Development of hemp seed oil nanoemulsions loaded with ascorbyl palmitate: Effect of operational parameters, emulsifiers, and wall materials

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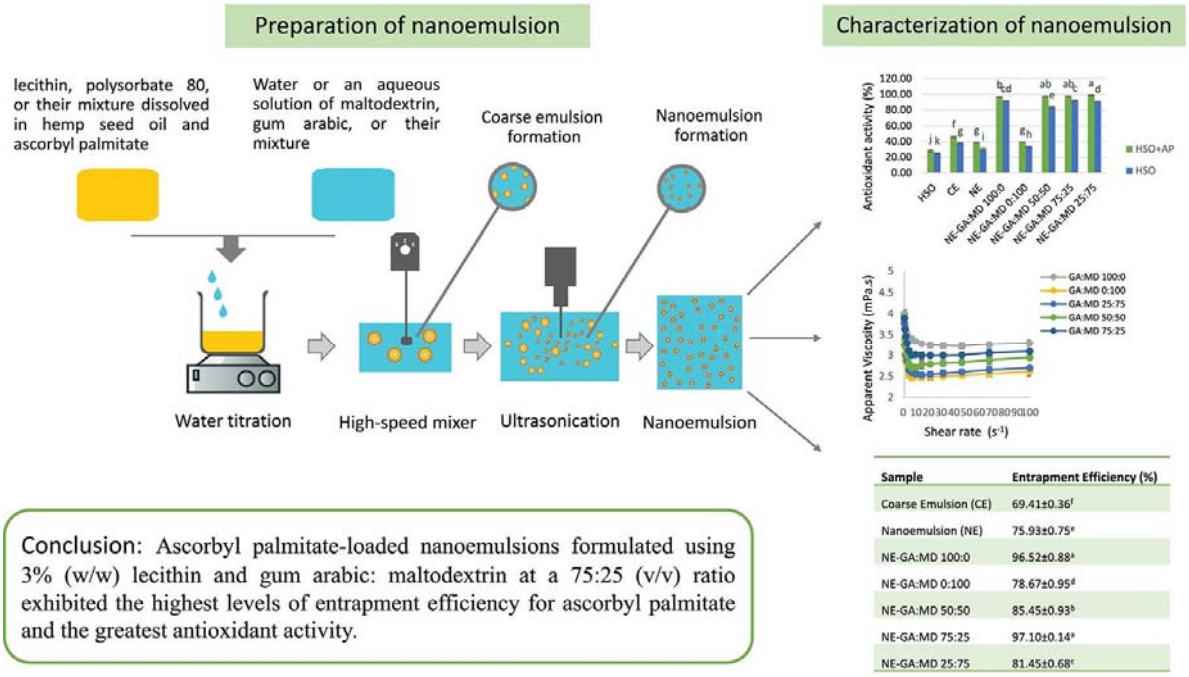
Highlights

- Gum arabic and maltodextrin are very efficient in entrapment of ascorbyl palmitate.
- Proper wall material can effectively preserve the antioxidant activity of the core.
- Nanoemulsions containing gum arabic have the highest antioxidant activities.
- Incorporation of ascorbyl palmitate can effectively retard the oxidation process.

Abstract

The perceived health properties of hemp seed oil, as one of the few plant-based sources of omega-3 and omega-6 fatty acids with an ideal ratio of 1:3, suggest its incorporation in food-grade emulsions to improve its water solubility and oxidative stability. The current research's main aim was nanoemulsification of hemp seed oil using the oil-in-water emulsification method followed by ultrasonication. The entrapment efficiency of the nanoemulsions for antioxidant ascorbyl palmitate and its impact on oxidative stability of the oil was also evaluated. Gum arabic: maltodextrin in 75:25 ratio could result in nanoemulsion with entrapment efficiency of 97.10 % for ascorbyl palmitate and radical scavenging activity of oil-soluble bioactives of 92.13 %. Moreover, incorporation of ascorbyl palmitate could effectively retard the oxidation, specifically in nanoemulsions containing gum Arabic. The optimum formulation of nanoemulsion having an average droplet size of 293 nm can be applied as an ideal vegetarian source of omega-3 fatty acids.

Graphical abstract



Sample	Entrapment Efficiency (%)
Coarse Emulsion (CE)	69.41±0.36^{f}
Nanoemulsion (NE)	75.93±0.75^{e}
NE-GA:MD 100:0	96.52±0.88^{a}
NE-GA:MD 0:100	78.67±0.95^{d}
NE-GA:MD 50:50	85.45±0.93^{b}
NE-GA:MD 75:25	97.10±0.14^{a}
NE-GA:MD 25:75	81.45±0.68^{c}

Keywords: Antioxidant; Ascorbyl palmitate; Entrapment; Hemp seed oil; Nanoemulsion; Ultrasound

Chemical compounds

Gum arabic (PubChem CID91333377)
Maltodextrin (PubChem CID68229136)
Polysorbate 80 (PubChem CID5284448)
Lecithin (PubChem CID10425706)
2,2-Diphenyl-1-picrylhydrazyl (PubChem CID129737978)
Acetic acid glacial (PubChem CID176)
Acetonitrile (PubChem CID6342)
Methanol (PubChem CID887)
Ethanol (PubChem CID702)

1. Introduction

Omega-3 polyunsaturated fatty acids are metabolized in the body by metabolic pathways into anti-inflammatory and anti-proliferative metabolites contributing to the reduced risk of various types of cancer, cardiovascular diseases, cognitive decline, depression, and inflammatory disorders of the skin (Abedi and Sahari, 2014, Innes and Calder, 2020, Shahidi and Ambigaipalan, 2018). Although fish oil contains considerable quantities of omega-3 polyunsaturated fatty acids, there are several limitations linked with its consumption due to the presence of toxic chemicals (dichlorodiphenyltrichloroethane and polychlorinated biphenyls), heavy metals (lead, mercury, arsenic, and cadmium) and high cost and complexity of

purification of omega-3 polyunsaturated fatty acids (Abedi and Sahari, 2014, Innes and Calder, 2020). Its limited availability and affordability in many regions, environmental sustainability, high susceptibility to oxidation, and undesirable tastes and odors are also matters of concern worldwide (Abedi & Sahari, 2014).

Hemp (*Cannabis sativa* L.) seed oil is known as a rich natural vegetable source of omega-3 and omega-6 fatty acids. It may be considered as a substitute source for conventional fish oil or a genuine alternative for vegetarians. Unsaturated fatty acids make up about 85 % of hemp seed oil, while its content of saturated fatty acids is very low. The hemp seed oil contains a unique fatty acid composition of linoleic (55 %), α-linolenic (17 %), oleic (12 %), and γ-linolenic acid (3 %). It also delivers some γ-tocopherol, β-sitosterol, phytol, campesterol, and cycloartenol, hence receiving growing attention in the formulation development research of healthy and nutritious food products (Jarzębski et al., 2021, Montserrat-de la Paz et al., 2014).

Besides the amount of omega-3 polyunsaturated fatty acids, the balanced ratios of omega-3 to omega-6 fatty acids are well documented to be of nutritional and pharmacological value. As biologically active lipids, eicosanoids have a fundamental impact on the regulation of inflammation and immune functions. They are derived primarily from arachidonic acid, whereas eicosapentaenoic acid (omega-3 fatty acid) is found to be in competition with arachidonic acid (omega-6 fatty acid), inducing the production of eicosanoids with less bioactivity (Abedi and Sahari, 2014, Montserrat-de la Paz et al., 2014, Shahidi and Ambigaipalan, 2018). The hemp seed oil has an ideal ratio of 1:3, whereas fish oils have a typical mean ratio of more than 6:1 of omega-3: omega-6 (Abedi & Sahari, 2014).

Despite all the rare and interesting biological attributes of hemp seed oil, its peroxidative deterioration by environmental stresses and its poor water-solubility and bioavailability remain major limitations in its application for the formulation of functional beverages and foods (Khalid et al., 2017, Mikulcová et al., 2017). Ascorbyl palmitate has triggered enormous research interest in recent years owing to its exceptional performance as an antioxidant and higher stability than vitamin C. Such nutraceutical shows similar efficiency to ascorbic acid in scavenging oxygen-derived free radicals and inhibiting the formation of hydrogen peroxide (Zhou et al., 2017). It is believed to inhibit oxidation and degradation and enhance oxidation stability of the oil during process and storage with a protection factor (the extent of oxidative stability improvement in the oil achieved after the inclusion of ascorbyl palmitate) of 1.31 (Senanayake, 2018, Yin et al., 2021). Since ascorbyl palmitate is a more stable hydrophobized derivative of ascorbic acid, it is easy to dissolve it in the oil to benefit from its strong antioxidant properties (Yin et al., 2021, Zhou et al., 2017).

Incorporation of ascorbyl palmitate in hemp seed oil-in-water emulsions can enhance its dissolution rate, oral bioavailability, permeation across the biological cell membrane, and therapeutic effects (Amiri-Rigi and Abbasi, 2016, Khalid et al., 2017, Zheng et al., 2020). When dissolved in the internal phase of a nanoemulsion, ascorbyl palmitate can be protected from environmental stresses such as UV or visible light and particularly oxygen brought in during emulsification. This protection provided by surfactant molecules at the interface and the aqueous phase would result in improved functionality of the ascorbyl palmitate. Moreover, nonpolar antioxidants can show higher capacity in a medium of relatively higher polarity (e.g., oil-in-water emulsion) than bulk oil, clearly attested by polar paradox theory that explains the paradoxical behavior of antioxidants as influenced by their own polarity and by that of the surrounding media (Aswathanarayan and Vittal, 2019, Zheng et al., 2020).

Emulsions comprised of smaller droplets (e.g., nanoemulsions) are usually of great scientific interest, not only due to their higher kinetic stability over time but also because bioactive compounds in smaller droplets show improved solubility, controlled/sustained release, digestibility, and functionality in the upper gastrointestinal tract (Aswathanarayan & Vittal, 2019). Nonetheless, the greater surface area provided by very small-sized droplets of a nanoemulsion can cause higher exposure of an oil droplet to the surrounding environment leading to more oxidative deterioration. A proper selection of coating material(s) may allow the formation of sufficiently vigorous interfacial layers to retard free radical penetration into the oil droplets, thus shielding the bioactive compounds from degradation when exposed to undesirable processing and storage conditions such as pH changes, heat stress, exposure to reactive oxygen and UV light (Firoozy and Anarjan, 2019, Premi and Sharma, 2017, Zheng et al., 2020). In addition to improving chemical stability, encapsulation of core lipophilic bioactives in nanoemulsion can enhance their water dispersibility, slow and controlled release, intestinal absorption, and bioavailability through efficient entrapment of the hydrophobic molecules in proper wall materials (coating agents) (Aswathanarayan & Vittal, 2019). Maltodextrin and gum arabic have been among the most commonly used wall materials for the encapsulation of oil-soluble bioactives. Maltodextrin has been demonstrated to provide highly effective protection against oxidative deterioration, even though it shows poor emulsifying properties. Thus, its application with coating materials of higher emulsifying ability such as gum arabic can provide both chemical stability and enhanced functionality for fat-soluble core bioactives (Premi & Sharma, 2017).

Few studies on hemp seed oil, as an ideal source of omega-3 fatty acids, have generally addressed hemp seed oil emulsification using synthetic non-ionic surfactants (Mikulcová et al., 2017) and refined lecithin and poloxamer 188 in a high concentration of about 6 % in the optimum formulation (Fathordoobady et al., 2021) without incorporating any antioxidant and coating material in the formulation that may enhance the oxidative stability of the hemp seed oil in tiny droplets of emulsion. Stability analysis of hemp seed oil emulsions prepared using a relatively high concentration of surfactant (5.2 %) has also been performed by Kowalska, Ziomek, and Zbikowska (2015) for industrial applications. Other studies have also revealed the antibacterial activity of hemp seed oil emulsions against *Staphylococcus aureus* subsp. *aureus* and *Micrococcus luteus* (Mikulcová et al., 2017).

However, currently, there is a notable absence of knowledge on nanoemulsification of hemp seed oil using food-grade emulsifiers and ascorbyl palmitate encapsulation in hemp seed oil-in-water nanoemulsions applying gum arabic and maltodextrin as coating materials. Optimization of main operational parameters and composition of hemp seed oil-in-water nanoemulsion as a potential delivery system for ascorbyl palmitate or other nutraceuticals has also been neglected but seems worth studying.

Therefore, the current study was conducted to fabricate hemp seed oil-in-water nanoemulsions and provide a better picture of the effect of main operational parameters, emulsifier type, and application of coating materials on the mean droplet diameter, polydispersity index, zeta potential, and rheological properties of hemp seed oil-in-water nanoemulsions. Loading and entrapment efficiency of the developed systems for ascorbyl palmitate as an antioxidant and its effect on overall radical scavenging activity was also evaluated.

2. Materials and methods

2.1. Materials

Unrefined additive-free cold-pressed hempseed oil was provided by Hemporium (Cape Town, Western Cape). Gum arabic (Acacia Gum), maltodextrin with dextrose equivalent of 10, polysorbate 80, refined soy lecithin and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were supplied through Merck (Darmstadt, Germany). Acetic acid glacial, acetonitrile, methanol, ethanol, and other solvents were of chromatography or analytical grade, acquired from Merck (Darmstadt, Germany), Sigma Aldrich Co. ltd (St. Louis, MO, USA) or other reputable suppliers.

2.2. Preparation of nanoemulsion

The importance of the addition sequence of the main components in the nanoemulsion preparation technique was taken into account (Meng et al., 2019). First, the coating materials of maltodextrin and gum arabic were homogenized in ultrapure water to a 4 % (w/v) aqueous solution. In the present study, gum arabic and maltodextrin were separately dissolved in ultrapure water, and then the resultant aqueous solutions were mixed to get different gum arabic: maltodextrin ratios of 0:100, 25:75, 50:50, 75:25, and 100:0. In order to ensure complete dissolution and hydration of biopolymers of maltodextrin and gum arabic, the prepared solutions were kept for at least 12 h before use (Meng et al., 2019, Zheng et al., 2020).

Subsequently, 5 % (w/w) hemp seed oil (with and without 10 mg of ascorbyl palmitate per 50 ml of nanoemulsion) was dissolved in 3 % (w/w) lecithin, polysorbate 80 or their mixture at ratios of 0:1, 1:2, 1:1, 2:1, and 1:0. Nanoemulsions were then prepared by water titration method using either deionized water (control, without coating material) or aqueous phase containing varying ratios of gum arabic: maltodextrin of 0:100, 25:75, 50:50, 75:25, and 100:0 as coating materials to evaluate the performance of coating materials in terms of emulsification process, entrapment of ascorbyl palmitate and preserving the oxidative capacity of oil-soluble bioactives.

A two-step procedure was applied to prepare hemp seed oil nanoemulsion comprising an oil-in-water emulsification step followed by ultrasonication. In the first step, the dispersed phase (hemp seed oil/ mixture of hemp seed oil and ascorbyl palmitate) was titrated dropwise with the aqueous phase under continuous stirring and then thoroughly mixed using a digital Ultra Turrax T25 homogenizer (IKA, Staufen, Germany) with an S25KV-25F model rotor head (IKA, Mission Viejo, California, USA) for approximately 5 min to prepare the coarse oil-in-water emulsion. Further, the mean droplet diameter of coarse emulsions was reduced using a Q700 sonicator system (QSonica LLC, Newtown, CT, USA) equipped with a cup horn at a frequency of 20 kHz and different amplitudes and durations of 50 % and 15 min, 50 % and 30 min, 80 % and 15 min, and 80 % and 30 min, respectively, to assess the effect of energy output on nanoemulsification process. The temperature during the sonication process was maintained near constant by immersing the sample in an ice bath, and the pulse cycled on and off every 1 min (Jarzębski et al., 2021, Mehmood and Ahmed, 2020, Meng et al., 2019).

The best formulations of the nanoemulsion were selected for further analysis of the effect of coating materials once different main operational parameters and surfactants were tested. The optimization process was based on the results of the mean droplet diameter, polydispersity

index (PDI), and zeta potential. First of all, the effect of main operational parameters (homogenization speed, sonication amplitude, and sonication time) was tested on mean droplet diameter (nm), polydispersity index (PDI), and zeta potential (mV) of nanoemulsions prepared using lecithin: polysorbate 80 at 1:1 ratio (selected based on preliminary experiments) without using any coating material. Subsequently, the effect of different proportions (0:1, 1:2, 1:1, 2:1, and 1:0) of surfactants lecithin and polysorbate 80 on mean droplet diameter (nm), polydispersity index (PDI), and zeta potential (mV) of nanoemulsions, prepared without coating material using the optimal main operational parameters (obtained in the previous stage) was assessed. Then, the wall materials (maltodextrin and gum arabic) and core (ascorbyl palmitate) were incorporated into the nanoemulsions prepared using optimum main operational parameters and optimum surfactants ratio. Finally, the effect of different proportions of coating materials (0:100, 25:75, 50:50, 75:25, and 0:100 of gum arabic: maltodextrin) on mean droplet diameter, polydispersity index (PDI), zeta potential, overall antioxidative activity, as well as entrapment efficiency (%), and loading efficiency for ascorbyl palmitate was studied.

2.3. Droplet diameter and polydispersity index

Mean droplet diameter (average peak maximum by intensity) and polydispersity index (PDI) analysis of the nanoemulsion were based on dynamic light scattering (DLS) by means of Zetasizer (Malvern Zetasizer Nanosizer®, Malvern Instruments Ltd, Worcestershire, UK) with a fixed light-scattering angle of 90 at ambient temperature (25 ± 1 °C). Hydrodynamic size and polydispersity index measurements were performed immediately following about 100 times dilution of aliquots of nanoemulsions with deionized water in 1 cm disposable cuvettes just before the analysis to avoid multiple scattering effects (Meng et al., 2019).

2.4. Zeta potential

The surface electrical charges (zeta potentials) of droplets were determined via laser Doppler electrophoresis by means of the Zetasizer. The multiple scattering effects were minimized by a 20-fold dilution of nanoemulsions with deionized water and vortex-mixing to ensure homogeneity of the sample before being loaded into a capillary cell to evaluate zeta potentials at ambient temperature (25 ± 1 °C) (Jarzębski et al., 2021).

2.5. Modified DPPH method

Antioxidant activities of the dispersed phase (hemp seed oil/ mixture of hemp seed oil and ascorbyl palmitate) of nanoemulsions were determined through the DPPH radical method of Song, Jang, Kim, Kim, and Lee (2016) with some modifications. Briefly, nanoemulsion samples were mixed with hexane in a 1:1 (v/v) ratio in centrifuge tubes and vortexed for 2 min. The mixture was then centrifuged at a centrifugal force of 6000×*g* at 4 °C for 20 min using Hermle Z366K centrifuge (HERMLE Labortechnik GmbH, Wehingen, Germany) before leaving under the hood for at least 24 h. When the hexane has been all evaporated, oil was diluted with methanol until a final concentration of 40,000 ppm was reached and vortexed for 1 min. The mixture of the sample and methanol was subsequently centrifuged at a centrifugal force of 9500×*g* for 3 min using a Hettich Zentrifugen Mikro 120 (Andreas Hettich GmbH and Co. KG, Tuttlingen, Germany). The alcoholic supernatant was collected and mixed with 1×10^{-4} mol/L DPPH in methanol at a 1:1 (v/v) ratio. Control was prepared by mixing methanol with DPPH radical solution in a 1:1 (v/v) ratio, and a blank was composed of the alcoholic supernatant of the sample and methanol at a ratio of 1:1 (v/v). After leaving

in darkness at room temperature with an incubation time of 30 min, the absorbances of the samples were read at 517 nm, the most sensitive wavelength for DPPH dissolved in methanol, using a FLUOstar Omega microplate reader spectrophotometer (BMG Labtech GmbH, Ortenberg, Germany). If the absorbance value was below 0.3, the sample was further diluted with DPPH radical solution to read an absorbance greater than 0.3 but lower than 0.7 (Song et al., 2016, Zheng et al., 2020). The antioxidant capacity of the samples was calculated and presented as the percentage of DPPH scavenged by the antioxidants present in the sample compared to the control by using Eq. (1).

$$\text{DPPH radical scavenging activity } (\%) = 1 - \frac{A_{sample} - A_{control}}{A_{blank}} \times 100 \quad (1)$$

where A_{sample}, A_{blank}, and $A_{control}$ stand for absorbance of sample, blank, and control, respectively.

2.6. Loading and entrapment efficiency

The amounts of free ascorbyl palmitate in the nanoemulsions were determined through the ultrafiltration technique employing centrifugal concentrator devices having 10 kDa MWCO (molecular weight cut-off) and high-performance liquid chromatography (HPLC). The reversed-phase HPLC was conducted with a Waters 1525 Binary HPLC pump and a Waters 2487 Dual Absorbance detector running on Breeze software (Waters Corporation, Milford, Massachusetts, USA). Chromatographic separation was achieved on a Phenomenex-Luna C18 HPLC column (250 × 4.6 mm, 5 µm, Torrance, CA, USA), and the detection was by UV absorbance at 243 nm. The injected volume was 20 µl, and analytes were eluted by a mobile phase of acetonitrile: 0.5 % acetic acid (95:5 v/v) at a 1 ml/min flow rate at 30 °C. The entrapment efficiency and loading efficiency of ascorbyl palmitate were obtained according to the following equations:

$$\text{Entrapment efficiency } \% = \left[1 - \frac{Weight\ \ of\ free\ ascorbyl\ palmitate}{Weight\ \ of\ the\ feeding\ Ascorbyl\ palmitate}\right] \times 100 \quad (2)$$

$$\text{Loading efficiency } \% = \left[\frac{Weight\ \ of\ entrapped\ ascorbyl\ palmitate}{Total\ \ weight\ of\ nanoemulsion}\right] \times 100 \quad (3)$$

(Chou et al., 2021, Zhou et al., 2017).

2.7. Viscosity analysis

Viscosity variation of the nanoemulsion samples as a function of shear rate was monitored applying a Physica MCR 101 rheometer (Anton Paar, Ostfildern, Germany) mounted with a concentric cylinder geometry operating in rotational mode. All rheological data were generated and recorded through Rheoplus software version 3.0x (Anton Paar, Ostfildern, Germany). For viscosity analysis, nanoemulsion samples (10 ml) were subjected to a steady-shearing ramp within the range of 0.01 to 1000 s^{-1} at a constant temperature of 25 °C (Teo et al., 2015).

2.8. Statistical analysis

One-way analysis of variance followed by post-hoc tests (Duncan multiple range test) was performed to investigate statistical differences between the individual treatments (Statistica, StatSoft, Inc., Tulsa, OK, USA). The p-value of ≤ 0.05 was regarded as the level of significance. All assays were carried out at least in triplicate in 3 independent experimental sets, the data were averaged, and the standard deviations were calculated. The result values are expressed as mean ± SD.

3. Results and discussion

The structure and subsequent stability of nanoemulsions are greatly affected by the main emulsification parameters such as homogenization speed, sonication energy, and sonication time and their prime components (Hamed and Abo-Elwafa, 2020, Zheng et al., 2020). First, the process was optimized by taking various homogenization speeds, sonication amplitudes, and sonication times based on physicochemical characterization of particle size, polydispersity index, and zeta potential. In these series of experiments, all samples were prepared using lecithin: polysorbate 80 at a ratio of 1:1 as surfactant selected based on preliminary experiments (data not shown) without using coating materials.

The surfactant type was then optimized by preparing five different formulations comprising lecithin: polysorbate 80 at ratios 1:0, 2:1, 1:1, 1:2, and 0:1 without coating material at optimum operational conditions. Further, various combinations of wall materials (maltodextrin and gum arabic) were applied to select the optimum wall material while keeping the operational parameters and surfactant type constant. Finally, the best formulation was selected based on characteristics such as mean droplet diameter, polydispersity index, zeta potential, antioxidant activity, entrapment efficiency, and viscosity for further studies. However, the emphasis was given to antioxidant activity and entrapment efficiency.

3.1. Effect of energy input

In optimizing the prime process parameters of nanoemulsion preparation, the primary purpose was to decrease the mean droplet diameter (average peak maximum by intensity) as it is well documented to be correlated with higher stability, absorption, bioavailability, and functionality (Mikulcová et al., 2017, Teo et al., 2015). Supplementary Table 1 represents the effect of homogenization speed, sonication amplitude, and sonication duration on mean droplet diameter (nm), zeta potential, and polydispersity index of nanoemulsions prepared using lecithin: polysorbate 80 at a 1:1 ratio without using any coating material. The mean droplet diameter was significantly controlled by the sonication process, while the effect of homogenization speed was not remarkable. Increasing the sonication time at a lower amplitude (50 %) led to the formation of nanoemulsions with smaller sizes, though a higher amplitude (80 %) significantly increased the mean droplet diameter (see Supplementary Table 1).

Homogenization as a low-energy technique uses mixing devices and mechanical energy to homogenize two immiscible phases of oil and water and form the coarse emulsion. It has been reported in previous literatures that the droplet size of the emulsions is not influenced by stirring speed (Santana, Perrechil, & Cunha, 2013). On contrary, ultrasound as a high-energy technique is remarkably efficient in droplet size reduction in emulsions containing comparatively low concentrations of surfactant(s) such as nanoemulsions (Santana et al.,

2013). Long sonication time at a relatively lower amplitude is usually more efficient in decreasing mean droplet diameter and providing a narrower distribution curve (Fathordoobady et al., 2021). In terms of short ultrasonication times, the mean droplet diameter of the developed system is mainly dictated by the specific energy input (Aswathanarayan & Vittal, 2019). Nevertheless, it should be noted that further transformation of droplet sizes can occur with increasing sonication power or time through shear or surface-induced coalescence, implying the importance of adjusting the optimum level of input energy (Fathordoobady et al., 2021, Hadžiabdić et al., 2017).

Sonication energy first disrupts the dispersed phase into fine droplets via mechanisms such as cavitation and shear forces (Amiri-Rigi, Abbasi, & Emmambux, 2022). Subsequently, amphiphilic molecules adsorb onto the newly formed droplet surface and form a protective interfacial layer, thus preventing recoalescence and stabilizing the droplets. However, the adsorption rate of surfactants required to reduce surface tension should be higher than the rate of favorable coalescence of the newly formed droplets (Aswathanarayan & Vittal, 2019). Therefore, the increase of droplet diameter with increasing time observed here can also be due to the higher rate of recoalescence of droplets than surfactant adsorption at the interface induced by shock waves generated by the tip of the sonotrode at a higher amplitude.

Three operating conditions that produced the lowest mean droplet diameters were homogenization speeds, sonication amplitudes, and sonication times of 3000 rpm, 80 %, 15 min; 6000 rpm, 50 %, 30 min; and 3000 rpm, 50 %, 30 min, respectively. Since there were no significant differences between these three treatments, homogenization speed of 3,000 rpm, sonication amplitude of 80 %, and sonication duration of 15 min were considered as the method of choice to proceed with throughout further experiments to shorten the whole process time.

3.2. Effect of surfactant

The suitable emulsifier(s) selection would be crucial in stabilizing the newly formed tiny oil droplets through sufficient interfacial coverage and preventing droplet aggregation during nanoemulsion preparation (Zheng et al., 2020). The preferred HLB value to formulate an oil-in-water emulsion lies between 8 and 13. Soybean lecithin (HLB 4–8), when used along with another surfactant of higher HLB in optimal proportions, might perform the desired function (Fathordoobady et al., 2021). Non-ionic surfactant polysorbate 80 (HLB 15) was selected as an auxiliary surfactant due to its documented potential for forming stable emulsions of bioactive hemp seed oil and improving the emulsification efficacy of lecithin (Mehmood and Ahmed, 2020, Mikulcová et al., 2017).

As can be seen from Supplementary Table 2, all the treatments prepared using lecithin and polysorbate 80, individually or combined in various ratios, satisfied the size criterion of nanoemulsion (mean droplet diameter < 500 nm). The least mean droplet diameter was observed in nanoemulsions prepared using lecithin: polysorbate 80 in both ratios of 1:1 (127.60 nm) and 2:1 (134.87 nm) with no significant difference ($p > 0.05$) followed by lecithin individually (147.73 nm) (see Supplementary Table 2).

Polysorbate 80 was efficient to some extent in decreasing droplet size at lower concentrations while increasing its concentration in the formulation led to an increment in mean droplet diameter. This could imply the importance of the critical level of polysorbate 80, which needs to be justified for its proper function as an auxiliary surfactant. The mixed interfacial film comprised of polysorbate 80, and lecithin formed around the dispersed oil droplets, tends to

show a more hydrophilic surface character due to the long chain of polyoxyethylene glycols present at polysorbate 80. This can somehow reduce interfacial tension and the growth and coalescence of oil droplets, preventing the enlargement of droplet size (Zheng et al., 2020).

Variations in mean droplet diameter may also be related to variations in the molecular weight of the emulsifiers, being 644 and 1310 g/mol for lecithin and polysorbate 80, respectively. Application of polysorbate 80 alone or at a high ratio (polysorbate 80: lecithin of 2:1) increased droplet sizes (Supplementary Table 2), which can be explained by the large molecular size of polysorbate 80 inhibiting its proper diffusion and efficiency at the interface (Zheng et al., 2020). Surfactants with lower molecular weights can easily absorb on the droplet surface compared with larger molecular weight ones. These findings agreed with previous investigations that reported higher efficiency of surfactants of lower molecular weight in developing smaller droplets (Hamed & Abo-Elwafa, 2020).

Polysorbate 80 combined with lecithin could reduce mean droplet diameter, in its best performance, only about 7 % (127.60 ± 0.26 nm) compared with lecithin alone (147.73 ± 1.07 nm). Hence, lecithin was selected as the surfactant of choice for further experiments out of all other combinations studied due to its satisfactory efficiency in generating the acceptable droplet size required by many products. Moreover, the use of polysorbate 80 in foods, beverages, and oral pharmaceutical formulations has recently come under scrutiny due to its health concerns (Jarzębski et al., 2021). Polysorbate 80 can significantly impact the microbiota composition of human skin and intestines and decrease the antibacterial efficacy of lipophilic antimicrobial components (Nielsen, Kjems, Mygind, Snabe, & Meyer, 2016). On the contrary, lecithin is universally accepted as an emulsifier, stabilizer, and nutritional supplement for medicinal and food use and has the potential to develop both oil-in-water and water-in-oil emulsions (Amiri-Rigi and Abbasi, 2018, Hamed and Abo-Elwafa, 2020, Xu et al., 2011).

3.3. Effect of coating material

The visual appearance of the prepared coarse emulsion after water titration method, coarse emulsion after homogenization and nanoemulsion after sonication, immediately after preparation, and nanoemulsions prepared using different proportions of coating materials of maltodextrin and gum arabic after three weeks of preparation is depicted in Supplementary Fig. 1. All nanoemulsions showed a turbid appearance before sonication and turned milky following sonication (Supplementary Fig. 1). Turbid and milky appearance after sonication for nanoemulsions prepared using thymol essential oil as oil phase and saponin as surfactant (Kumari et al., 2018) and nanoemulsions developed using hemp seed oil as oil phase, *Aesculus hippocastanum* L. as stabilizer and whey protein isolate as co-surfactant (Smułek et al., 2021) has been already reported.

From the results obtained, it was concluded that comparatively homogenous nanoemulsions could be produced, using either maltodextrin or gum arabic individually or combined in various ratios, without severe energy input or long process time. Nevertheless, the application of coating materials as large molecular biopolymers led to a remarkable increase in mean droplet diameter compared to control (Table 1). The lowest mean droplet diameter was observed using a mixture of gum arabic: maltodextrin at a 25:75 ratio (165.77 ± 3.53) followed by maltodextrin alone (244.63 ± 1.56). These findings indicate maltodextrin potential in developing relatively smaller droplets that can be explained in light of the prior literature reporting its surface activity contributing to reduced interfacial tension (Firoozy & Anarjan, 2019).

Table 1. Effect of coating material on total energy consumption of ultrasonic (J) and physicochemical properties of nanoemulsions prepared using lecithin as surfactant and homogenization at 3,000 rpm for 5 min followed by sonication at 80 % amplitude for 15 min.

Gum arabic: Maltodextrin	Mean droplet diameter (nm)	Polydispersity index (PDI)	Zeta potential (mV)	pH	Energy (J)
0:100	244.63 ± 1.56^{d}	0.46 ± 0.01^{b}	-0.66 ± 0.27^{ab}	6.53	68,268
25:75	165.77 ± 3.53^{e}	0.36 ± 0.02^{c}	-3.31 ± 0.77^{d}	6.10	73,170
50:50	366.40 ± 6.10^{a}	0.51 ± 0.04^{a}	-1.79 ± 0.92^{c}	5.76	70,023
75:25	293.13 ± 6.70^{c}	0.50 ± 0.07^{a}	-1.37 ± 1.00^{c}	5.56	71,222
100:0	315.80 ± 12.68^{b}	0.46 ± 0.01^{b}	0.05 ± 0.03^{a}	5.44	73,908
Control	147.73 ± 1.07^{f}	0.47 ± 0.01^{b}	-0.95 ± 0.74^{ab}	6.65	67,383

Different superscript letters (a-f) indicate significantly different means between each column's values at $p < 0.05$.

A rise in mean droplet size with the incorporation of more gum arabic in the formulation (Table 1) can be due to its higher molecular weight (250,000 g/mol) compared to maltodextrin (1,700 g/mol). Maltodextrin exhibits a rather low viscosity at high concentration but has a limitation of low emulsifying efficiency on its own. Hence, it is recommended to be applied only in combination with other coating materials having somewhat higher emulsifying ability and stabilizing properties, such as gum arabic (Premi & Sharma, 2017).

Moreover, the larger droplet diameter of all nanoemulsions fabricated using wall materials (maltodextrin and gum arabic) compared to control (Table 1) could be explained by an increase in viscosity of the developed formulations that lower the efficiency of high-energy techniques in minimizing droplet sizes (Artiga, Guerra-Rosas, Morales-Castro, Salvia-Trujillo, & Martín-Belloso, 2018) and the incorporation of more core materials (Firoozy and Anarjan, 2019, Premi and Sharma, 2017). A remarkable increase in droplet diameter from 350 nm to 850 nm with increasing the concentration of biopolymer pectin in nanoemulsion formulation from 1 % to 2 % (w/w) has been previously reported by Artiga et al. (2018) for essential oils-loaded nanoemulsions stabilized using Tween 80. Kowalska et al. (2015) studied the optimal composition in preparing hemp seed oil emulsion using computer simulation based on Kleeman's method. They achieved a stable dispersion system with a mean droplet diameter of almost 6 μm using 5.2 % lecithin as surfactant and 0.6 % carboxymethylcellulose solution as the aqueous phase. Taking into account that small size and low surfactant-to-oil ratio are typically preferred, in the present study nanoemulsion system could be developed using 3 % lecithin as surfactant and hydrated solutions (4 % w/v) of gum arabic and maltodextrin alone or their mixture as aqueous phase having mean droplet diameter of <366 nm in all treatments.

Typically, charge stabilization of a colloidal system tends to be effective if the zeta potential, a measure of electrochemical equilibrium at interfaces, is higher than + 30 mV or lower than − 30 mV. The relatively low absolute values recorded for zeta potential in the current study (−13.93 mV to + 1.23 mV) may be induced by adsorption of hydroxyl (OH^-) groups from the aqueous medium to the polar head of surfactants, forming a hydrogen bonding. Dissociation of H+ (hydrogen ions) from the droplet surface into the surrounding solution may also lead to new hydrogen bonds and the formation of hydronium ions (H_3O^+) to a lower extent. Therefore, the rather low absolute values of the nanoemulsions' zeta potential (Tables 1 and Supplementary Tables 1 and 2) may arise from a charge neutralization among OH^- and H_3O^+ to some extent (Teo et al., 2015).

The relatively low values for zeta potential may also be attributed to the application of lecithin and non-ionic surfactant polysorbate 80 in the formulations. Among lecithin's main components, it is only phosphatidylinositol (PI) that contributes to the negative charge, whereas phosphatidylcholine (PC) and phosphatidylethanolamine (PE) contribute insignificantly to the surface charge of emulsion droplets prepared using lecithin at physiological pH (Xu et al., 2011, Teo et al., 2015). However, steric repulsion is known to be the primary stabilization mechanism in both oil-in-water and water-in-oil nanoemulsions prepared using non-ionic surfactants, like polysorbate 80 or polymers (Rocha-Filho et al., 2020, Teo et al., 2015).

The influence of the main operational parameters (homogenization speed and sonication amplitude and time), type of surfactant (different proportions of lecithin and polysorbate 80) and coating materials (various combinations of maltodextrin and gum arabic) on PDI was also evaluated. Supplementary Tables 1 and 2 and Table 1 present values for PDI of nanoemulsions ranging from 0.22 to 0.51, indicating uniformity in droplet size and narrow distribution within all formulations where values higher than 0.7 have been documented as broad-size distribution (Hamed & Abo-Elwafa, 2020). Moreover, all the nanoemulsions showed a pH value between 5.4 and 6.6 (Table 1) which is favorable for food and pharmaceutical applications (Teo et al., 2015). Nanoemulsions formulated using different proportions of gum arabic: maltodextrin were further applied to encapsulate the ascorbyl palmitate to investigate and correlate the effect of various coating materials on the entrapment efficiency for ascorbyl palmitate, the antioxidant activity of core material and viscosity of the developed system.

3.4. Entrapment and loading efficiency

In this study, ascorbyl palmitate was incorporated in the coarse emulsion, a nanoemulsion without coating material, and six different nanoemulsions prepared using different proportions of coating materials of maltodextrin and gum arabic. The high entrapment efficiency of more than 69 % and loading efficiency of higher than 1.4 % were achieved for all the samples irrespective of the type of emulsion and coating material, indicating extremely efficient entrapment of ascorbyl palmitate within the developed formulations (Table 2). One can see that the free ascorbyl palmitate was lower than 25 % in all nanoemulsion formulations, which is in agreement with the poor solubility of ascorbyl palmitate in water (Zhou et al., 2017).

Table 2. Entrapment efficiency (%) and loading efficiency (%) of coarse emulsion (CE), nanoemulsion without coating material (NE) and nanoemulsions prepared using different ratios of gum arabic (GA) and maltodextrin (MD) as coating materials for ascorbyl palmitate.

Sample	Entrapment Efficiency (%)	Loading Efficiency (%)
CE	69.41 ± 0.36^{f}	1.39 ± 0.01^{f}
NE	75.93 ± 0.75^{e}	1.52 ± 0.01^{e}
NE-GA:MD 100:0	96.52 ± 0.88^{a}	1.93 ± 0.02^{a}
NE-GA:MD 0:100	78.67 ± 0.95^{d}	1.57 ± 0.02^{d}
NE-GA:MD 50:50	85.45 ± 0.93^{b}	1.71 ± 0.02^{b}
NE-GA:MD 75:25	97.10 ± 0.14^{a}	1.94 ± 0.02^{a}
NE-GA:MD 25:75	81.45 ± 0.68^{c}	1.63 ± 0.01^{c}

Different superscript letters (a-e) indicate significantly different means between each column's values at $p < 0.05$.

Nonetheless, as shown in Table 2, core retention was strongly impacted by the emulsion type and the coating material composition. All nanoemulsions presented higher entrapment efficiency and loading efficiency than the coarse emulsion. This can be attributed to the high energy generated by the collapsing of cavitation bubbles during sonication, which could rupture viscous oil droplets, thus facilitating the emulsification and micelle formation (Chou et al., 2021).

Furthermore, core-in-wall nanoemulsions (formulated using wall materials of maltodextrin and gum arabic in different proportions) showed increased entrapment efficiency and loading efficiency compared to control (nanoemulsion stabilized using just surfactant). The larger droplet diameter of nanoemulsions formulated using wall materials (section 3.3.) could also be the reason behind their higher entrapment efficiency.

The highest values for loading and entrapment efficiencies were found for the nanoemulsions formulated using gum arabic: maltodextrin at ratios of 75:25 (loading efficiency 1.94 % and entrapment efficiency 97.10 %) and 100:0 (loading efficiency 1.93 % and entrapment efficiency 96.52 %), showing no significant difference ($p > 0.05$). The enhanced value of entrapment efficiency and loading efficiency with increasing gum arabic concentration in nanoemulsions formulations (Table 2) may be accounted for by the thickening property of gum arabic. The long and highly branched structure of gum arabic enables it to form a solid protective coating over the bioactive core materials and interact with the ascorbyl palmitate (Premi & Sharma, 2017). Another reason behind this could be emulsifying capacity and encapsulation efficiency of gum arabic, leaving a lower amount of uncovered oil droplets (Premi & Sharma, 2017).

At the same time, increasing the amount of maltodextrin reduced the entrapment efficiency and loading efficiency to as low as 78.67 % and 1.57 %, respectively, in the nanoemulsions prepared using gum arabic: maltodextrin at a 0:100 ratio. This decreased core retention may rise from leaching out of ascorbyl palmitate when using a high concentration of maltodextrin. In other words, ascorbyl palmitate could easily release from the inner core into the external phase of the emulsion with no shielding effect from the biopolymer coating film of maltodextrin (Assadpour, Maghsoudlou, Jafari, Ghorbani, & Aalami, 2016).

The result was in line with previously published findings that suggested high encapsulation efficiency of maltodextrin and gum arabic in combination with each other in the developed drumstick (*Moringa oleifera*) oil emulsion (Premi & Sharma, 2017) and *β*-carotene emulsion (Dłużewska, Florowska, Domian, Wojciechowska, & Maszewska, 2020). Moreover, increased encapsulation efficiency has been reported for a higher proportion of gum arabic (Premi & Sharma, 2017).

Additionally, the entrapment efficiency was found to be higher at lower pH. The highest values of 96 % and 97 % for entrapment efficiency of nanoemulsions formulated using gum arabic: maltodextrin at 100:0 and 75:25 ratios, respectively, coincided with the lowest pH of 5.44 and 5.56, respectively (Table 1, Table 2). This can be an implication of reduced solubility of ascorbyl palmitate in the external aqueous phase of relatively lower pH values resulting in improved entrapment efficiency of ascorbyl palmitate (Khalid et al., 2017, Teo et al., 2015).

3.5. DPPH radical scavenging activity

In the current research, the incorporation of bulk hemp seed oil in nanoemulsion formulation was first carried out to explore the impact of nanoemulsification on the oxidative stability of the oil. As a second step, coating materials were used to study their efficiency in protecting oil and oil-soluble bioactives from oxidation. Finally, ascorbyl palmitate was added to the oil phase prior to emulsification to extend its stability against oxidation during the procedure and over storage.

Levels of antioxidative activity were measured for ascorbyl palmitate-loaded and non-loaded samples of bulk hemp seed oil, coarse emulsion, nanoemulsion formulated without wall material (maltodextrin and gum arabic), and nanoemulsions produced using gum arabic: maltodextrin in various proportions after entire storage of 10 days at ambient temperature (25 ± 1 °C) (Fig. 1). The lowest radical scavenging activity was observed in crude hemp seed oil (25.11 %), followed by nanoemulsion (30.11 %), nanoemulsion developed using just maltodextrin as coating material (33.40 %), and coarse emulsion (37.79 %).

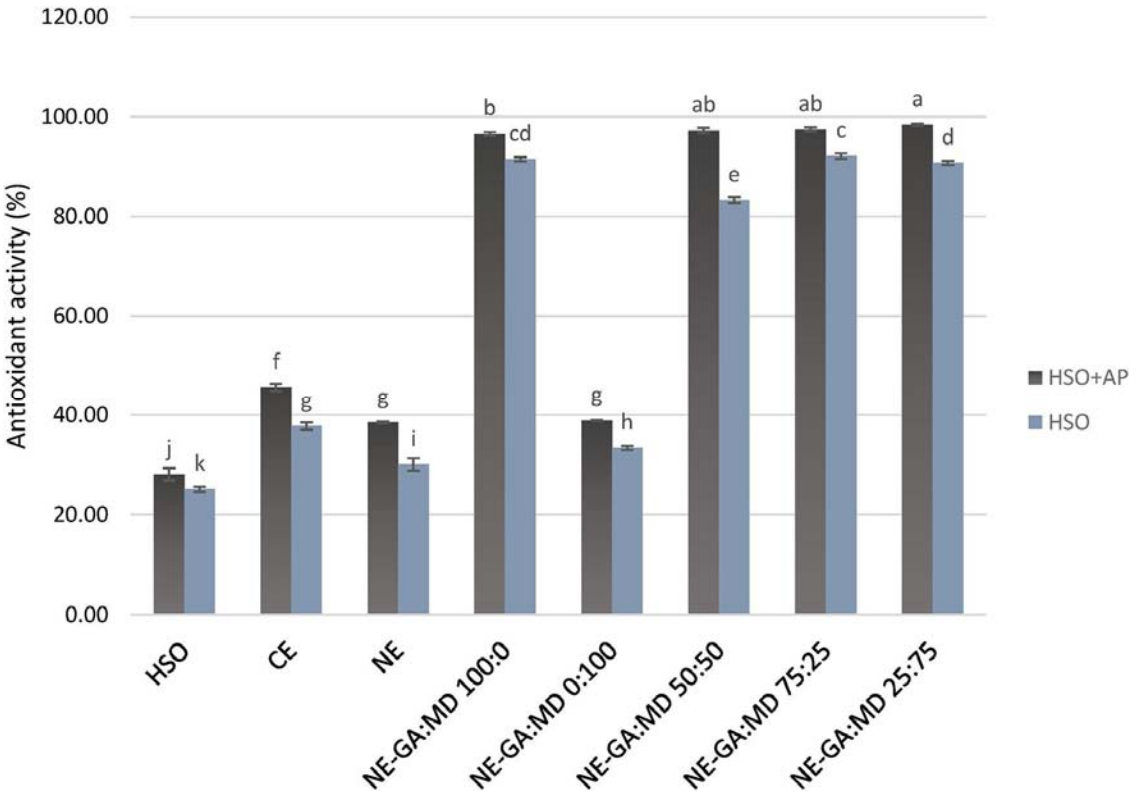


Fig. 1. Changes in antioxidant activity in a 10-day storage test at ambient temperature (25 ± 1 °C) in ascorbyl palmitate (AP)-loaded and non-loaded samples of hemp seed oil (HSO), coarse emulsion (CE), nanoemulsion without wall material (NE) and nanoemulsions developed using gum arabic (GA) and maltodextrin (MD) at different ratios. Different lowercase letters above the error bars represent significant differences among samples ($p < 0.05$).

Higher oxidative stability of all developed emulsions compared to bulk oil can be attributed to the interfacial properties of the developed formulations, whereas the oxidative stability of bulk oils may be affected just by their considerable quantities of polyunsaturated fatty acids (Amiri-Rigi et al., 2022). In addition, the rates of diffusion and invasion of oxygen from the

surrounding into the unsaturated sites at the air/oil interface in crude oil are far faster than the oil/water interface in emulsions (Hamed & Abo-Elwafa, 2020). Higher antioxidant activity of all developed emulsions compared to crude oil can also be explained in terms of the shielding effect of the emulsifiers (lecithin and polysorbate 80), which could adsorb to the surface or interface of oil droplets acting as a barrier (Xu et al., 2011).

Nanoemulsions developed using gum arabic: maltodextrin at 100:0, 75:25, 50:50, and 25:75 ratios contributed to the highest DPPH radical scavenging activity (96.58–98.39 %) with no significant difference ($p > 0.05$). These formulations had larger mean droplet diameters and hence may have a higher local concentration of lipophilic ascorbyl palmitate in bigger oil droplets compared with smaller ones. Antioxidant molecules can also diffuse faster and more uniformly at the comparatively smaller interface of the larger droplets. Previous research also indicated higher susceptibility of dispersed oil droplets to autooxidation in nano-sized emulsions than in coarse emulsions (Tan and Nakajima, 2005, Yi et al., 2014, Zheng et al., 2020).

Antioxidative activity of oil droplets embedded throughout maltodextrin as the wall matrix was lower compared with those composed of gum arabic alone or in combination with maltodextrin. This can be an implication of the role of other factors, including interface structure and composition. Higher antioxidant activities coincided with increasing gum arabic concentration in the formulation, which can be due to the thickening and stabilizing properties of gum arabic. Gum arabic-stabilized nanoemulsions may form a thicker and more compact interfacial film around oil droplets than maltodextrin (Zheng et al., 2020). The solid protective layer formed by gum arabic around oil droplets can prevent oxidation from occurring (Premi & Sharma, 2017). Moreover, gum arabic exhibits higher viscosity than maltodextrin in the same concentration, which supports the conclusion made in terms of the thick and compact film formed using gum arabic (Firoozy and Anarjan, 2019, Premi and Sharma, 2017). The findings of the present study were in line with those reported by Firoozy and Anarjan (2019), in which DPPH radical scavenging of α-tocopherol nanoemulsions increased in the presence of coating material.

Ascorbyl palmitate could increase antioxidant activity in all the samples irrespective of the type of emulsion and formulation. However, ascorbyl palmitate was more efficient in nanoemulsion formulation than bulk oil. This finding can be interpreted based on the paradoxical behavior of antioxidants (polar paradox theory) that explains nonpolar antioxidants are more active in relatively more polar matrices. Ascorbyl palmitate, as a nonpolar antioxidant, shows higher activity in oil-in-water emulsion than in bulk oil because of a better tendency for the oil-water interface, the crucial site where oxidation occurs, forming an interfacial layer around the individual oil droplets (Aswathanarayan and Vittal, 2019, Zheng et al., 2020). Such an enhanced effect of ascorbyl palmitate on antioxidant capacity could be supported by the findings of previous studies (Zhou et al., 2017).

3.6. Viscosity analysis

As can be seen from Supplementary Fig. 2, all the nanoemulsions exhibited two different types of flow behaviors, shear-thinning behavior at lower shear rates changing to Newtonian behavior somewhere beyond 15 s^{-1}. Shear-thinning behavior could be taken as an indication of the weak interactions between the dispersed oil droplets and the surrounding aqueous media, which initially induce resistance to flow under low shear rates. In higher shears, hydrodynamic forces imposed by the fluid shearing prevailed over the viscous effect allowing the fluid to flow

more easily in the same direction as the applied shearing force. The observed shear thinning behavior of the hemp seed oil-in-water nanoemulsions at lower shear rates and transition to Newtonian flow behavior at high shear rates is comparable with the flow behavior of several other nanoemulsions prepared using various amounts and types of oil phases and different surfactants (Jarzębski et al., 2021, Jaworska et al., 2014, Teo et al., 2015).

It is worth pointing out that the apparent viscosity of nanoemulsions increased with increasing the gum arabic concentration in formulations (Supplementary Fig. 2). The higher entrapment efficiency and antioxidant capacity of samples prepared using gum arabic alone and in combination with maltodextrin can be explained by the higher apparent viscosity of the stabilizing interfacial film in formulations containing gum arabic forming a solid protective layer around the bioactive core material (Premi & Sharma, 2017).

4. Conclusion

The present study established a simplified and efficient procedure to develop ascorbyl palmitate-loaded hemp seed oil-in-water nanoemulsion for oral delivery purposes. Considering that food-grade nanoemulsions in the literature are mostly developed using either synthetic surfactants or relatively high concentrations of natural ones, ascorbyl palmitate-loaded nanoemulsions were successfully formulated using 3 % (w/w) lecithin in the final formulation. The optimum nanoemulsion formulated by gum arabic: maltodextrin at a 75:25 ratio as coating materials has an average droplet size of 293 nm. These systems exhibit the highest levels of entrapment (97 %) and loading efficiency (1.94 %) for ascorbyl palmitate and a remarkably greater antioxidant activity even without antioxidant ascorbyl palmitate (92 %) than bulk oils in the presence of antioxidant (28 %). The results obtained clearly indicated the functionality of gum arabic and maltodextrin as encapsulating agents and confirmed the importance of careful screening of the composition of core-in-wall nanoemulsion. The experimental results obtained in the present study would be beneficial in developing bioactive hemp seed oil-in-water nanoemulsions, which can be incorporated into formulations of functional food and pharmaceutical supplements. The formulated hemp seed oil nanoemulsion may be applied as a nanocarrier to modify the dispersant state and oral delivery of ascorbyl palmitate or other poor aqueous-soluble nutraceuticals. Further work may focus on the stability and best storage condition of the developed hemp seed oil nanoemulsion loaded with ascorbyl palmitate. The effectiveness of prepared nanoemulsions in preserving antioxidant activity in more realistic environments, such as food matrices, also requires further investigation.

CRediT authorship contribution statement

Atefeh Amiri-Rigi: Investigation, Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Sreejarani Kesavan Pillai:** Data curation, Formal analysis. **Mohammad Naushad Emmambux:** Supervision, Project administration, Funding acquisition, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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