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RESEARCH ARTICLE
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ASSESSMENT OF OXIDATIVE STABILITY OF MICROENCAPSULATED FLAXSEED OIL VIA FREEZE DRYING USING VARIOUS WALL MATERIAL COMBINATIONS

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Abstract
Flaxseed oil is a rich source of omega-3 fatty acids, particularly alpha-linolenic acid, but its high content of polyunsaturated fatty acids makes it prone to oxidation. This study aimed to enhance the oxidative stability and shelf life of flaxseed oil through microencapsulation using freeze-drying with different combinations of guar gum and whey protein concentrate as wall materials. Emulsions were evaluated for physical stability, viscosity, droplet size, and peroxide value. The resulting powders were assessed for encapsulation efficiency, moisture content, bulk density, morphology, and oxidative stability. The combination of guar gum and whey protein concentrate (1:1 ratio) showed the highest encapsulation efficiency and oxidative stability, confirming its suitability for flaxseed oil encapsulation.

Keywords: Flaxseed oil, wall material, Emulsion, Freeze Drying, Encapsulation

INTRODUCTION

Flaxseed oil is one of the numerous foods high in alpha-linolenic acid (ALA), which has already been proposed to have certain health benefits by lowering LDL, the low-density lipoprotein responsible for the cardiovascular disorder (Bekhit *et al*., 2018). Flaxseed oil has recently become popular as a dietary supplement due to increased public perception of the rich amount of ALA content in flaxseed oil. The rapid growth of linseed oil not only enhances the level of activity of linseed oil extraction but also increases the volumes of by-products derived from the extraction method (e.g., flaxseed meal and cake). Linseed oil is obtained by exerting pressure on the whole flaxseed, a process known as oil pressing, which can be done with or without heating. When whole flaxseed is pressed, flaxseed oil separates from the compact linseed mass, which is commonly referred to as "linseed cake." If not discarded, linseed cake is ground into meal and used in animal feed as a by-product (Bekhit *et al*., 2018).

Antioxidants are currently used to avoid the degradation of oil-based food products. Oxidative degradation is higher in fats and oils because the abundance of PUFAs can create toxic compounds that have adverse health effects. The anti-inflammatory properties of omega-3 FAs can aid in the reduction of the hyperactive immune system associated with depression. EFAs include gene transcription regulators in the central nervous system and may have to be impersonated by neural membrane fluidity and receptor binding (Smith *et al*., 2011).

Fats and oils deteriorate due to lipid oxidation, which causes a loss of quality and fatty acids as well as nutritional value and the development of off-flavors. The term "oxidative stability" refers to a substance's resistance to oxidation under laboratory conditions and is typically expressed as the time needed to reach an end point that can be chosen according to varying criteria (such as the onset of rancidity) but typically corresponds to a sharp increase in the oxidation rate. Since oxidation often moves at a snail's pace up to this moment, we call it the induction stage (IP). There are a plethora of techniques available for determining oxidative stability, most of which include the use of accelerated oxidation conditions. Results are obtained in relatively short times by applying elevated temperatures in the presence of abundant oxygen. The Rancimat method, also known as the oil stability index (OSI) approach, provides for the automatic determination of oxidative stability under controlled, standardised settings (AOCS, 2006). Encapsulation is a procedure in which solids, liquids, and gaseous materials are contained in small capsules. Capsules can be prepared with polysaccharides, proteins, and lipid-based materials. Encapsulation aims to enhance the oxidative stability of the oil and the targeted delivery of the food formulation. It also protects items with sensory qualities and consumer loyalty. Various polysaccharides (modified starch, gum arabic, and maltodextrin) may be used as the core material (Carneiro *et al*., 2013).

Microencapsulation is a technique used to protect fragile food particles, lessen nutritional losses, increase the usefulness of vulnerable materials, attach specific food particles to other products, cover up odor and flavors, and transform liquid foods into solids for more convenient storage and preparation. In addition to preventing the rapid loss of volatile substances due to evaporation, microencapsulation can shield them from radiation and oxidative destruction. Microencapsulation has many benefits, including extending the shelf life of a product, hiding off-putting aromas and flavors, making it more manageable, more transparent, and easier to handle. This includes fluidized bed drying, spray drying, freeze drying, inclusion complexion, extrusion, and entrapping liposomes. Spray drying is the most often used encapsulation technology because of its low cost and convenience of application, especially in the food business. In addition, before being encapsulated, certain parts underwent spray drying processes. Microencapsulating thermo sensitive core materials is best achieved using freeze drying, as opposed to spray drying, which is less efficient. As a result of their potential impact on core material stability and microencapsulation efficiency, twists in the wall material also play a significant role in the microencapsulation process. The cost and improvement of the wall material's properties influence the variety of wall material used in encapsulation. Carbohydrates like maltodextrin and modified starch are the preferred wall materials due to their film formation characteristics and low density. Gum arabic, guar gum, and modified starches have been used to encapsulate the flaxseed oil to prevent oxidation. All types of gums have been used in the food industry as emulsifiers, stabilizers, gelling agents, thickeners and texture modifiers (Liu *et al*., 2021).

Materials and Methods

Encapsulation of Flaxseed oil by emulsion

Flaxseed oil encapsulates were prepared through emulsion method. In this regard, guidelines of Carneiro *et al*. (2013) were followed with slight modification. Table 3.1 illustrates the treatment plan for encapsulation of the oil sample. Purposely, flaxseed oil (20% of the wall material) was captured in guar gum and whey protein concentrate blends. Emulsion was prepared that consisted of different proportions of polymers, prepared by a dispersing mixture of guar gum and whey protein concentrate (10g) in distilled water up to a final volume of 100 mL. Prepared slurries were mixed with a magnetic bar stirrer for 3 min at 1200 rpm. The solution was then given an overnight stay time at 8 to 10^{o}C to get completely hydrated. Tween 80 (0.1 g/100 mL emulsion) was then added to the emulsifying solution along with flaxseed oil (with 20% of the encapsulating material). The mixture was again stirred with the help of a magnetic stirrer. The mixture was further homogenized at 3000 rpm for 5 to 7 minutes using a homogenizer followed by sonication at a frequency of 37 kHz and 80-Watt power for 5 min in an ultrasonic bath at room temperature. The prepared

emulsions were frozen at -40°C for 24 hours before lyophilization. Finally, the resulting flocks were finely ground with mortar and pestle and stored in the desiccator for further analysis.

Table1. Treatment plan for encapsulation of flaxseed oil by emulsion method (g/100 mL)

Treatments	Guar gum-Whey protein concentrate's ratio
T_o	Flaxseed oil
T_1	1:1
T_2	1:2
T_3	1:3
T_4	2:1
T_5	2:3
T_6	3:1
T_7	3:2

Core material: flaxseed oil
Wall material: whey protein and guar gum

Physicochemical characterization of emulsion

Emulsion stability

Physical stability was analyzed as creaming index by adopting the method devised by Goyal *et al*. (2015). Immediately after preparation, 25 mL of each emulsion was poured into a calibrated cylindrical glass tube, sealed with a plastic cap. The initial height of the emulsion layer was recorded and stored at low temperature (7±1°C) for a period of 28 days. The stability of the emulsion was calculated by the change in height of the lower serum phase (H) with storage time, using the following equation:

$$\text{Percent of separation} = \frac{H}{Ho} \times 100$$

Where:
H_o = Initial height of the emulsion
H = Height of the emulsion after phase separation.

Emulsion viscosity

Viscosity of the emulsion was determined by steady-state flow curves using Rheometer (ARES G2 Rheometer, TA Instrument, USA). The guidelines of Carneiro *et al*. (2013) were followed in this regard. 20 mL of oil sample was taken in concentric cylinder probe. Temperature was regulated at 25°C. Rheograms were analyzed through analytical models and viscosity of the emulsions was determined as a relationship between shear stress and the shear rate.

Emulsion droplet size

The distribution of the droplet size was measured through Dynamic light scaterring instrument. In this regard, polydisparity model was used. Purposely, a small sample was suspended in water by magnetic agitation, and the distribution of the droplet size was controlled during each measurement before subsequent measurements became constant. The emulsion droplet size was represented as D50 (Almasi *et al*., 2021).

Peroxide value

In order to determine the peroxide value, method no. Cd 8-53, proposed by AOCS (2017) was adopted. Purposely, oil was extracted from the emulsion. Then, twenty grams of the extracted oil sample were mixed in a 200 mL cold mixture of chloroform-methanol (with a ratio 2:1) in a separate funnel. After shaking gently for 3 minutes, the mixture was allowed to stand for 10 minutes. The lower chloroform layer was extracted separately. The upper layer was washed with 100 mL of chloroform-methanol (ratio 2:1) mixture and the lower chloroform layer was removed.

The remaining was blended with the previous mixture along with the addition of 40 mL of distilled water. Following phase separation, the lower chloroform layer was collected, passed through anhydrous sodium sulphate and dried using a flash evaporator (Metrex Science Instruments, India). Drying was done under vacuum at 40^{o}C. Peroxide value of extracted oil was measured on weekly basis during a total storage period of 28 days.

Freeze drying of the emulsion

The prepared emulsion was first frozen at-40^{o}C for 24 hours. After freezing temperature was further lowered to -80^{o}C for a second interval of 24 hours, according to the directions of Fioramonti *et al*. (2017). After completion of twenty-four hours period, the frozen sample was lyophilized and grounded into coarse powder.

Powder analysis

Encapsulation efficiency

The powder encapsulation efficiency was determined by following the protocol of Bae and Lee (2008). Accordingly, 15mL of hexane were added to 1.5g of powder in a glass tube. The tube was covered with lid and the mixture was well shaken by hand for two minutes at room temperature for the extraction of free oil. The mixture was filtered with Whatman filter paper and powder that retained on filter paper was rinsed 3 times with 20 mL hexane. The leftover solvent was evaporated at room temperature by putting in oven at 60^{o}C. During evaporation, mixture was weighed at different intervals until constant weight attained. The surface oil was analyzed by the difference between initial flask weight and extracted oil residue. Encapsulation efficiency was calculated by using following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{TO-SO}}{\text{TO}} \times 100$$

TO= Total oil content
SO= Surface oil content

Peroxide value

The peroxide value of the powder was estimated as milliequivalent per kilogram of fat/oil. The peroxide value of the encapsulated flaxseed powder was assessed on weekly basis for a total storage period of 28 days. In this regards, method no. Cd 8-53 of AOCS (2017) was followed as mentioned in one of the above sections.

Moisture content

Powder's moisture content was determined by adopting the protocol of AOAC (2006). Powder moisture was determined gravimetrically by drying in vacuum oven at 105^{o}C until constant weight was noticed.

Bulk density

The bulk density of the powder was determined following the guidelines of Goula and Adamopoulos (2004). Firstly, a considerable quantity of powder was passed through a sieve (aperture size: 1.0 mm) to break the agglomerates. Afterwards, the powder was gently introduced into a graduated cylinder (250 mL) without getting compacted. 100 g of sample was weighed and carefully leveled. The apparent volume (unsettled) was noted. The bulk density was calculated using following formula:

$$\text{Bulk density (gram per mL)} = m/V_o$$

Where:
m = weight of test sample
V_o = Unsettled apparent volume

Particle mean diameter

The average particle diameter of the powder was measured by scanning electron microscope (EDX-Leo 440i) as per the protocol of Carneiro *et al*. (2013). Microcapsules were analyzed by scanning electron microscope detector through energy dispersive V-rays at 15kv and beam of electron current of 100 pA. The sample was static on door metallic specimens of 12mm diameter. It was then subjected to metallization with a thin layer of gold coating through a sputter coater at a coverage rate of 0.51 A/s for 180 seconds, with a current of 3.5 mA, 1V and 2×10^{-2} Pa. After metallization, the samples were analyzed with different magnifications *i.e*., 4000, 5000 and 10,000. Image acquisition was performed by LEO software.

RESULTS AND DISCUSSION

Physical Stability

The type of encapsulating agent plays an important role in emulsion stability. During emulsion formation, an oil droplet is broken up into tiny droplets, and the encapsulating agent forms a shield around the oil droplet, preventing aggregation.

Statistical analysis of the physical stability of the emulsion showed non-significant variations between the control treatments, whereas other treatments showed significant variations. Table 3.2 shows the physical stability of all treatments prepared as emulsions. It is obvious from the mean table that the maximum value of physical stability was found for control treatment T_0 at 0, 7, 14, 21, and 28 days as 99.99±0.005, 99.98±0.015, 99.98±0.015, and 99.99±0.010%, respectively. T_1, T_2, T_3, T_4, T_5, T_6, and T_7 had the greatest physical stability variation from 0 to 28 days, with maximum values of 99.98±0.015, 99.99±0.005, 99.99±0.010, 99.99±0.010, 99.99±0.010, and 99.99±0.010%, respectively, and minimum values of 93.46±0.015, 93.66±0.025, 93.34±0.030, 93.55±0.030, and 93.74±0.025%, respectively. The results were consistent with the findings of the researcher Fustier *et al*. (2015), who discovered the stability of an emulsion with coating material whey protein isolate and fish gelatin amalgamation at pH 6.5.They compared the stability of emulsions of whey protein isolate with those of fish gelatin at different storage conditions and finally concluded that WPI, which was 0.5 to 1% concentrated, had highly stable emulsions that delivered good protection, whereas in the case of fish gelatin, the stability ranged from 0.1 to 0.75%, which indicated that the reduction of backscattering increased the destabilization rate. They investigated the slight difference in initial height due to the tiny particles that destabilised during emulsion formulation.

Peroxide Value

The peroxide value is a perseverance of the concentration of hydroperoxides in tested samples.

The mean value Peroxide value of emulsion illustrated in Table 3.3 revealed significantly variations of peroxide value among all treatments. T_0 control treatment mean value of oil at 28^{th} day was found to contain highest peroxide quantity 93.367±0.90 meq/Kg, However the minimum value was noted T_0 at 0 days 3.453±0.15 meq/kg. Likewise, the 7, 14, 21 days control sample was significantly different mean values was noted as 10.950±0.53, 36.133±1.00 and 62.367±0.85meq/kg respectively. The maximum mean value of T_1, T_2, T_3, T_4, T_5, T_6, and T_7 were found at 28^{th} day noted as 8.967±0.35, 9.400±0.30, 9.900±0.40, 9.167±0.35, 9.767±0.35, 10.433±0.25 and 10.300±0.30 meq/kg respectively, where's the minimum value of T_1, T_2, T_3, T_4, T_5, T_6, and T_7 was found at 0 day 6.300±0.2, 7.100±0.3, 6.433±0.20, 6.733±0.20, 5.667±0.15, 7.167±0.35 and 6.833±0.50 meq/kg respectively.

The current study agreed with a group of researchers Kuhn and Cunha (2012) who stated that the evolution of oxidation was investigated using primary oxidative products in an oil-water emulsion during high pressure homogenization. However, the low peroxide value was discovered at a lower pressure (20 MPa) than the pure oil after 30 days of storage. The emulsifier plays a significant role in preventing pro- and post-oil oxidant impurities. while the peroxide value only rose in the range of 0.42 to 0.71 meq/kg oil for 30 days of storage. They found that if pressure was up to 80 MPa, it increased the rate of primary oxidative products. Another researcher discovered that

homogenization of the emulsion at 80 MPa increased the peroxide value from 0 to 1.77 meq/kg after I pass, while 4 and 7 passes increased the peroxide value by 0.84 and 2.9 meq/kg, respectively.

Emulsion viscosity

The statistical analysis of emulsion viscosity is illustrated in Table 3.4. It is evident that emulsion apparent viscosity were significantly effected by different treatments, but no variations was noted for control treatment T_0 at 0 and 28th days followed by 0.0230±0.003, 0.0230±0.003 Pa.s, respectively. The significant variation were noted for treatment T_1, T_2, T_3, T_4, T_5, T_6 and T_7 at 0 days followed by 0.0337±0.003, 0.0723±0.002, 0.0517±0.001, 0.0427±0.003, 0.0923±0.002, 0.1167±0.015 and 0.1747±0.021 Pa.s, respectively, where at 28th days apparent viscosity noted as 0.0337±0.002, 0.0430±0.003, 0.0617±0.002, 0.0827±0.002, 0.0550±0.004, 0.0923±0.002 and 0.1233±0.002 Pa.s, respectively.

Similarly in case of shear rate no variation was noted Table 3.5 for control treatments, T_0 at 0 and 28th days as 400±2 and 400±2 shear rate/s respectively. The significantly variation were noted for treatment T_1, T_2, T_3, T_4, T_5, T_6 and T_7 at 0 days followed by 637.7±3.05, 1245±4, 749±4, 1474±5.56, 1643.3±6.50, 1142±7 and 967±4.58 shear rate/s, respectively, where at 28th days shear rate noted as 637.7±3.51, 1245±6.65, 1273.3±6.02, 1755.3±5.13, 1850±8, 1438.7±6.11 and 1006.3±8.02 shear rate /s, respectively. Kuhn and Cunha (2012) produced a stabilized flaxseed oil emulsion using whey protein isolate at high pressure with various passes through the homogenizer. All of the emulsions had a viscosity of 100 s-1 shear rate and a flow behavior index ranging from 0.78 to 0.95. They observed that the homogenizer's number of passes increased the consistency index, while the average flow index decreased. The number of passes of emulsion through the homogenizer and pressure steering to increase the viscosity could be related to droplet size and a high amount of protein content.

Droplet size of emulsion

The mean values recorded for droplet size of emulsion are mentioned in Table 3.6. It is evident that droplet size of emulsion was significantly affected by different treatments, but no variations was noted for control treatment T_0 at 0 and 28th days followed by 254±3.60 and 254±3.60 respectively. The significantly variation were noted for treatment T_1, T_2, T_3, T_4, T_5, T_6 and T_7 at 0 days followed by 396.66±5.50, 564.66±3.21, 790.66±3.05, 604.66±2.51, 705.66±3.78, 435.33±5.85 and 884.66±2.08 nm respectively. Where T_1, T_2, T_3, T_4, T_5, T_6 and T_7 at 28th days droplet size noted as 521±3, 1011.66±15.53, 955.66±4.93, 784±4, 880.66±7.7, 603.66±4.16 and 612.66±2.51 nm respectively.

Our investigations were supported by the group of scientists (Abbasi *et al*., 2019) who determined the particle size of flaxseed oil emulsion through dynamic light scattering. They discovered that the emulsion made from whey protein and sodium alginate had a larger particle size (464 nm), whereas the particle size of the separately prepared emulsion was WP 214 nm and SA 196 nm. The DLS analyzed data described that the emulsion prepared from the combination of WP and SA was uniformly distributed and had a size less than 1000 nm.

Microencapsulation efficiency (ME)

The powder's oil content as a proportion of the powder's total weight indicates that the encapsulation procedure was highly effective. Since emulsion created with whey protein and guar gum produced particles with considerably reduced surface oil, the type of encapsulating material utilized had a significant effect on the encapsulation efficiency of the sample. Table 3.7 represents the mean values of the studied treatments among different ratios of the wall material used (GG:WPI) for the making of emulsion. T_1 treatment showed the highest encapsulation efficiency (98.17±0.22 %), followed by T_2 as 98.15±0.19% and T_3 as 96.17±0.17%. Whereas the other T_4, T_5 was found as 94.12±0.20% and 93.12±0.13% respectively. T_6 exhibited the lower value as 94.29±0.30%. T_7 treatment showed the lowest encapsulation efficiency among all treatments as 92.41±0.18%. The

outcomes acquired be similar to the data profound by other researchers (Gallardo *et al*., 2013) reported that the emulsion prepared from spray drying with core material flaxseed oil and wall material was whey protein isolate and maltodextrin mix with Arabic gum surface oil, total oil and resolute the efficiency process in the arrays *i.e.,* 1.9–2.7, 22.1–32.9 and 87.8–91.4% (*w*/*w*) respectively.

Encapsulated Powdered

Moisture content of encapsulated powder

Moisture content plays an important role in the shelf life of encapsulated powder. Physical and chemical stability may be distressed due to higher moisture content, which caused caking and fungal growth that affected overall acceptability. Table 3.8 presented the value of moisture content of current studied encapsulated powder prepared from different ratio of wall material were used. The higher value was noted as T_4 3.63±0.036 followed T_3 and T_5 was 2.73±0.045% 2.53±0.35% correspondently. The mean value of T_1, T_6 was 2.36± 0.045% and 1.48± 0.33%, respectively. Lower value of moisture content of powder was noted as T_7 1.22±0.045%. T_2 shown the lowest mean value among all above treatments was 1.21±0.036%. Our current outcomes, which were in close agreement with the group of researchers Pham *et al*. (2020), revealed that the moisture content of powder was 2.660.09%. The moisture content of all encapsulated powders was below 3%, which is considered stable moisture content in food encapsulated powders.

Carneiro *et al*. (2013) also supported our current studies; they reported that the properties of encapsulated powder particles prepared with a combination of wall materials The powder's moisture content was found to be 1.540.060. Maltodextrin-based wall materials have a lower moisture content than other wall materials. (Hogan *et al*., 2001) discovered that different wall materials had no effect on the moisture content of soya bean oil encapsulated powder, which ranged from 1 to 3%.

Bulk Density

To a certain extent, the physical parameter of microencapsulated powder is bulk density, which reflects the oxidative stability and flow ability of microcapsules. Table 3.9 represented the monotonous value of bulk density of different treatments. T_4 as 0.56±0.02g/cm^3 followed by T_6 and T_5 as 0.55±0.02 g/cm^3, 0.53±0.02 g/cm^3 correspondently. The other treatments showed lower bulk density T_3, T_7 and T_2 as 0.52±0.02 g/cm^3, 0.52±0.02 g/cm^3 and 0.50±0.02 g/cm^3 respectively. T_1 exhibited the lowest bulk density between all treatments as 0.45±0.03g/cm^3.

Our current findings were also supported by Zhang *et al*. (2021), who investigated that the bulk density of the wall material used with SC-IMO encapsulated powder was found to be 0.456 g/cm^3, whereas the control sample density was 0.224 g/cm^3. They also observed that the microcapsule that is coated with MRPs has a more compact, round structure and is smoother. The smaller particle size is another reason for the higher bulk density of wall material made of SC-IMO encapsulated powder. The current fines are closely related to the above investigation.

Scanning Electron Microscope (SEM)

The morphology of microencapsulated powder produced with the freeze-drying technique was analyzed by scanning electron microscopy. SEM revealed that the morphology of powder varied significantly across treatments Table 3.10, that enclose the highest value of particle size was found T_2 as 12.94±0.04µm followed by T_4 and T_3 as 11.45±0.29µm and 10.54±0.38µm, respectively. Where the other treatment is was found T_5 and T_6 as 9.40±0.36µm, 8.64±0.36µm, correspondently. The lower value of particle size of powder T_1 was found 8.43±0.47µm. T_7 noted the lowest value of among all treatments was 7.6±0.28µm.

Carneiro *et al*. (2013) reported that the powder morphology analyzed by SEM (microstructure external and internal morphology) of powder obtained from different combinations of wall material They discovered powder particles measuring 17.98±0.88 µm in size.The external morphology of the particle presented a variety of sizes with a spherical shape and no fissures, which means the

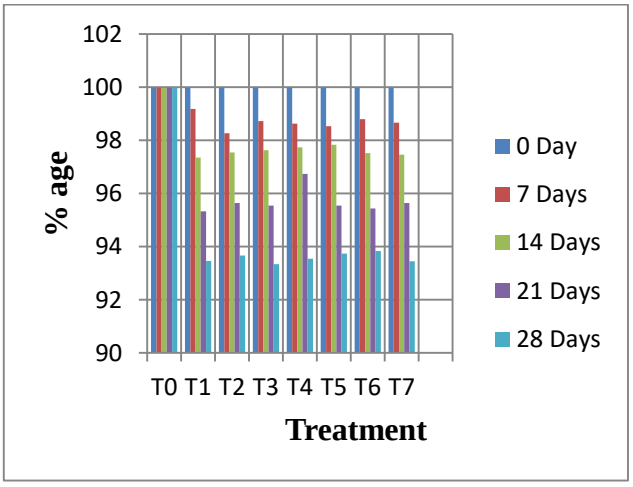
microcapsule has lower gas penetrability, protects from oxidation, and has active material retention. Furthermore, the variation and features of particle size obtained from spray drying. The combination of wall material influenced on microstructure of capsule. They also observed that the image comparison of maltodextrin:Hi-Cap with the other wall materials produced microspheres with a rougher and smoother surface. Re, (1998) discovered that roughness produced by the slow process of film formation during the drying process of atomized droplets correlated the presence of superficial miseries to the droplet's agony during the initial stage of drying. Carneiro *et al*. (2013) demonstrated a third internal morphology; active material was adhered to the surface and all microspheres were hollow-shaped, with small droplets entrenched in matrix. Another feature was found to be bareness due to fast expansion during the final stage.

Peroxide value of Encapsulated Powder

Peroxide value analysis over 4 weeks confirmed that flaxseed oil capsules made with Guar gum and whey protein isolate. Table 3.11 presented the control sample T_0 peroxide value mean value at 0 days as 1.52±0.16 meq/kg followed as T_7, T_5 was the significantly higher value then other treatments as 5.34±0.16 meq/kg and 4.68±0.30 meq/kg, respectively. Another treatments T_4, T_6, T_2 was calculated as 3.75±0.18 meq/kg, 3.52±0.20 meq/kg and 3.41±0.22 meq/kg, correspondently. The mean value of different treatments are illustrated in (Figure 4.10), lower value noted as T_1 was 2.45±0.30 meq/kg. The lowest value was noted among all encapsulated treatment was T_3 as 1.44±0.15 meq/kg. Encapsulated powder was stored at room temperature for 28 days. The peroxide value was noted T_0 showed the peroxide value of pure oil was significantly higher as 33.16±0.68 meq/kg, followed as T_7, T_4 was 15.71 ±0.43 meq/kg and 13.59±0.31 meq/kg respectively. Where T_6, T_5, T_1 is as the treatments was 12.55±0.26 meq/kg, 11.44±0.30 meq/kg and 10.50±0.32 meq/kg, respectively. The lower mean value was observed as T_3 was 10.24±0.24 meq/kg. T_2 showed the lowest peroxide mean value between all encapsulated treatments after 28th day storage was 9.88±0.28 meq/kg.

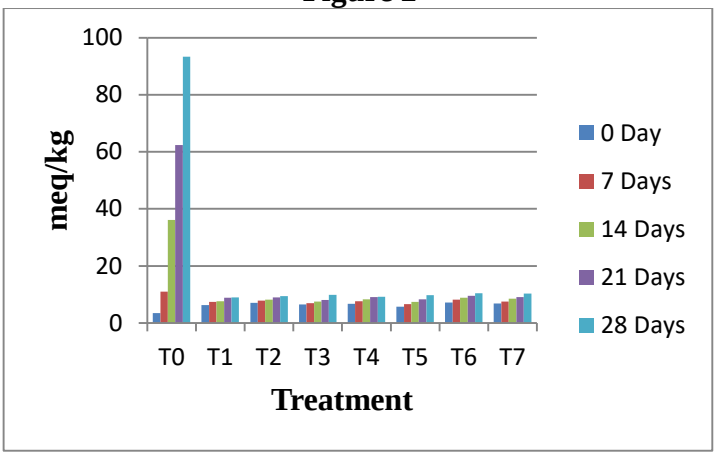
Zhang *et al*. (2021) reported that the oxidative stability of encapsulated flaxseed oil with wall material was determined by headspace chromatography and peroxide value at 50 ºC for 4 weeks, which is similar to our current values. At 0 weeks, all samples ranged from 0.79 to 1.04 meq/kg oil, with no significant difference between samples. Flaxseed oil coated with Millard reaction products had lower meq/kg of oil (0.79 to 13.10 meq/kg compared with other control groups' 0.75 to 23.07 meq/kg and 1.04 to 24.68 meq/kg). One possible explanation was that the encapsulated powder derived from the wall material SC-IMO contained less surface oil glycosylated protein, which adsorbed surface oil from oil droplets. As a result, the peroxide value was greatly reduced.

Figure 1



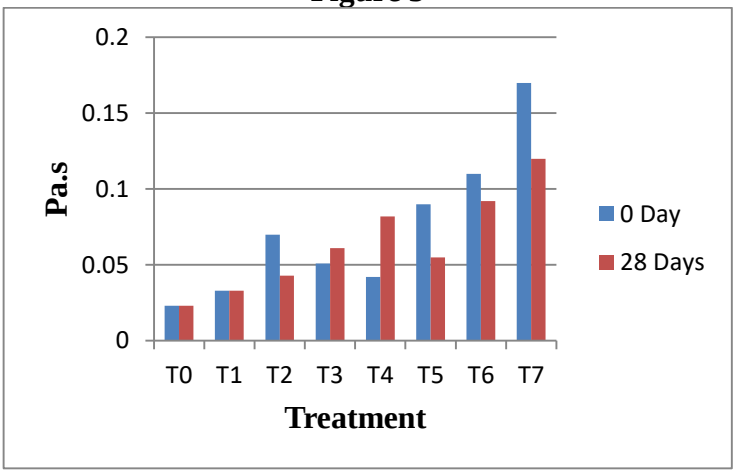
Physical stability

Figure 2



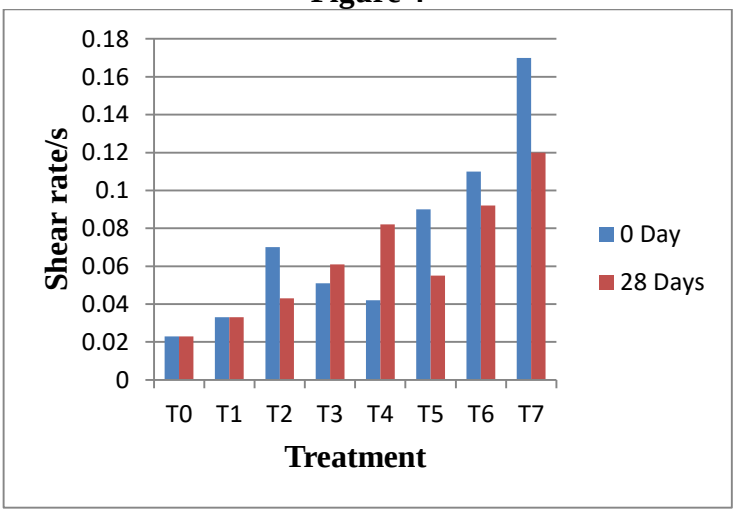
Peroxide value

Figure 3



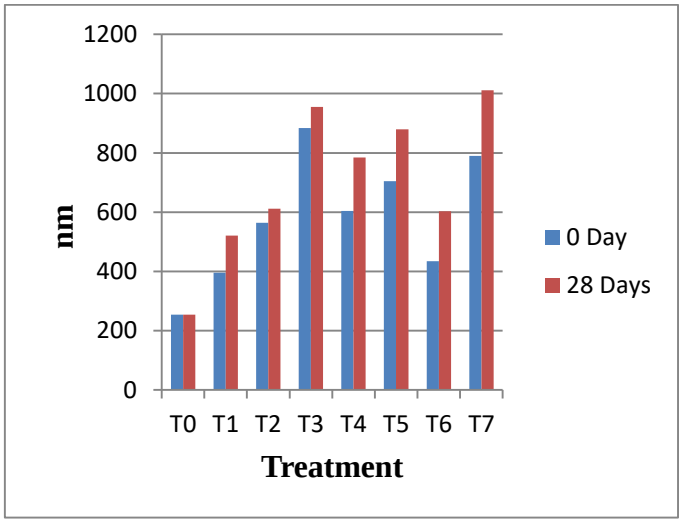
Apparent viscosity

Figure 4



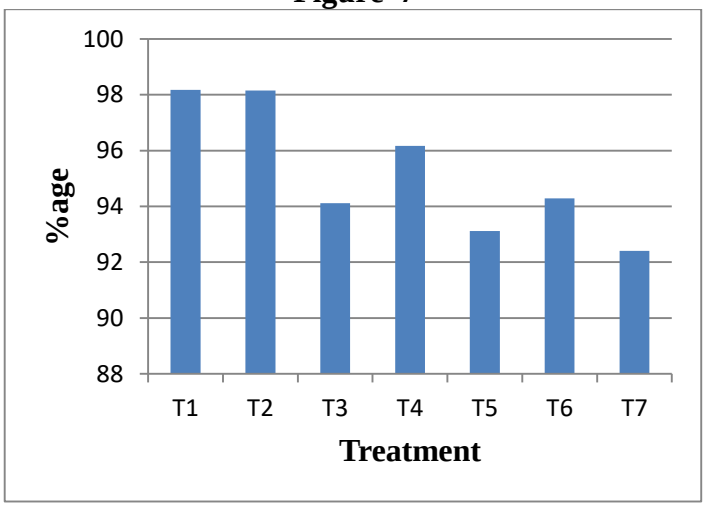
Shear rate

Figure 5



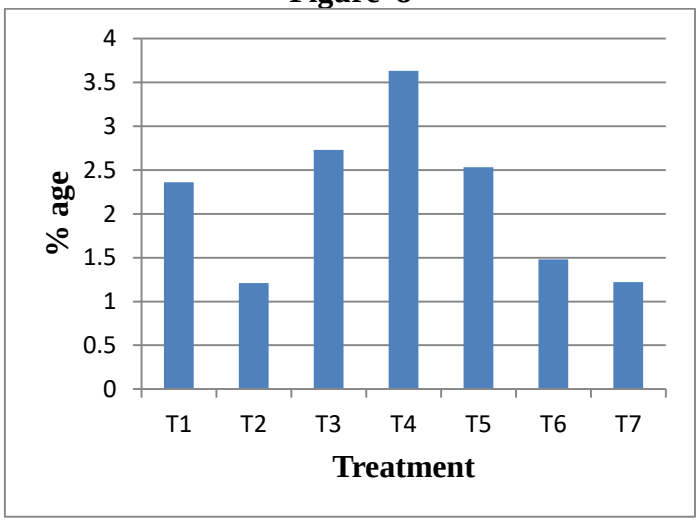
Emulsion droplet size

Figure 7



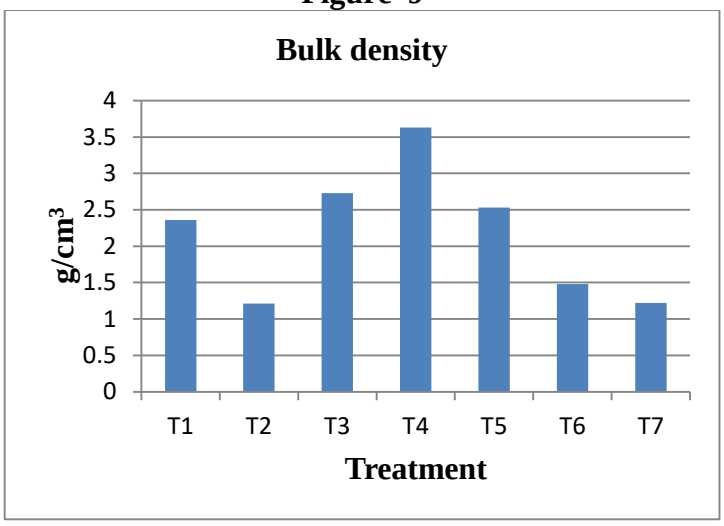
Encapsulation efficiency

Figure 8



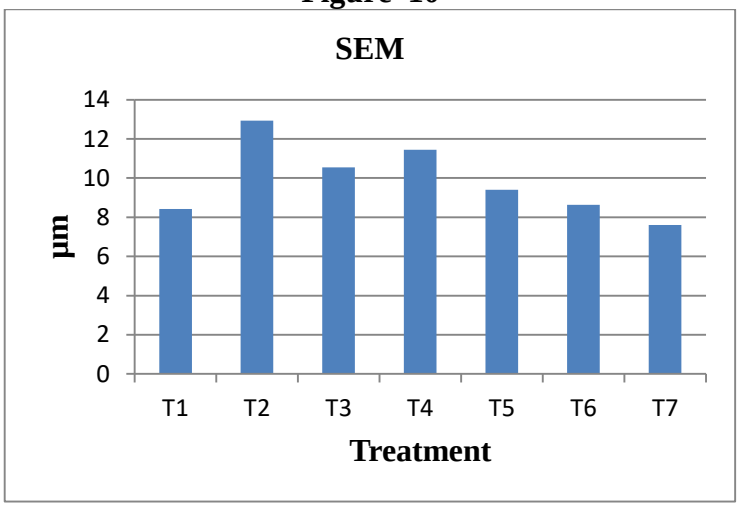
Moisture content

Figure 9



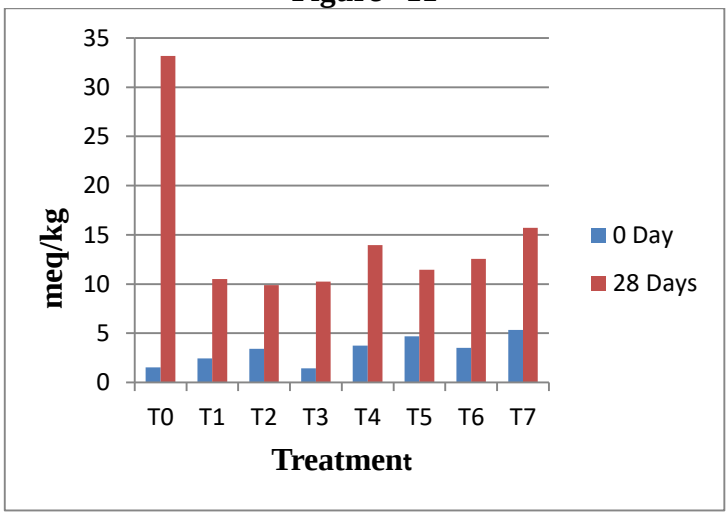
Bulk density

Figure 10



SEM

Figure 11



Peroxide value

Conclusion

Overall, microencapsulation through freeze drying represents a promising approach to stabilize sensitive oils such as flaxseed oil, extending their shelf life and broadening their application in the nutraceutical and functional food industries. Future studies should focus on scaling up the process, assessing sensory acceptability, and exploring long-term stability under real-time storage conditions.

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Literature Cited

1. Almasi, K., Esnaashari, S. S., Khosravani, M., and Adabi, M. (2021). Yogurt fortified with omega-3 using nanoemulsion containing flaxseed oil: Investigation of physicochemical properties. Food Sci. Nutr, *9*(11), 6186-6193.
2. AOAC. 963.15 Fat in cacao products, Soxhlet extraction method. In Association of Official Analytical Chemists, Official Methods of Analysis of AOAC International; AOAC International: Rockville, MD, USA, 2010.
3. AOCS official method Ca 2b-38, Moisture and Volatile Matter in Butter, Fats, Margarines, and Oils, Hot Plate Method. Official Methods and Recommended Practices of the American Oil Chemists' Society, 7th ed., AOCS Press, Champaign, IL, 2017 .
4. Bekhit, A. E. D. A., Shavandi, A., Jodjaja, T., Birch, J., Teh, S., Ahmed, I. A. M., ... and Bekhit, A. A. (2018). Flaxseed: Composition, detoxification, utilization, and opportunities. Biocatal. Agric. Biotec, *13*, 129-152.
5. Carneiro, H.C., R.V. Tonon, C.R. Grosso and M.D. Hubinger. 2013. Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. J Food Eng. 115: 443-451.
6. Fioramonti, S. A., Stepanic, E. M., Tibaldo, A. M., Pavón, Y. L., and Santiago, L. G. (2019). Spray dried flaxseed oil powdered microcapsules obtained using milk whey proteins-alginate double layer emulsions. Food Res. Int, *119*, 931-940.
7. Fustier, P., Achouri, A., Taherian, A. R., Britten, M., Pelletier, M., Sabik, H., ... and Mondor, M. (2015). Protein–protein multilayer oil-in-water emulsions for the microencapsulation of flaxseed oil: Effect of whey and fish gelatin concentration. J. Agric. Food Chem, *63*(42), 9239-9250.
8. Gallardo, G., Guida, L., Martinez, V., López, M.C., Bernhardt, D., Blasco, R., Pedroza-Islas, R., Hermida, L.G. (2013). Microencapsulation of linseed oil by spray drying for functional food application. Food Res. Int, *52*(2), 473–482.
9. Goula, A. M., Adamopoulos, K. G., and Kazakis, N. A. (2004). Influence of spray drying conditions on tomato powder properties. Dry. Technol, *22*(5), 1129-1151.
10. Goyal, A., Sharma, V., Upadhyay, N., Singh, A. K., Arora, S., Lal, D., and Sabikhi, L. (2015). Development of stable flaxseed oil emulsions as a potential delivery system of omega-3 fatty acids. J. Food Sci. Technol, *52*(7), 4256-4265.
11. Kuhn, K. R., and Cunha, R. L. (2012). Flaxseed oil–whey protein isolate emulsions: effect of high pressure homogenization. J Food Eng, *111*(2), 449-457.
12. Smith, M.A., L.J. Beilin, T.A. Mori and W.H. Oddy. 2011. Essential fatty acids and mood: a systematic review of observational studies. Am. J. Clin. Nutr. 1: 14-27.
13. Tonon, R. V., Grosso, C. R., and Hubinger, M. D. (2011). Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. Food Res. Int, *44*(1), 282-289.
14. Zhang, Y., Zhou, Y., Song, Z., Jin, J., Tang, J., Wang, X., ... and Jin, Q. (2021). A chemometrics approach comparing characteristics and free radical scavenging capacity of flax

(Linum usitatissimum L.) oils obtained from seeds and cakes with different extraction methods. J. Sci. Food Agric, *101*(13), 5359-5367.