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ORIGINAL ARTICLE

Storage Stability and Physicochemical Properties of Flaxseed Oil Microemulsions Stabilized with *N*-Octenylsuccinate-Derived Starch and Sodium Caseinate

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ABSTRACT

Background: Flaxseed oil is one of the richest sources of omega-3. The aim of this study was to investigate the effects of different wall materials, weight ratio (WR) and storage time on physicochemical properties and storage stability of flaxseed oil microemulsions.

Methods: Encapsulation efficiency and emulsion oxidative stability were measured. Fatty acid composition of oil extracted from emulsions was analyzed by gas chromatography (GC). Particle size distribution and morphology of microemulsions were measured by dynamic laser scattering (DLS) technique and scanning electron microscopy (SEM), respectively.

Results: The maximum encapsulation efficiency of 95.8% was obtained in the emulsion with the highest *n*-octenylsuccinate-derivatized (*n*-OSA) starch content. Increasing *n*-OSA starch concentration led to a higher microencapsulation efficiency and a lower lipid oxidation. Increasing *n*-OSA starch/sodium caseinate ratio led to a decrease in peroxide values and thiobarbituric acid contents. GC-FID results showed a superior stability of ω-3 fatty acids and improved nutritional quality in microencapsulated flaxseed oil upon storage. The average droplet size distribution of emulsions ranged from 428 to 728 nm. An increase in total solid content with the same oil concentration led to smaller droplets size. Morphological study performed by SEM confirmed the results obtained by DLS technique.

Conclusion: Our findings have important implications for the design and utilization of emulsions as delivery systems for food enrichment.

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Introduction

Flaxseed oil is one of the richest sources of omega-3 in the human diet due to its substantial amounts of unsaturated fatty acids. Oils rich in polyunsaturated fatty acids play an important role in the prevention of coronary artery disease (arteriosclerosis),

arthritis, hypertension, cardiovascular and immune response disorders, besides reducing the risk of stroke and cancer (1, 2). Flaxseed oil has a great potential as a supplement in functional food by increasing the omega 3 content. However, flaxseed oil due to its poly unsaturated fatty acids, has a high susceptibility to oxidation during processing and storage leading to loss of essential compounds (3).

Lipid oxidation leads to a reduction in shelf life, because of undesirable changes in the flavor, appearance and nutritional quality. Moreover, the generation of free radicals with negative physiological impacts on the organisms is enhanced (4). In order to increase the shelf life of oils, microencapsulating in a polymeric matrix to protect unsaturated fatty acids against lipid oxidation has been extensively investigated (5, 6). Interestingly, microencapsulation of oils not only can protect them against oxidative damages, but also can control the release of functional lipophilic components leading to an opportunity to formulate foods with polyunsaturated fatty acids (7).

Proteins and polysaccharides are used safely for microencapsulation, while changing the interface properties of the emulsion droplet, reduces the oxidative stability of oil-in-water emulsions (8). Researches have shown that oil droplets coated by protein-polysaccharide complexes have better physical and oxidative stability (9). Protein-coated droplets can be created by adding a polysaccharide phase that adsorbs onto the droplet surface and creates a protective layer, preventing droplets coalescence and aggregation (10). *n*-octenylsuccinate-derivatized (*n*-OSA) starch has also been widely used for oil microencapsulation and exhibits increasing the physicochemical stability of emulsions when compared to other polysaccharide. It is a modified starch with some side chains of lipophilic succinic acid with good emulsifying properties, which promotes good oil retention and higher encapsulation efficiency (11, 12).

Sodium caseinate can prevent lipid oxidation in emulsions when it is either at the surface of the emulsion droplet or in the aqueous phase (13). The droplet stability from aggregation increased by increasing the repulsive colloidal interactions between them, lowering interfacial tension and forming protective membranes around the oil (14). These wall materials are considered ideal to the encapsulation of lipid droplets, as they fulfill the roles of both surface-active agent and drying matrix (15). Encapsulation efficiency enhancement through preventing lipid oxidation and volatile losses as well as product shelf life extension have been particularly studied in recent years (16).

It is noteworthy to mention that the type of wall and core materials and properties (e.g. concentration), emulsion characteristics (e.g. droplet size, viscosity), and conditions of spray drying (e.g. inlet air temperature, air flow) are major parameters affecting the microencapsulation efficiency (MEE) of oils and flavors (17). Tonon et al. (18) studied the effect of combination of maltodextrin with different wall materials (gum arabic, whey protein, *n*-OSA starch and HiCap starch) on the microencapsulation of flaxseed oil by spray drying. The authors fixed the spray drying operational conditions and the emulsion composition, and evaluated the influence of relative humidity on the encapsulation efficiency (95%) and lipid oxidation of particles during storage. Barroso et al. optimized the microencapsulation process of flaxseed oil as a function of wall (*n*-OSA starch) to core material ratios and implicated that this factor and microencapsulation technique affected MEE to achieving the highest encapsulation efficiency (19).

Due to its high unsaturated fatty acids content, flaxseed oil can be easily oxidized during processing, handling, and distribution, leading to the formation of unpleasant tastes and odors and, consequently, to a reduction of product shelf life. Thus, the objective of this study was to study the influence of total solid content, wall material weight ratios and storage time on the flaxseed oil-in-water microemulsions stabilized by sodium caseinate and *n*-OSA starch to better understand the differences in the oxidative stability of emulsions. Encapsulation efficiency, fatty acids profile, emulsions morphology and particle size distribution were evaluated.

Materials and Methods

Flaxseed was dried in a spouted bed drier equipped with IR lamp at 80°C for 30 min. The flaxseed oil was extracted by a cold press using a screw press expeller (Cold press, D50 model, Esfahan, Iran). The resulting oil, which was used as a core material, had a fatty acid composition of 5.77% C16:0, 4.57% C18:0, 20.36 C18:1, 14.21% C18:2 and 53.12% C18:3. Sodium caseinate (Iran Caseinate co. Tehran. Iran) and the *n*-OSA starch (National Starch, Sao Paulo, Brazil) were used as wall materials. The powder composition (% w/w) of sodium caseinate was 89% protein and 5.4% moisture on wet basis. The *n*-OSA starch claimed to contain 0.5% protein and 3% moisture. Double distilled water was used in the preparation of all dispersions. Trichloroacetic acid (TCA) was provided by Samchun Pure Chemicals Co. Ltd. (Seoul, Korea). All other chemicals and solvents used in this study were of analytical reagent grade and purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO).

In order to prepare the emulsions, the wall

materials were dissolved in distilled water at 25°C containing 0.02% (w/w) sodium azide as an antimicrobial agent. The obtained mixture was stirred magnetically at 600 rpm for 3 h. To ensure complete hydration, it was kept at refrigerator (4°C) overnight. The flaxseed oil was slowly added into the aqueous phase with stirring until completely dissolved. The mixture was homogenized (T18 digital, ULTRA TURRAX, Deutschland, Germany) at a speed of 15000 rpm for 5 min followed by homogenization at 20000 rpm for 2 min. Table 1 shows six emulsion preparations with 10 and 15% total solids having different ratio of wall materials. The amount of oil was fixed at 5% (w/w) in all emulsions.

In order to measure the surface oil, 15 mL of hexane was added to 2 g of emulsion and was vortexed for 2 min at room temperature. Then, the obtained mixture was filtered via Whatman filter paper No. 1, and was rinsed three times with 20 mL of hexane (20). The filtrate containing the extracted oil was concentrated at 40°C using a vacuum rotary evaporator (DENA Co. Tehran. Iran). Then the low residual solvent was evaporated using a stream of nitrogen (17). As the flax seed oil was not volatile and there was no possibility of evaporation during the process, for measuring the microencapsulation efficiency, the total oil of final emulsions was considered as the initial amount of flaxseed oil used in the preparation of emulsions. Encapsulation efficiency (EE) was calculated as follows:

$$EE\% = Total\ oil - Surface\ oil/Total\ oil \times 100$$

The extraction of oil from emulsions for analysis was carried out by mixing a chloroform/methanol solvent (2:1, v/v) with the emulsion in a shaker for 1 min, followed by 2 min centrifugation at 3400 g (SW14R, Froilabo, Meyzieu, France). The nitrogen stream was used for evaporating solvent and purified the low layer extracted oil. The PV was measured spectrophotometrically at 500 nm by UV–VIS instrument (VIS-7220G/UV-9200, Rayleigh, China) after 5 min incubation at room temperature. To prepare the iron (II) chloride solution, 0.4 g barium chloride erlenmeye was dissolved in 50 ml water. This solution was added slowly and with constant stirring to an iron (II) sulfate solution (0.5 g FeSO4.7H2O dissolved in 50 ml water). Totally, 2 ml of 10 M HCl was added to the resulting solution.

The barium sulphate precipitate was filtered off to give a clear iron (II) solution, which was stored in a brown bottle and kept in the dark. To prepare the ammonium thiocyanate solution, 30 g ammonium thiocyanate was dissolved in water, and the volume was made up to 100 ml. To determine the peroxide value, the oil sample was mixed with 9.8 ml chloroform– methanol (7:3, v/v) on a vortex mixer for 2-4 s. Ammonium thiocyanate solution (50 µl) was added, and the sample was mixed on a vortex mixer for 2-4 s. Then, 50 µl iron (II) solution was added, and the sample was mixed on a mixer for 2-4 s. Hydroperoxide concentrations were determined using a Fe3 standard curve with iron concentration varying from 1 to 25 µg (21).

Thiobarbituric acid-reactive substances (TBARS) were considered as the indicator of secondary product formation via lipid oxidation. This value was determined according to the method described by Lee et al. (14). First, 100 ml of the prepared solution (75 g of TCA, 1.68 g of TBA, 8.8 ml of 12 M HCl, and 414 g of distilled water) and 3 ml of 2% (w/w) BHT in ethanol were successively added, and then 2 ml of the emulsion was mixed with 2 ml of this solution. The obtained mixture was heated in a boiling water bath for 15 min; then cooled to room temperature and finally centrifuged (SW14R, Froilabo, Meyzieu, France) at 1000 g for 10 min. The values of TBARS were measured using a spectrophotometer at 532 nm after 10 min and through a standard curve prepared by 1,1,3,3-tetraethoxypropane in a similar manner.

To determine fatty acids profile, 500 mg of the freeze- dried powder of each emulsion were mixed with 10 ml of methanol/acetyl chloride (95:5, v/v) solution. The mixture was sealed in a screw-capped test tube and heated to 85°C for 1 h. After cooling, 5 mL of distilled water was added and shaken for 5 min. Thereafter, 2 mL of hexane containing 0.01% tertiary butylhydroquinone (TBHQ) was added into the sample and finally centrifuged at 2000 g for 20 min. TBHQ was added to prevent the oxidation of double bonds during the isolation procedure. The hexane-containing lower phase, which contained most of the total lipids was transferred into a clean vial and injected to the gas chromatograph for quantitative and qualitative determination of FAME (22).

The GC chromatographic (B420A, BEIFEN, China) conditions was capillary column (BPX70, 60 m length, 0.25 mm inner diameter, and 0.25 mm film thickness) and a flame ionization detector (FID). The stationary and carrier phases were polymer biscyanopropylsiloxane- silphenylene and nitrogen gas, respectively. The column temperature was programmed from 90 to 210°C at 5°C/min and then remained constant for 25 min. Detector and injector temperatures were set at 300 and 250°C, respectively. In order to analyze the obtained chromatograms, the Chromatography Data Handling System software was applied.

To assess dynamic light scattering (DLS), droplet size was measured immediately after emulsion preparation. The droplet size distributions of the emulsions were measured using a laser light

diffraction instrument (Laser Diffraction Particle Size, SALD-2101, Shimadzu, Japan). A small amount of emulsion was suspended in deionized water (1:49 w/w) under agitation at ambient temperature. The droplet mean diameter was expressed as the volume weighted mean diameter (17). Droplet size and morphology of microemulsions were characterized using scanning electron microscopy (TESCAN Vega 3 model, Czech Republic) (18). Emulsions (1 g) were suspended under agitation at 25°C.

The results were reported as the mean±standard deviation using the SPSS software (ver. 22, IBM, New York). An Analysis of variance (ANOVA) was performed using the general linear models procedure to determine significant differences among the samples. Means were subjected to Duncan's multiple ranges tests. Differences of $P<0.05$ were considered to be significant. All experiments were carried out two times.

Results

Depending on the experimental run, surface oil content ranged from 0.21 to 0.34 g/100 g emulsion, which should be as low as possible. As emulsion was not dried to create capsule, total oil content was assumed to remain at 5%, equal to the initial oil content. The degree of oil entrapment in the emulsion was characterized by microencapsulation efficiency at a level from 93.24 to 95.84% (Table 2). An increase in the total content of carrier in the emulsions from 10 to 15%, resulted an increase in the MEE value. The MEE obtained in this study which was between 84.51- 93.25% was much higher than those reported by Carneiro et al. for flaxseed oil microencapsulation (6).

Figure 1 shows peroxide value of bulk flaxseed oil and microencapsulated flaxseed oil during storage at 4°C for 30 days. The peroxide values ranged between 0.67 to 0.78 mEq/kg oil at the first day, which continuously increased to 2.39 mEq/kg at the end of day 30th for sample 1. It is evident that there was a significant difference ($P<0.05$) amongst the PV of different emulsions and free flaxseed oil during storage. All the emulsions showed a gradual, but significant increase in PV during the storage, varying from 0.98 to 1.23 mEq/kg and 1.81 to 2.39 mEq/kg in 15 and 30 days, respectively. Figure 1 also shows that the PV of bulk oil increased at a faster rate as compared to the encapsulated oil. Lower solid content (10%) led to higher peroxide values (Figure 1).

The oxidative stability of emulsions during 30 days of storage at 4°C was determined through TBARS values (Figure 2), which demonstrated the formation of secondary reaction products (especially aldehydes e.g. malondialdehyde). A significant increase in TBARS values of emulsions was observed during the first day of storage followed by an increase in longer storage times, confirming the decomposition of primary oxidation products into secondary ones.

Table 3 shows the changes in the fatty acid profile of flaxseed oil, identified by GC-FID during storage time. The α-Linolenic acid (ALA) was the major fatty acid in flaxseed oil (49.48%) followed by oleic acid (21.65%). Regarding fatty acid composition, the flaxseed oil had high amounts of unsaturated fatty acids, which is much more susceptible to lipid oxidation. Fatty acid composition of flaxseed oil

Table 1: Phase separation of the emulsions prepared with different wall materials

Emulsion	%Wall material		
	%Total solid	Sodium caseinate	*n*-OSA starch
10% (2Cs/1Hp)	10	3.33	1.67
10% (1Cs/1Hp)	10	2.50	2.50
10% (1Cs/2Hp)	10	1.67	3.33
15% (2Cs/1Hp)	15	6.66	3.34
15% (1Cs/1Hp)	15	5.00	5.00
15% (1Cs/2Hp)	15	3.34	6.66

Table 2: Encapsulation efficiency of microcomplexes produced with different wall materials

Emulsion	%Surface oil	%Microencapsulation efficiency
10% (2Cs/1Hp)	0.338 ± 0.04^{a}	93.24 ± 0.82^{c}
10% (1Cs/1Hp)	0.249 ± 0.03^{c}	95.01 ± 0.66^{a}
10% (1Cs/2Hp)	0.214 ± 0.01^{c}	95.71 ± 0.24^{a}
15% (2Cs/1Hp)	0.330 ± 0.03^{ab}	93.40 ± 0.76^{bc}
15% (1Cs/1Hp)	0.269 ± 0.00^{bc}	94.62 ± 0.05^{ab}
15% (1Cs/2Hp)	0.208 ± 0.00^{c}	95.84 ± 0.08^{a}

For each sample, means in each column with different superscript letters are significantly different ($P<0.05$).

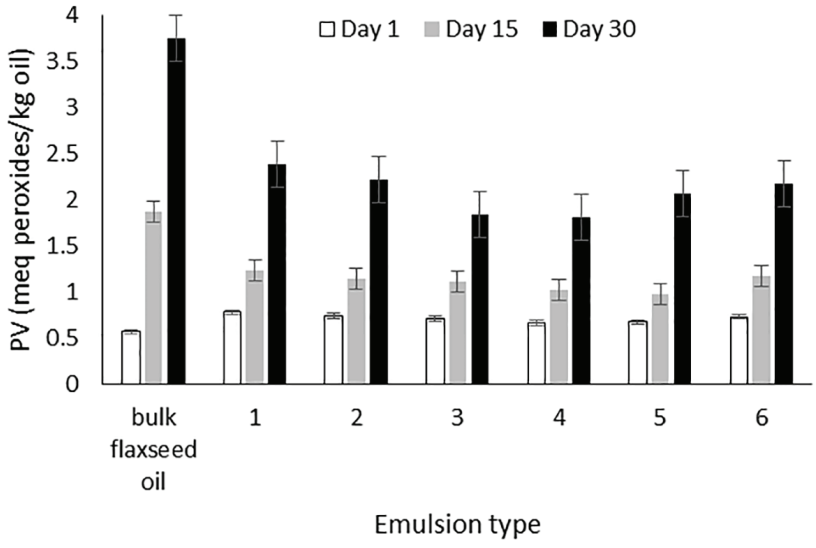

Figure 1: Peroxide values of bulk flaxseed oil and microcomplexes prepared with 10% (2Cs/1Hp), 10% (1Cs/1Hp), 10% (1Cs/2Hp), 15% (2Cs/1Hp), 15% (1Cs/1Hp) and 15% (1Cs/2Hp) summarized as 1-6, respectively, during 1 month storage at 4°C. Data are mean of triplicate runs. Error bars indicate SD values.

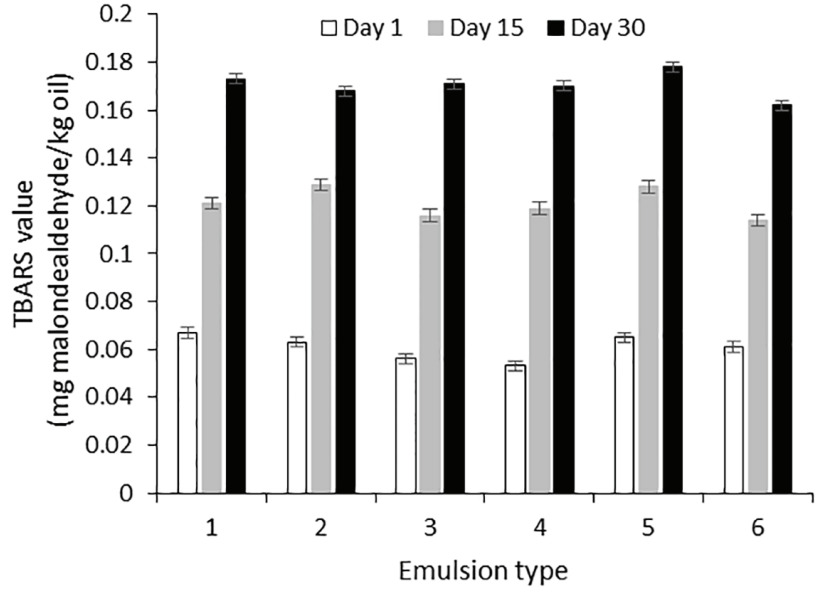

Figure 2: TBARS values of microcomplexes prepared with 10% (2Cs/1Hp), 10% (1Cs/1Hp), 10% (1Cs/2Hp), 15% (2Cs/1Hp), 15% (1Cs/1Hp) and 15% (1Cs/2Hp) summarized as 1-6, respectively, during 1 month storage at 4°C. Data are mean of triplicate runs. Error bars indicate SD values.

extracted from emulsions is shown in Table 3. As can be seen, an increase in the relative total amount of saturated fatty acids (ΣSFA) was observed during storage, which was promoted by slight decreases in ω-3 and ω-9 fatty acids, probably due to oxidation upon storage time. According to our result, 15% (1Cs/1Hp) and 15% (1Cs/2Hp) emulsions were the highest ω-3/ω-6 ratio and polyene index (3.08 and 6.65), respectively, at the end of storage period.

The average droplet size distribution after emulsions preparation ranged from 428 to 728 nm (Table 4). Because droplet diameter was higher than 100 nm, prepared dispersion called microemulsion. Table 4 shows the decrease of *n*-OSA starch content that may have promoted higher droplets coalescence in the emulsions studied, resulting in the formation of larger droplets. The increase in total solid content resulted in smaller droplets size, which can be attributed to the emulsion viscosity, i.e. more viscous emulsions were obtained with higher solid content. According to Table 4, all the emulsions showed a gradual but significant increase in droplets size during 30 days of storage.

The microstructure of the emulsions was analyzed at days 1 and 30th of storage using scanning electron microscopy (SEM). The emulsion droplets were characterized by spherical shape, smooth surface with no visible cracks, which causes better protection and retention of flaxseed oil. The results are shown in Figures 3(a) and 3(b) exhibited the micro-sized

Table 3: Changes in fatty acids profiles of bulk flaxseed oil and microcomplexes prepared during 1 month storage

Sample	Days	C16:0	C18:0	C18:1	C18:2	C18:3	ΣSFA	ΣUFA	UFA/SFA	ω-3/ω-6
Bulk flaxseed oil	D_1	7.21^{kl}	4.19^{l}	21.65^{l}	17.66^{a}	49.48^{a}	5.70^{l}	88.80^{a}	15.56^{a}	2.79^{g}
	D_{15}	8.66^{a}	6.65^{b}	24.95^{b}	14.83^{h}	45.01^{j}	7.26^{k}	84.79^{j}	11.06^{b}	3.03^{cd}
	D_{30}	8.84^{a}	7.36^{a}	25.48^{a}	13.75^{i}	44.60^{k}	8.10^{j}	83.84^{k}	10.34^{c}	3.24^{a}
10% (2Cs/1Hp)	D_1	7.72^{ghi}	5.12^{hi}	23.69^{hi}	15.56^{cde}	47.92^{d}	12.85^{fgh}	87.17^{cd}	6.78^{fgh}	3.07^{cd}
	D_{15}	7.89^{efg}	5.55^{bcd}	24.71^{bcd}	15.15^{gh}	46.69^{h}	13.45^{b}	86.56^{h}	6.44^{ij}	3.08^{cd}
	D_{30}	8.10^{cde}	5.68^{bcd}	24.70^{bcd}	15.21^{efg}	46.27^{i}	13.78^{a}	86.18^{i}	6.25^{j}	3.04^{cd}
10% (1Cs/1Hp)	D_1	7.21^{kl}	5.03^{kl}	22.77^{kl}	16.47^{b}	48.54^{b}	12.24^{i}	87.79^{b}	7.17^{d}	2.94^{e}
	D_{15}	8.11^{cde}	5.05^{jk}	23.30^{jk}	16.45^{b}	47.12^{f}	13.16^{cde}	86.88^{efgh}	6.60^{fghi}	2.86^{f}
	D_{30}	7.90^{efg}	5.25^{hi}	23.70^{hi}	16.33^{b}	46.79^{gh}	13.15^{cde}	86.82^{efgh}	6.60^{fghi}	2.86^{fg}
10% (1Cs/2Hp)	D_1	7.96^{efg}	4.86^{k}	23.13^{k}	15.76^{c}	48.24^{c}	12.82^{gh}	87.13^{cde}	6.79^{fg}	3.05^{cd}
	D_{15}	8.02^{def}	5.08^{ij}	23.48^{ij}	15.80^{c}	47.61^{e}	13.11^{def}	86.90^{efgh}	6.63^{fghi}	3.01^{de}
	D_{30}	8.20^{bcd}	5.17^{ijk}	23.40^{ijk}	15.70^{cd}	47.48^{e}	13.37^{bcd}	86.58^{gh}	6.47^{hij}	3.02^{d}
15% (2Cs/1Hp)	D_1	8.15^{bcde}	5.26^{ijk}	23.44^{ijk}	15.53^{cdef}	47.67^{e}	13.40^{bc}	86.64^{fgh}	6.46^{ij}	3.06^{cd}
	D_{15}	8.33^{bc}	5.53^{cde}	24.58^{cde}	15.16^{gh}	46.34^{i}	13.87^{a}	86.08^{i}	6.20^{j}	3.05^{cd}
	D_{30}	8.40^{b}	5.46^{bc}	24.79^{bc}	15.20^{fg}	46.11^{i}	13.86^{a}	86.10^{i}	6.21^{j}	3.02^{cd}
15% (1Cs/1Hp)	D_1	7.63^{hij}	5.09^{gh}	23.87^{gh}	15.25^{efg}	48.15^{cd}	12.71^{h}	87.28^{c}	6.86^{ef}	3.15^{b}
	D_{15}	6.98^{l}	5.74^{ef}	24.34^{ef}	15.33^{efg}	47.61^{e}	12.72^{h}	87.28^{c}	6.86^{ef}	3.10^{bc}
	D_{30}	7.83^{fgh}	5.52^{bcd}	24.70^{bcd}	15.15^{gh}	46.81^{gh}	13.35^{bcd}	86.67^{fgh}	6.48^{ghij}	3.08^{cd}
15% (1Cs/2Hp)	D_1	7.14^{l}	5.17^{hij}	23.62^{hij}	15.71^{cd}	48.36^{bc}	12.31^{i}	87.69^{b}	7.12^{de}	3.07^{cd}
	D_{15}	7.43^{jk}	5.58^{fg}	24.14^{fg}	15.39^{defg}	47.43^{e}	13.01^{efg}	86.97^{cdef}	6.68^{fghi}	3.07^{cd}
	D_{30}	7.53^{ij}	5.53^{def}	24.43^{def}	15.46^{cdefg}	47.01^{fg}	13.06^{efg}	86.91^{defg}	6.65^{fghi}	3.03^{cd}

For each sample, means in each column with different superscript letters are significantly different (P<0.05)

Table 4: Mean diameter (nm) of microcomplexes during 1 month storage

Emulsion	Day		
	1	15	30
10% (2Cs/1Hp)	728±9.89^{de}	776±8.48^{bc}	871±5.65^{a}
10% (1Cs/1Hp)	603±18.38^{h}	648±16.97^{g}	697±12.72^{f}
10% (1Cs/2Hp)	542±2.82^{i}	568±12.72^{i}	612±5.65^{h}
15% (2Cs/1Hp)	702±8.48^{ef}	755±19.79^{cd}	788±28.28^{b}
15% (1Cs/1Hp)	440±9.19^{k}	474±6.36^{j}	545±8.48^{i}
15% (1Cs/2Hp)	428±2.02^{k}	441±9.89^{k}	489±12.72^{j}

For each sample, means in each column with different superscript letters are significantly different (P<0.05)

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structures of emulsions during 30 days storage and particle size in total of samples increased because of flocculation phenomenon occurrence in emulsions.

Discussion

PV of flaxseed oil was 0.57 mEq/kg oil, which was lower than the maximum acceptable level (2 and 5 mEq/kg of solid fats and liquid oils, respectively) recommended by Horwitz (23). Linolenic and linoleic acids were the main fatty acids (53.12 and 14.21%, respectively) identified by GC-FID in flaxseed oil. These results showed similar trends as those reported by Barroso et al. (19); Popal et al. (24); and Condori et al. (25). The microencapsulation efficiency was mainly determined by carrier type and content (sodium caseinate and *n*-OSA starch) in the emulsion. It was observed that increasing in *n*-OSA starch concentration, increased the microencapsulation efficiency. This phenomenon is related to the high emulsifying capacity of *n*-OSA starch, which resulted in slightly lower mean diameters of emulsions droplets (6).

The oil droplet size is associated with the microencapsulation efficiency, and the emulsion

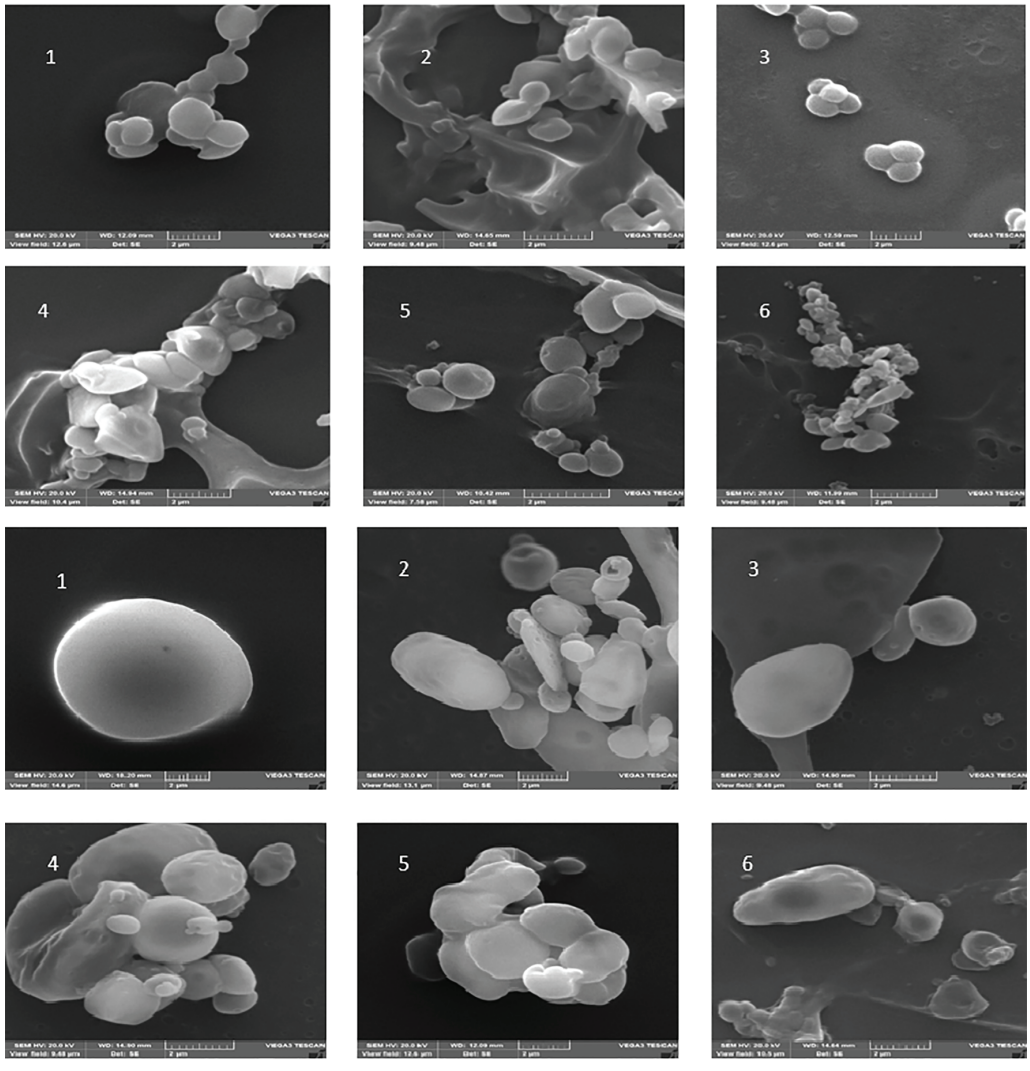

Figure 3: Microstructures of complexes prepared with 10% (2Cs/1Hp), 10% (1Cs/1Hp), 10% (1Cs/2Hp), 15% (2Cs/1Hp), 15% (1Cs/1Hp) and 15% (1Cs/2Hp) as summarized 1-6, respectively, at (a) the first day and (b) 30 days after preparation and storage at 4°C.

with smaller droplet size was exhibited to have higher MEE (17). Increasing oil droplet size in the emulsion could result in a higher amount of surface oil, which further causes the acceleration of the oil oxidation in the microcapsules (26). Tonon et al. (18) used combinations of maltodextrin with different wall materials (*n*-OSA starch, Hi-Cap starch, gum arabic and whey protein) as the carrier for flaxseed oil microencapsulated and observed that the *n*-OSA starch/ maltodextrin wall (in the 3:1 ratio) had highest encapsulation efficiency (95%).

According to previous results, in emulsions with lower solid content, MEE decreased and oil located outside the capsule was exposed to change upon the atmospheric influence, which lead to a decreased shelf life and stability during storage. Due to being highly polyunsaturated fatty acids (~75% PUFAs), flaxseed oil is extremely prone to oxidation caused by high temperature, atmospheric oxygen, and metal ions (27). Hydroperoxide, a highly toxic component causing a reduction in bioavailability of fatty acids, is the main reaction product of lipid oxidation. According to the literature, oxidation susceptibility of α-Linolenic acid (ALA) is 20 times of oleic acid (28). However, PVs in all cases were acceptable for human consumption (28).

The antioxidative properties of casein owing to free-radical scavenging and metal ion chelation properties give rise to the higher oxidative stability of encapsulated flaxseed oil than free oil. The presence of different amino acids including tyrosine, phenylalanine, cysteine, tryptophan, and histidine as well as free sulphydryl groups in milk proteins lead to their free-radical scavenging properties (27). A good oxidative stability showed similar trends as those reported by others (18, 29) that oxidative stability of emulsions was dependent on the nature of biopolymer present in the emulsion. The *n*-OSA starch has excellent emulsifying properties, which can cause smaller particle size in the emulsion, which influences surface area and thickness of the emulsifier layer at the interfacial region of the

emulsion droplets, leading to higher oil protection against oxidation (29).

The amounts of TBARS developed during 1-month storage were less than the maximum allowable levels (5mg malondialdehyde/kg oil) according to Codex Alimentarius Commission (28). Zhong et al. (30) showed correlation between TBARS and the other secondary reaction product indicators (e.g. specific aldehydes) produced during lipid oxidation in emulsions and bulk oils. The type and amount of wall materials cannot significantly ($P<0.05$) influence TBARS values of emulsions at 4-week storage time at 4°C. It is noteworthy to mention that non-covalent interactions such as hydrophobic bonds, are likely to occur between hydrophobic parts of casein and volatile oxidation products. The amount of produced lipid oxidation products could be underestimated due to the occurrence of these particular interactions (29).

The relative peak area in GC-FID analysis can be used to calculate the relative amount of each fatty acid. The degradation of omega-3 and omega-6 essential fatty acids as well as the rate of their degradation in flaxseed oil was higher than in emulsions. This better maintenance indicate that microemulsions have been shown to be mainly appropriate for protection of omega-3 (19, 31) and oxidatively stable to decrease during storage. The PUFA/SFA ratio (polyene index) is shown the extent of poly unsaturation of flaxseed oil followed by suitable shelf life, which is an important factor for marketing of oil. For investigating the nutritional value of flaxseed oil emulsions, the ω-3/ω-6 ratio is calculated. Similar results have been reported by Nejadmansouri et al. (31).

The droplet mean diameter of *n*-OSA starch and sodium caseinate emulsions was smaller in samples prepared with higher concentration of *n*-OSA starch, because of the excellent emulsifying properties of this modified starch. Consequently, less efficient emulsification may be obtained using the lower concentration of this particular wall material. In order to protect emulsion droplets from flocculation and/or coalescence, an emulsifier is applied, which is a surface-active substance and is capable of adsorbing to an oil–water interface. Of course during homogenization, proteins are capable of rapidly absorbing to the surface of oil droplets, where they lower interfacial tension and inhibit droplet coalescence by forming protective membranes around the droplets (32).

The rate of particle sedimentation and creaming is reduced with an increase in the viscosity, and as a result a better emulsion stabilization was obtained and therefore the droplets coalescence was avoided (31). These results were in line with those reported by Tonon et al. (26), in which different total solid contents and oil concentrations were used to produce emulsions. The mean diameter of droplets were in the range of 2.27 and 4.77 μm. An increase in total solid content with the same oil concentration led to smaller droplets size. Flocculation of microemulsions with lower *n*-OSA starch content were higher than those of emulsions; which confirmed the results of DLS. Koocheki et al. (33) explain the similar results. Tonon et al. (18) implies that microcapsules produced with modified starch had a larger number of particles with smooth surfaces, which shows faster film formation and better flow properties.

Conclusion

Microemulsions showed lower oxidation rate as compared to bulk flaxseed oil at 4°C. GC-FID results showed ω-3 fatty acids better protected in microencapsulated flaxseed oil and improved the nutritional quality of the flaxseed oil in storage period. *n*-OSA starch resulted in the highest encapsulation efficiency and lowest lipid oxidation and is highly indicated for microencapsulation of flaxseed oil. The increase in droplet size led to lower encapsulation efficiency and, consequently, higher lipid oxidation. The results reported in this study have important implications for the design and utilization of emulsions as delivery systems for food enrichment. Flaxseed oil emulsions can be used as an ingredient in sauces، cereal and dairy products.

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Conflict of Interest

None declared.

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