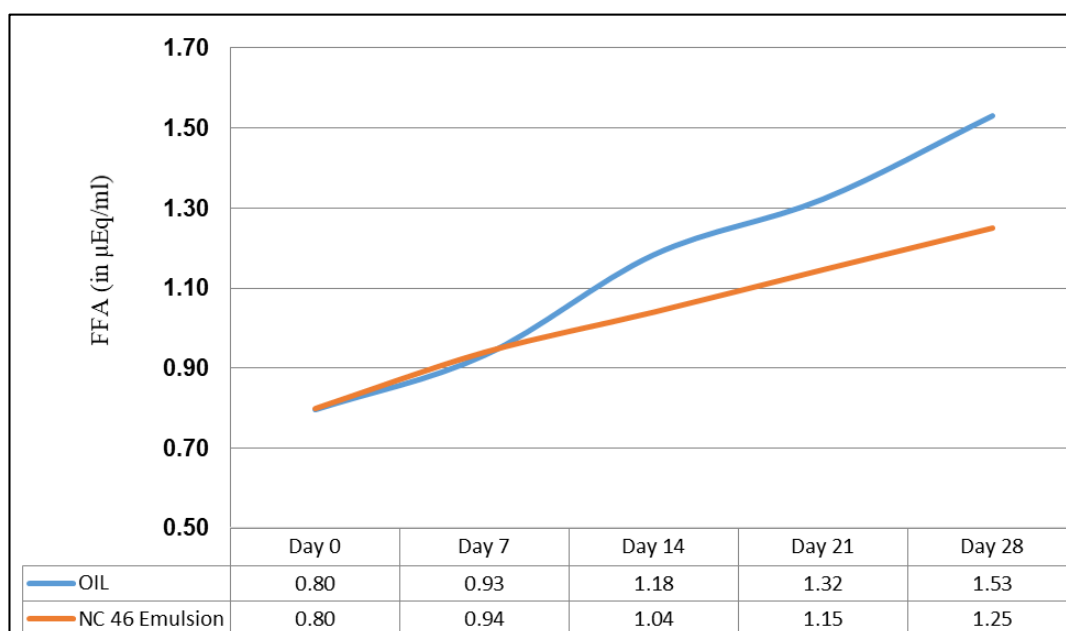


Fig 3: Effect of storage (at 4-7 °C) on FFA (in µEq/ml) of flaxseed oil and emulsions

4. Conclusion

It can be concluded from the study that flaxseed oil emulsions which are stabilized by soy protein isolates and modified starches at a level 30% oil load of 30% TS showed good physical stability with no sign of phase separation, when homogenized at 18000 rpm for 5 min and stored at low temperature (4-7 °C) for 28 days. A significant difference in peroxide and free fatty acid contents of free oil and the oil encapsulated by SPI and modified starches indicated better protection of flaxseed oil, which was not only due to the formation of interfacial film around the oil droplet, but also due to the antioxidant properties of SPI. Results indicated that emulsion containing 30% oil load of 30% TS showed the best results among all the emulsions with lowest droplet size and highest ζ potential. Rheological data revealed that all the emulsions showed shear thinning behaviour, which is a characteristic of food emulsions. Pseudoplastic behaviour

suggested the stability and suitability of emulsions during processing conditions.

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