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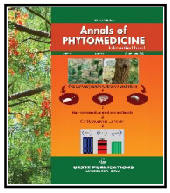

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Effect of liposomal encapsulation on oxidative stability of cold-pressed *Nigella sativa* L. seed oil and virgin coconut oil

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Abstract

An essential quality parameter of vegetable oils is their oxidation stability. In addition to determining their suitability for technological applications, it also determines storage stability. In the present study, effect of liposomal development on the oxidative stability of cold-pressed *Nigella sativa* L. seed oil (NSO) and virgin coconut oil (VCO) was investigated. Both the oils were studied for physiochemical properties and their stability studies were performed. Oils were entrapped in liposomes and evaluated for the stability study. The stability study of both the free oils and the oil entrapped liposomes was performed at 25^{0}C for 30 days. The study findings revealed that the encapsulation of the oils in liposomal formulation retarded oxidative deterioration. Oxidative stability of oils was improved by the encapsulation into liposomes.

1. Introduction

Oils are employed extensively to prevent or treat human diseases, especially when applied topically to promote skin health, heal injuries and burns, reduce scarring, improve cosmetic outcomes, and reduce social stigmatizing. Plant oils can penetrate quickly through the skin's lipid layers and interact with the cell membrane proteins to alter their conformation. The unique physicochemical properties of these compounds are utilized during transdermal drug delivery to enhance skin penetration (Moore *et al.*, 2020).

Black seeds or kalonji, also known as *Nigella sativa* L. seeds, are among the most commonly used herbal medicines due to their wide range of biological and pharmacological properties. It consists of several bioactive constituents which are present in both fixed and essential oils (Camlicau *et al.*, 2019). As an alternative folklore medicine, it has been used for centuries to treat a wide variety of conditions. It has shown effectiveness in treating both infectious as well as non-infectious skin disorders. The dermatological and cosmeceutical applications of *N. sativa* seed oil (NSO) are attributed to its powerful antioxidant, anti-inflammatory, antimicrobial, and immunomodulating properties, making it a promising skincare candidate (Khare *et al.*, 2004; Ahmad *et al.*, 2013; Yimer *et al.*, 2019).

Virgin coconut oil (VCO) is the highest quality coconut oil that can be obtained from the coconut fruit. VCO shares similar chemical properties as coconut oil with added benefits, that is, higher phenolic content and antioxidant activity. Ferulic acids and p-coumaric are major substances that contribute to the antioxidant properties of VCO. It is already being extensively used in tropical areas as homeo medicine and is traditionally used to improve skin health. However, there is limited literature available on the performance of VCO exploited as a topical cosmetic product. Since the topical administration of bioactive compounds often results in poor absorption and limited bioavailability, therefore studies on VCO-based topical cosmeceutical are necessary. The externally applied cosmeceuticals (drugs/skincare products) may be targeted to remain at the epidermis (Mansoor *et al.*, 2012; Marina *et al.*, 2009; Ghani *et al.*, 2018).

In comparison to virgin oils, refined oils are much more processed, and therefore provide fewer nutrients. The oil is treated with a chemical solution (typically sodium hydroxide) and then deodorized and bleached as part of the refining process. To keep costs down, refined oils may be combined with cheaper oils. Refined oils lack many of the nutrients and antioxidants that are present in virgin oils, so they cannot provide the full benefits of these oils. Although, refined oils might be cheaper, they might not provide the results you were hoping for because the potent antioxidants have been stripped from the oil (Mustafa, 2014). Using cold-pressed oils is an extra step we take to ensure maximum results from our skincare products. A cold press compacts seeds and plants between two plates, releasing their natural oil. Applying heat to the seeds and nuts as they are being pressed can speed up the process and produce more oil; the downside is that this heat can also break down the nutritional content and weaken the aroma (Teh *et al.*, 2013).

However, these oils are rather susceptible to lipid oxidation, due to their composition, especially the presence of unsaturated molecules, which can undergo degradation reactions, leading to nutritional losses and the formation of compounds that are toxic to human health (Maszewska *et al.*, 2018; Ozdemir *et al.*, 2018; Chandran *et al.*, 2017).

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The entrapment of oils into a stable formulation has emerged as an alternative to increase stability. Liposomes are an emerging encapsulating vector. According to the literature, many components that are sensitive to oxygen, temperature and light are protected by liposome encapsulation to extend their shelf life. Liposomes are spherical natural phospholipids vesicles consisting of one or several lipid bilayers. They can enclose both hydrophobic and hydrophilic compounds, avoiding the decomposition of the entrapped molecules and releasing them to designated targets. The main structural components of the liposome are lipids such as egg or soybean phosphatidylcholine, similar to those found in biological membranes (Le *et al.*, 2019; Yeligarυ *et al.*, 2021). The physicochemical characteristics of liposomes are majorly regulated by the incorporated phospholipid. Cholesterol, a sterol, is most commonly used thereafter, in liposome formulations. The empty spaces between the phospholipid molecules are filled with cholesterol which strengthens the liposomal membrane and provides structural stability (Ahmad *et al.*, 2020; Nakhaei *et al.*, 2021)

NSO and VCO are of paramount importance in pharmaceutical and cosmetic areas due to their crucial properties. However, stability and bioactivity determine the effectiveness of these oils. These oils are easily oxidized, leading to rancidness and a decline in quality. Therefore, these oils have a high risk of oxidation in direct applications. Hence, the present study aims to investigate effect of liposomal encapsulation on oxidative stability of cold-pressed *N. sativa* seed oil and virgin coconut oil.

2. Materials and Methods

2.1 Materials

Nigella sativa L. seed oil (NSO) was procured from Safa Honey cooperation, Bengaluru, and Virgin coconut oil (VCO) was procured from Coco soul Marico, Mumbai, India. Cholesterol Extra Pure was purchased from E. Merk (India) Limited. Soya-L- α-Lecithin was procured from Himedia Laboratories Pvt Limited. Chloroform and methanol were purchased from Qualigens Fine Chemicals, Mumbai, India. All the chemicals were of analytical grade. Double distilled water (DDW) was used throughout the study.

2.2 Methods

2.2.1 Preparation of liposome

The liposomes were prepared by using a modified thin-film hydration method. Various ratios of cholesterol and soya lecithin were weighed and dissolved in a chloroform and methanol (2:1 v/v) mixture in round bottom flasks. The flask was rotated continuously at room temperature in such a way that it formed a thin layer around the flask and chloroform was evaporated. A perforated aluminum foil cover was placed over the flask for 12 h to ensure complete chloroform removal. The dry film was hydrated with a 20 ml phosphate buffer solution (pH 7.4) (Wang *et al.*, 2020; Yeligarυ *et al.*, 2021**)**.

2.3 Physiochemical screening

The physicochemical tests were performed for both the oil samples, *i.e.*, NSO and VCO. All the tests were conducted following the Food Safety and Standards Authority of India (FSSAI), American Oil Chemists Society (AOCS), Asian Pacific Coconut Community (APCC), and Indian standards (IS).

2.3.1 Iodine value

The iodine value of both the oils and liposomes were determined by Wijs method (AOCS, 2004). After mixing 4.0 g of sample with 20 ml cyclohexane to dissolve the fat content, 25 ml of Wijs solution was added. The flask was closed and shaken continuously for 30 min. The flask was closed and the mixture was shaken continuously for 30 min. At the same time, 20 ml of KI solution (15% v/v) was added to the mixture, which was then titrated against 0.1 N $Na_2S_2O_3$ until the disappearance of yellow color. The samples were then exposed to a few drops of starch solution, turning the solution blue, and the titration continued until the blue color diminished. The $Na_2S_2O_3$ consumption was recorded and represented as S. For the analysis, the same process was repeated with a blank sample and the volume of $Na_2S_2O_3$ consumed was recorded as B. The IV was calculated using the following formula:

$$\text{IV} = (\text{B}–\text{S}) \times \text{Sodium thiosulphate} \times \frac{12.69}{\text{Weight of sample (gm)}}$$

2.3.2 Peroxide value

The peroxide value is a parameter specifying the content of oxygen as peroxide, especially hydroperoxides in a substance. The peroxide value is a measure of the oxidation present.

The peroxide value (PV) of both the oils and liposomes were determined by the International Union of Pure and Applied Chemistry (IUPAC) 2.201(1979)/ISO 660:1996). Diethyl ether and ethanol of equal volume (25 ml) were mixed and 1ml of 1% phenolphthalein indicator solution was added. The mixture was then neutralized with 0.1M potassium hydroxide solution and the oil sample (between 1-10 g) were dissolved in the neutralized solvent mixture. The sample mixture was then titrated against 0.1 M potassium hydroxide solution with constant shaking until the appearance of pink color that persisted for about 15 seconds was obtained.

$$\text{PV(mEq per 1000 g)} = (V_s – V_b) \times F \times N \times 1000$$

where V_s = titration volume of sample (ml); V_b = titration volume of blank (ml); F = factor of 0.01N $Na_2S_2O_3$ solution; W = weight of fat in volume of extract used (g); N = normality of $Na_2S_2O_3$ solution (in this case N/100) (IFRA 2019).

2.3.3 Moisture content

Moisture content and volatile components are important factors to determine the oil quality. Moisture content (MC) of the oils was determined based on the American Oil Chemists Society (AOCS) (Firestone *et al.*, 2009) method. About 5.0 g of oil sample was weighed in a crucible with a lid which was preheated, weighed, and dried. It was then heated at 105°C for about 24 h until there is no change in the successive observations. The crucible with the sample was then placed in the desiccator and allowed to cool to room temperature. The crucible with oil sample was then re-weighed (Mansor *et al.*, 2012). The moisture content was then calculated using the following formula:

$$\%\ \text{Moisture content} \frac{\text{Initial weight} – \text{Final weight}}{\text{Initial weight}} \times 100$$

2.3.4. Refractive index at 40 °C (RI)

The refractive index (RI) of the oil samples was measured using precision Abbe's refractometer based on AOAC official method and

ISI having a measuring range of refractive index of 1.300-1.700 with the accuracy within ± 0.0002 (Ghani *et al.*, 2018). The refractive index was calculated by sandwiching 1 or 2 drops of oil sample between illuminating and refracting prisms of Abbe's refractometer using the syringe.

2.3.5 Density

The density of edible was calculated by mass of sample per unit volume (Ghani *et al.*, 2018).

Density = mass of the oil (g) / volume of the oil (ml)

2.3.6 Specific gravity

Specific gravity of oil is determined as the ratio of the density of oil into the density of water at the same temperature (Ghani *et al.*, 2018).

Specific gravity = Density oil/ Density of water

2.4 Stability studies

The stability of both the free oils and the oil entrapped liposomes were performed at 25^{0}C for 30 days. Samples were stored in the ambered color bottle with a cap covered with aluminium foil. The iodine value and peroxide value of free oils and liposomes were measured and compared to determine the oxidative stability of the free oil vs the developed liposomes. Moisture content in free oils was also determined to study the effect of moisture content on the oxidation of oils.

2.5 Statistical analysis of data

Dunnett's t-test was used for the analysis of data. Results are presented as a mean ± SD, whereas $p<0.05$ was considered statistically significant.

3. Results

3.1 Physicochemical screening

The physicochemical tests were carried out for both the oil samples, *i.e.*, NSO and VCO. Both the oils were evaluated for its organoleptic characters, refractive index, specific gravity, iodine value, peroxide value and moisture content. Results of the physicochemical evaluations are presented in Table 1 and Table 2.

Table 1: Physicochemical properties of *N. sativa* oil (NSO)

Sl. No.	Parameters	Values obtained
1.	Organoleptic characters	
	Color	Orangish brown
	Odor	Characteristic
	Taste	Characteristic
2.	Refractive index	1.4671 ± 1.23
3.	Specific gravity	0.982 ± 1.12
4.	Iodine value (g I_2/100 g)	128 ± 1.42
5.	Peroxide value (mEq/kg)	5.24 ± 1.31
6.	Moisture content (% w)	0.54 ± 0.02
7.	Density (g/cm^3)	0.86 g/ml

Table 2: Physicochemical properties of virgin coconut oil (VCO)

Sl. No.	Parameters	Values obtained
1.	Organoleptic characters	
	Colour	Colourless
	Odour	Characteristic
	Taste	Characteristic
2.	Refractive index	1.4581 ± 1.43
3.	Specific gravity	0.921 ± 1.16
4.	Iodine value (g I_2/100 g)	5.891± 0.86
5.	Peroxide value (mEq/kg)	0.63 ± 0.34
6.	Moisture content (% w)	0.11 ± 0.01
7.	Density (g/cm^3)	0.88 g/ml

3.2 Stability studies

The stability study of both the free oils and the oil entrapped liposomes were performed at 25^{0}C for 30 days. Figure 1 represents the effect of thermal oxidation on the peroxide value of free NSO, VCO and oils entrapped liposomes. Influence of thermal oxidation on iodine value of free NSO, VCO, and oils entrapped liposomes are shown in Figure 2. Effect of stability conditions on the moisture content of oils was also studied. Results obtained are shown in Figure 3.

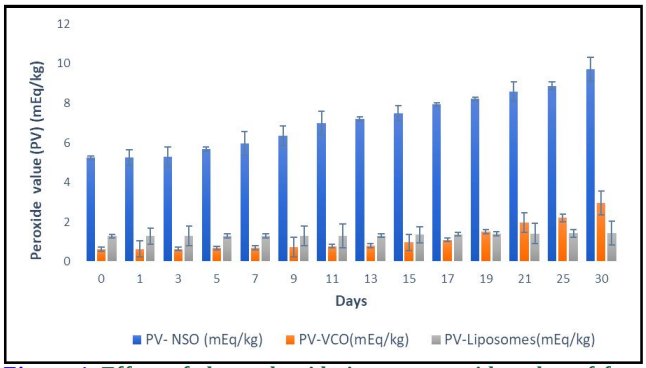

Figure 1: Effect of thermal oxidation on peroxide value of free NSO, VCO, and oils entrapped liposomes. All values are expressed as Mean ± SD (n = 3).

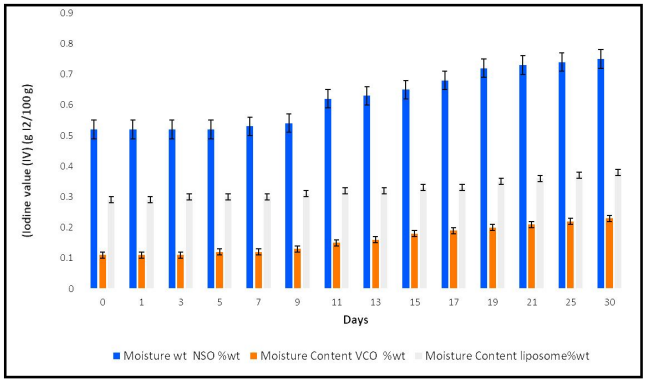

Figure 2: Effect of thermal oxidation on iodine value of free NSO, VCO, and oils entrapped liposomes. All values are expressed as Mean ± SD (n = 3).

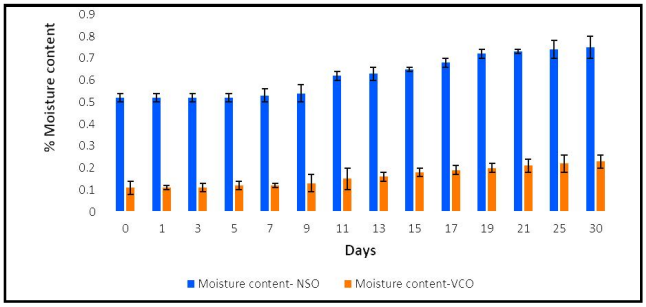

Figure 3: Effect of stability study on moisture content of free oils. All values are expressed as Mean ± SD (n = 3).

4. Discussion

In the present study, oxidative stability of free NSO and VCO were investigated and compared with oils entrapped liposomes. Both the oils and oils entrapped liposomes were stored at 25^0C for 30 days. The physicochemical properties of both the oils were studied, and compared with the oils entrapped liposomes. The peroxide value, iodine value, and moisture content were analyzed to determine the quality of the oil.

The peroxide value of NSO was greater than VCO, this could be due to its structural features the presence of ether linkages. The peroxide value in the initial phase was found to be 5.24 mEq/kg and 0.63 mEq/kg of NSO and VCO, respectively and at the end of 30 days, the values attained were 9.72 and 2.96, respectively. After 30 days, there was a noticeable increment in peroxide values of the free oil. It complies with the increase in free radicals. On introducing the mixture of two oils together in liposomal formulation, there was a significant decrease in the peroxide value of the NSO. This compiles that the oils entrapped in liposomes were protected from free radical mechanism to a certain extent as compared with the free oils. As illustrated in Figure 1, on the first day, all the samples showed a low level of oxidation, ranging from 0.77–0.82 mEq peroxide/kg oil. The study revealed after 30 days of storage, free oils presented a higher level of peroxide value whereas oils entrapped liposomes showed very less value. Encapsulation of oils in liposomes protected the unsaturated fatty acids from oxidation. The findings of the study correlate with the previously reported works. Previous studies have reported that phospholipid possess high antioxidant potential which might be responsible for protecting the NSO and VCO from oxidation which is in agreement with our findings (Anwar *et al.*, 2003). Due to the antioxidant potential of the phospholipid present in the liposomal structure, oxidation of oil was reduced by encapsulation.

The iodine value is a measure of the relative degree of unsaturation in oil. The greater the iodine value, the more unsaturation and the higher the susceptibility to oxidation. The iodine value of NSO was greater than VCO, this could be due to the presence of a greater number of double bonds. The iodine value in the initial phase was found to be 5.891 (g I_2/100 g) and 122 (g I_2/100 g) for VCO and NSO, respectively. After storage for 30 days, free oils showed an increase in the IVs whereas liposomes containing oils did not show any noticeable change which concludes the stability of oils in the liposomal entity. The encapsulation of the oils in liposomes retarded the oxidative deterioration.

The amount of water in the oil allows hydrolysis reaction that attributes to the formation of free fatty acids and causes rancidity. After 30 days of storage, the moisture content in free oils was observed. Results showed that the moisture content in NSO was higher which indicated the increased chances of rancidity in NSO than VCO.

5. Conclusion

In the present study, effect of liposomal encapsulation on the oxidative stability of cold-pressed *N. sativa* seed oil and virgin coconut oil was investigated. Both the oils were studied for physiochemical properties and their stability studies were performed. The oxidative stability of the *N. sativa* oil and virgin coconut oil was found to be increased by entrapping them in the liposome. Hence, the study findings conclude that the oxidative stability of vegetable oils could be enhanced by encapsulation into liposomes.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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