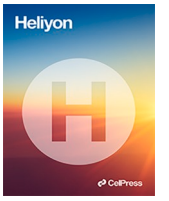
Heliyon 10 (2024) e39387

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Nanoencapsulation of limonene in octenyl succinic anhydride-modified starch (OSA-ST) and maltodextrin: Investigation and comparison of physicochemical properties, efficiency and morphology of nanoparticles

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ARTICLE INFO

Keywords:
Emulsifier
Encapsulation
Limonene
Maltodextrin
Modified starch

ABSTRACT

In recent years, the application of nanoencapsulation has attracted enormous attention for various food and pharmaceutical purposes. In this study, a functional powder containing limonene (the nutraceutical at concentration of 5 and 10 %) was prepared using octenyl succinic anhydride-modified starch (OSA-ST) and maltodextrin as carriers at 15 and 30 %. The emulsions were sonicated at a frequency of 30 kHz and a power of 100 W for 9 and 18 min, and the final nanoparticles were prepared through freeze-drying. The particle sizes were in the ranges of 62–248 and 10–24 nm in the suspensions of OSA and maltodextrin, respectively. The smaller particles of the maltodextrin-prepared sample resulted in more transparency. The zeta potential and consequently the stability of the maltodextrin-prepared emulsions were higher than those of the OSA-ST-prepared ones. As the maltodextrin concentration increased, this parameter was elevated from −42 to −36 as a result of the coverage of the surface-active lipids. The results of solubility correlated with those of the zeta potential (89.21 % for maltodextrin-prepared and 82.51 % for OSA-ST-prepared samples). The highest encapsulation efficiency (EE = 0.9) belonged to the samples prepared with OSA-ST. Comparison of the scanning electron microscopy (SEM) images revealed that the type of the wall material influenced the physical structure of the nanoparticles which were mostly porous and flake-like. Considering its encapsulating-emulsifying properties, OSA-ST can be suggested as a carrier for limonene with the need for emulsifiers.

1. Introduction

Flavor is highly vital and effective for the marketability of food products. Most aromatic compounds are unstable and generally volatile, due to their special chemical structures. They sometimes undergo loss and undesirable changes during the product processing and storage [1]. The low stability of natural flavorings and challenges encountered in their application in the food industry encourages

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https://doi.org/10.1016/j.heliyon.2024.e39387
Received 22 July 2024; Received in revised form 13 October 2024; Accepted 14 October 2024
Available online 15 October 2024

the use of artificial flavorings for industrial applications. However, due to increasing health conscious issues amongst population, the demand for the use of natural ingredients is on the rise [2].

One of the most common ways of meeting this challenge is the encapsulation of natural aromatic compounds with suitable wall materials prior to use in the food formulae. This not only protects the compounds from outside harsh environment during processing as well as digestion, but also may to a great extent control their release during the while such substances with wall materials that can control their release in addition to protecting them against environmental and processing factors [3]. This contributes to the targeted delivery of such components which usually have pharmacological effects. Encapsulation is divided into several techniques in terms of particle preparation method and the final size of the carriers. In fact, encapsulation can be regarded as a method through which solid, liquid, or gaseous ingredients are entrapped in tiny capsules and released under specific conditions [4].

Limonene is widely utilized as a flavoring agent in various industries including the food and pharmaceutical industries. It is the major aromatic compound of citrus fruits. Despite having technological (odor and flavor), anticancer and antimicrobial properties [5], application of limonene faces many challenges because of technological limitations such as its hydrophobicity, high reactivity, susceptibility to oxidation and volatility. Encapsulation is a technique which can contribute to the targeted and effective application of this valuable component [6].

Carrier size and type are two factors influencing EE. This process can be performed on nano-, micro- and macro-scales, each having its own advantages. Carrier type varies depending on the core material and the process purpose. In food and pharmaceutical systems, carriers are typically made from protein, polysaccharide or a complex of them [7].

Polysaccharides, including starch, gums, maltodextrin and a variety of other compounds, are one of the most useful groups of carriers in the encapsulation of food ingredients. Modification of polysaccharides, especially starch, raises their EE. OSA-ST is a cheap encapsulating agent/emulsifier, which is considered generally recognized as safe (GRAS) by food and drug administration (FDA), if modified up to 3 % relative to the total weight of the starch. Among alkenyl succinic anhydrides, only OSA ($C_{12}H_{18}O_3$) can be used for food purposes. As result of esterification, its hydrophobic sites bind with the hydrophilic molecules of hydrocolloid, leading to the development of emulsifying properties in the hydrocolloid in addition to the stabilizing and thickening ones. Indeed, the hydrocolloid converts into a amphiphile and acts as a surfactant [8]. Maltodextrin can also form a proper shell around the core material, owing to its hydrocolloid chemical structure, which can act as a barrier against environmental and processing factors and restrain the destruction of the core material to a high degree [9].

In recent years, utilization of physical methods (without the use of chemicals) has received considerable attention for the preparation of carrier particles, particularly on nano- and micro-scales. One of these physical methods is the application of ultrasound which is actually a form of energy generated by sound waves at frequencies above the hearing ability of human (>20 kHz). Ultrasound is broadly applied in encapsulation, due to its low energy consumption and processing time as well as high efficiency [10]. Ultrasonic waves produce their effect through cavitation which is effective in size reduction and achieving a homogenous particle size distribution in processes like encapsulation [11]. Furthermore, the cavitation's high energy can destroy strong tissues and dissociate bonds needing high mechanical stress for hydrolysis. The aim of the present study was to employ the physical technique of sonication to prepare limonene nanoparticles using OSA-ST and maltodextrin as carriers, as well as comparing their physicochemical and structural properties.

2. Materials and methods

2.1. Preparation of amylose nanoparticles containing limonene

First, the solutions of maltodextrin (Sigma-Aldrich, the USA) and OSA-ST (National starch, the UK) as a carrier, were prepared in water at 15 and 30 % and set aside at ambient temperature overnight for complete hydration. Afterwards, limonene (citrus concentrate Co., Ramsar, Iran) was added in multiple steps to the solutions at 5 and 10 % (relative to the carrier concentration) while stirring at 70 °C. Tween 80 was added as emulsifier to the maltodextrin solutions. Stirring continued for 30 min, and the prepared emulsions were immediately sonicated using the UP100 ultrasonic homogenizer (Hielscher, Germany) at 100 W and 30 kHz for 9 and 18 min. Eventually, the nanoparticles were prepared [12]. The ultrasonic waves were transmitted from the piezoelectric field to the sample through a sonotrode with a diameter of 19 mm, which had been immersed in the emulsion to a depth of 1 cm. An ice bath was used for temperature control. The obtained emulsions were powdered using a freeze-drier (Dena vacuum, Iran) and stored in sealed containers at refrigerator temperature for further analyses.

2.2. Determination of amylose nanoparticle sizes and zeta potential

The size and zeta potential of the nanoparticles were quantified through dynamic light scattering (DLS) using Nanotrac Flex In-situ Particle Size Analyzer and Microtrac ZETA-check respectively. These parameters were measured without any dilution 24 h after preparation.

2.3. Analysis of the nanoparticle's microstructure

The morphology of the nanoparticles was examined through scanning electron microscopy (SEM) (TESCAN-Vega3, Czech Republic). To that end, some of the freeze-dried powder was spread onto an aluminum plate and covered with a thin layer of gold by a sputter coater (Quorum Technologies- Q150R- ES, the UK). Finally, the SEM images were captured.

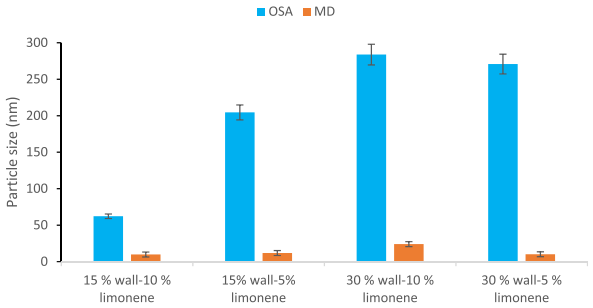

Fig. 1. Particle size of nanoemulsions prepared with maltodextrin and OSA-ST.

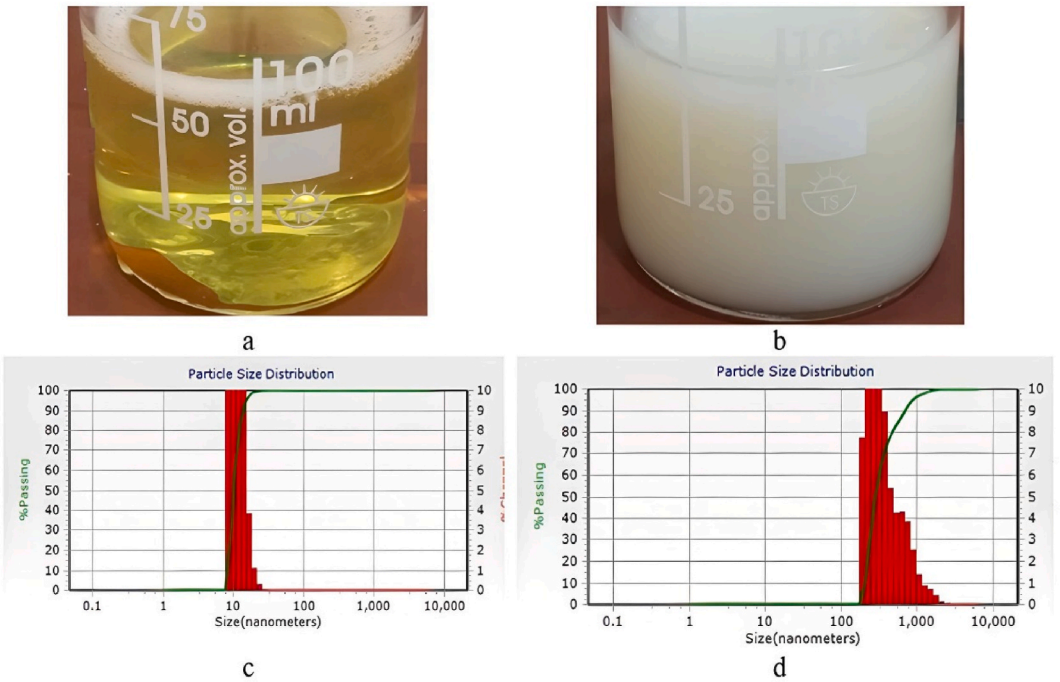

Fig. 2. Effect of particle size distribution on transparency of emulsion prepared with a) OSA-ST and b) maltodextrin. Particle size distribution of emulsion prepared with c) OSA-ST and d) maltodextrin.

2.4. Measurement of solubility

1 g of the powder was dissolved in 100 mL of distilled water while stirring at 385 rpm for 5 min. The resulting dispersion was centrifuged at 3000 g for 5 min. 25 mL of the supernatant was drawn, poured onto a pre-weighed petri-dish and dried in a hot-air oven at 105 °C for 5 h. The percentage of the weight difference between the dry matter in the dish and the powder was used to determine the powder solubility [13].

2.5. Encapsulation efficiency

5 mg of the freeze-dried limonene-containing nanoparticles was dispersed in 5 mL of hexane (Merck Co., Germany). After the dispersion was passed through Whatman No. 1 filter paper, the absorbance value of the filtrate was measured at 252 nm using a spectrophotometer (ModelVIS-7220G/UV-9200, Rayleigh, China) and reported as the free (non-encapsulated) limonene. Encapsulation efficiency was calculated using equation (1) [14].

$$EE = \frac{\text{total limonene} - \text{free limonene}}{\text{initial limonene}} \times 100 \qquad \text{Eq. 1}$$

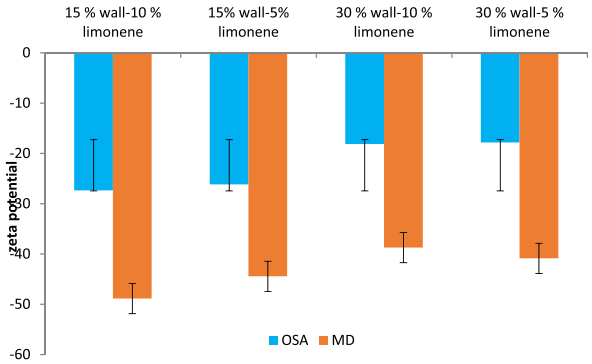

Fig. 3. Zeta potential of emulsions prepared with OSA-ST and maltodextrin.

2.6. Statistical analyses

A three-factor full factorial completely randomized design was created to investigate the effects of the independent variables (starch concentration, limonene concentration and sonication time) on the responses. All the experiments were carried out in triplicate. Using the SAS software, analysis of variance (ANOVA) and Duncan's multiple range test was conducted to analyze the obtained data at 95 % confidence level. All the graphs were drawn using Excel 2010. To test the correlation between variables, the Pearson analysis method was used.

3. Results and discussion

3.1. Particle size

Measurement of emulsion particle size is one of the most useful and reliable methods for studying emulsion formation and stability as well as determining the interactions between the emulsion components. Measurement of the particle size of emulsions is one of the most applicable and reliable methods [4]. In the samples prepared with OSA-ST, the particle sizes varied between 62 and 284 nm, while they were in the range of 10–24 nm for those containing maltodextrin (Fig. 1). An increase in the concentration of the wall materials led to a rise in the particle size. This may be attributed to the reduction in the destructive effect of ultrasound at higher concentrations. Contrary to the carrier concentration, limonene percentage did not significantly affect the particle size. Cavitation could be the major reason behind the reduction in the particle sizes. In fact, bubbles are continuously formed and collapsed as a result of sonication. Therefore, the wall materials are severely mechanically damaged. This phenomenon, in addition to the collision between the particles, causes their breakdown to nano-sizes [15,16]. Some researchers reported the particle sizes of 200–300 nm for the powder of Q10-coenzyme encapsulated using OSA-ST through high-pressure homogenization [17]. The interactive effect of OSA-ST and Tween 80 was also investigated on the properties of cardamom oil-in-water emulsion and the microcapsules resulting from it. It was observed that the mean droplet sizes of the different emulsion samples ranged from 182 to 383 nm after sonication [18].

Transparency is the most profound effect that particle size has on emulsion appearance. As can be seen in Fig. 2(a and b), there was a considerable difference in the appearance of the emulsions prepared with OSA-ST and maltodextrin, which was due to the major difference between the particle sizes of the two systems. After sonication, the maltodextrin emulsions became much more transparent than the OSA-ST ones, as their particle sizes were approximately equal to 10 nm. In addition to particle size reduction, the particles homogeneity also increased as their sizes decreased. It is obvious in Fig. 2 c and d that particle size distribution was narrower in the maltodextrin solutions compared with the OSA-ST ones.

An emulsion will be considered transparent if its turbidity is less than 0.05 cm^{-1} [19]. The turbidity of a nanoemulsion will be less than 0.05 cm^{-1} if its mean particle diameter is smaller than 80 nm, and its particle diameter distribution is narrow [19,20]. Consequently, the emulsion will be transparent and appropriate for transparent drinks which could be enriched without any alterations in their appearance [21].

3.2. Zeta potential

As it can be observed in Fig. 3, zeta potential was much higher in the samples prepared with maltodextrin than in those with OSA-ST. Our impression is that the application of Tween 80 could be the reason behind this difference. The literature has shown that the small molecules of the surfactant bind with maltodextrin through the entrapment of the non-polar moieties of the molecules in the helices of the maltodextrin chain [22,23].

The electric flux of the particles stabilized by non-ionic surfactants is associated with the absorption of H_3O^+ (low pH) or OH^- (high pH). As the maltodextrin or OSA-ST concentration increased, the system zeta potential lowered, which is in line with the results reported by Ref. [24] who stabilized corn oil using maltodextrin as wall material. In that study, with a rise in the maltodextrin concentration from zero to 35 % in the presence of Tween 80, mean zeta potential was elevated from −42 to −36. One of the reasons

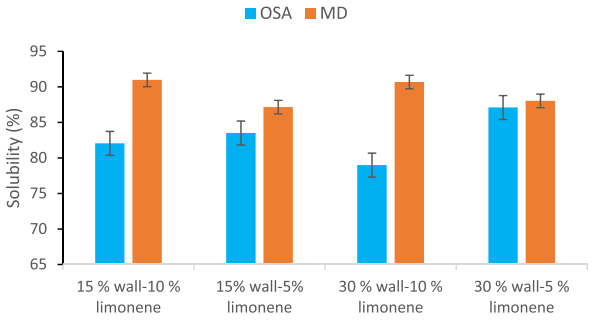

Fig. 4. Solubility percentage of various powders prepared with maltodextrin and OSA-ST.

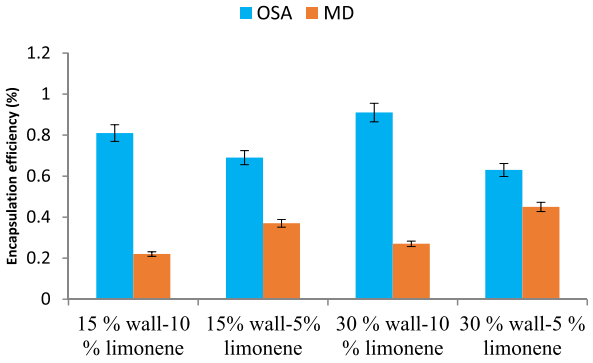

Fig. 5. Encapsulation efficiency of emulsions prepared with maltodextrin and OSA-ST.

behind the decrease in zeta potential with an increase in the maltodextrin concentration is that the maltodextrin molecules have more chance to adsorb onto surface active lipids which are actually the sites for the adsorption of the target compound [25,26]. This results in the negative charge of the particle surfaces [27,28].

The negative charge of the OSA-ST molecules is ascribed to the anionic carboxyl groups present on their surfaces [29,30]. Other researchers have also acquired Comparable results for OSA-ST and related its negative charge to the type of the polysaccharide chain substitutions (OSA basis). No statistically significant change was observed ($p > 0.05$) with a rise in the OSA-ST concentration, and the slight reduction in zeta potential could be attributed to the larger degree of overlap between the starch molecules at higher concentrations, which reduced or neutralized the electrical effect of the anionic carboxyl groups [31,32].

3.3. Solubility

Nanoencapsulation is one of the common methods for overcoming the low solubility and bioavailability of many nutraceuticals. Indeed, the bioavailability of these compounds as well as their applications in the formulation of food products is raised by using polysaccharide carriers [33,34].

Solubility is one of the most important indices for verifying powder efficiency in many industries, in particular the food industry [35,36]. This parameter can be intensely impacted by the drying process, particle sizes and zeta potential [25]. Given the results illustrated in Fig. 4, the solubility of the maltodextrin-prepared samples (89.21 %) was higher than that of the OSA-ST-prepared ones (82.51 %). In the maltodextrin-containing samples, as the limonene concentration was elevated, solubility increased, too (mean solubility of 90.83 and 87.59 % for 10 and 5 % limonene respectively). At the same time, this correlation was reversed in the case of the samples containing OSA-ST, and the ones containing the lower concentration of limonene showed higher solubility (mean solubility of 80.52 and 85.30 % for 10 and 5 % limonene respectively). These values are proportional to the surface charge or zeta potential of the prepared particles such that the higher the zeta potential of the particles, the more pronounced the electrostatic repulsion between them, leading to enhanced solubility [37,38]. The effect of the carriers' concentration on solubility was not significant and did not follow a specific trend. In similar studies, researchers have observed a decrease in the solubility of mango juice powder with a rise in the maltodextrin concentration [39]. However, some other papers have declared that powder solubility was improved after increasing the carrier concentration [40,41]. Considering the observed trends, particle sizes and zeta potential could be regarded as two major factors affecting the solubility of the powders.

Esterification of natural starch with OSA causes it to become hydrophobic while retaining its hydrophilic basis [42,43]. This results in emulsifying properties for the modified starch and makes it suitable for the preparation of oil-in-water emulsions [44,45]. Moreover, the low viscosity of the aqueous solutions prepared with this compound, makes it very suitable for use as emulsifier in emulsion

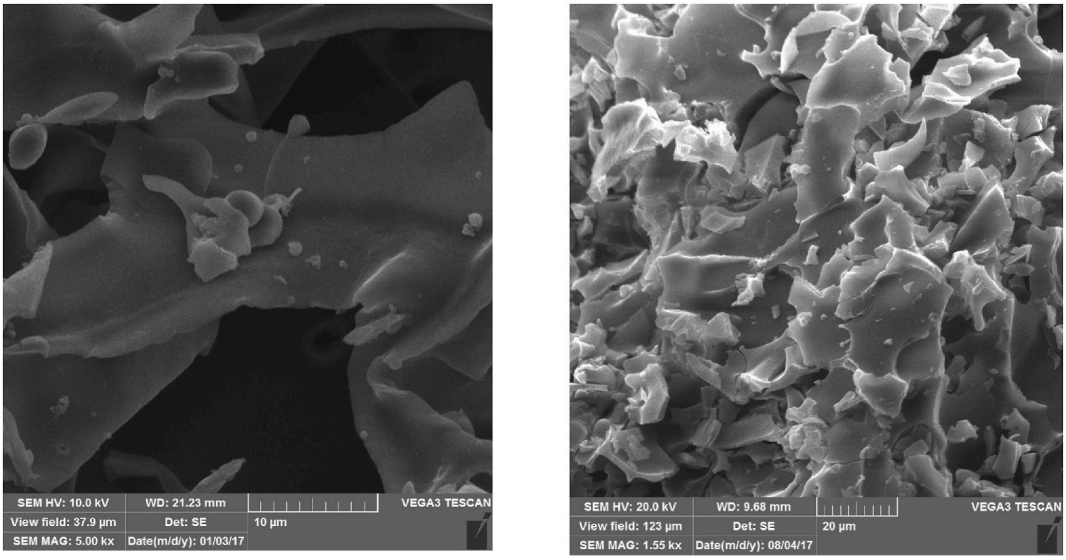

Fig. 6. Scanning electron microscopy images of emulsions prepared with a) OSA-ST and b) maltodextrin.

preparation and as carrier in encapsulation [46].

3.4. Encapsulation efficiency (EE)

As shown in Fig. 5, the highest EE belonged to the samples prepared with OSA-ST. EE was maximized in the samples containing 10 % limonene and both 15 and 30 % of OSA-ST. It seems that the OSA-ST molecules were adequately able to adsorb and entrap the limonene. In the first stage, a reduction in concentration of OSA-ST from 30 to 15 % at a constant concentration of limonene (10 %) resulted in 10 % reduction in EE from 91 to 81 % ($P < 0.05$). In the second stage of EE reduction, as the limonene concentration lowered from 10 to 5 %, EE decreased more regarding the more distribution of this compound, and the OSA-ST concentration did not have a significant effect (at 5 % limonene). In other words, as the limonene concentration was elevated, its molecules were more likely to be adsorbed onto and entrapped in the OSA-ST structure [38]. [47] Luo et al. (2011) claimed that with an increase in the concentration of zein-chitosan loaded with α-tocopherol, EE was raised from 76.5 to 86.5 % [47].

Conversely, maltodextrin exhibited behavior that was contrary to that of OSA-ST. As the concentration of the core material (limonene) rose, EE decreased, which could be associated with the limited capacity of maltodextrin to entrap limonene. In fact, the denominator of the EE equation (Equation (1)) was raised with an increase in the limonene concentration, whereas the numerator, which denotes the encapsulated limonene, was constant or reduced. This caused the EE to lower. Other researchers have employed OSA-ST along with β-cyclodextrin for the encapsulation of allicin and observed an EE of 91 %. Furthermore, allicin resistance to heat, pH, light and oxidation was improved [48]. In addition, some studies have so far been conducted on the encapsulation of juices, lycopene and menthol using OSA-ST. In all the cases, EE was considered acceptable [49].

3.5. Nanoparticles microstructure

The SEM graphs of the nanoparticles are depicted in Fig. 6. Composition of wall material, drying method and drying rate, especially in the early stages of drying, are included in the factors which can influence the surface properties of nanoparticles [50]. The microscopic images of a freeze-dried product are used for directly observing its matrix structure in addition to its structural integrity and changes [4]. Nanocapsules obtained through freeze-drying typically exhibit irregular geometric shapes and a flake-like structure. Indeed, the structure of the products resulting from this drying method is mainly porous and flake-like, owing to the nature of this process where the material is first frozen followed by the sublimation of the frozen water because of pressure decrease. Some researchers have pointed to the asymmetrical and irregular structure of freeze-dried products. In fact, these irregular structures protect the core material from destructive environmental factors such as heat and oxygen [51,52].

Comparison of the SEM images revealed that the type of wall material affected the physical structure of the nanoparticles. As illustrated in Fig. 6a, OSA-ST brought about the development of more angular shapes than maltodextrin. The rougher the surface of particles, the more difficult the powder flowability. The literature has shown that nanoparticles with rougher surfaces are more prone to decomposition reactions like oxidation, caused by their larger contact areas [53].

Application of maltodextrin led to the preparation of more homogenous capsules (Fig. 6b). The morphology of the particles denoted

Table 1
Correlation between measured characteristics.

	Encapsulation efficiency	Zeta potential	Particle size	Solubility index
Encapsulation efficiency	–	+0.856	+0.771	−0.919
Zeta potential	+0.856	–	+0.916	−0.741
Particle size	+0.771	+0.916	–	−0.640
Solubility index	−0.919	−0.741	−0.640	–

The data includes the degree of correlation and the + and - signs respectively indicate the relationship between the features in terms of positive and negative correlation.

that the formed matrices were rather smooth and fragile and had porous structures. The porosity of the matrices differed, probably due to the pores remaining from ice crystals or air bubbles. This difference could be ascribed to the high dextrose content of maltodextrin [54]. These properties are effective in the formation of more spherical and smoother particles [55].

3.6. Investigating the correlation of the measured characteristics

Table 1 shows that all the measured characteristics have a positive correlation with each other except the Solubility index, which indicates that all the studied parameters, radiuses the solubility of emulsion. On the other hand, it was found that among the characteristics measured, the highest correlation was between the two characteristics of Solubility index and the encapsulation efficiency, and this correlation was negative (−0.919). The lowest correlation was between the Solubility index with the particle size (−0.640), which also exhibited a negative value.

4. Conclusions

The limonene nanoemulsion prepared with OSA-ST was compared with that prepared with maltodextrin. Favorable results were obtained in the present study. The particle sizes of both emulsions were on nanoscale. The maltodextrin-prepared emulsion was more transparent than the OSA-ST-prepared one, as the particles of the former were smaller than those of the latter. In addition, the zeta potential of the former was considerably higher than that of the latter. The outcome was interesting, as a stable emulsion achieved solely with OSA-ST, eliminating the need for conventional emulsifying agents. The solubility of the powders produced from the OSA-ST-emulsion was comparable with those resulting from the maltodextrin-prepared emulsion. This proved that esterification of natural starch with OSA made it hydrophobic while keeping its hydrophilicity. Furthermore, the higher EE of OSA-ST than maltodextrin demonstrated that this compound had much more capacity to entrap limonene than maltodextrin. In conclusion, OSA-ST can be utilized as a carrier for limonene without the need for emulsifiers, due to its encapsulating-emulsifying properties.

CRediT authorship contribution statement

Mohammad Ganje: Software, Methodology, Formal analysis, Data curation, Conceptualization. **Raziyeh Jamalifard:** Writing – review & editing, Project administration. **Sajad Ghaderi:** Investigation, Funding acquisition. **Mehrdad Niakousari:** Writing – original draft, Supervision.

Ethical review

This study does not involve any human or animal testing.

Data availability

Data will be made available on request.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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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