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Simple physicochemical characterization of example emulsions with oleosomes for cosmetic and dermatologic product applications

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Abstract: Oleosomes are lipid carriers and they can carry and protect hydrophobic compounds in hydrophilic environments. They present unique properties and may be applicable in cosmetics and pharmaceutical emulsion. Due to the unique structure, they function as natural emulsifying system which operates over a wide HLB range, allowing you to make sustainable emulsions. Tested formulations with oleosomes showed some interfacial and emulsifying activity. Oleosomes were isolated from safflower seeds (Carthamus tinctorius) and used as an emulsifier for the stabilization of O/W interfaces. Investigated the potential of oleosomes as carriers of active agents' compounds, including Coenzyme Q10, Omega-3, and Linoleic acid. The study focused on developing natural cosmetic formulations and determining general physicochemical properties as particle size distribution, ζ potential, interfacial tension at the O/W interfaces and evaluating the stability of the resulting formulations.

Keywords: oleosomes, emulsions, stability, cosmetics

1. Introduction

Cosmetics, dermatological and pharmaceuticals products are formed in emulsions form often. This form allows for achieve a homogeneous systems of many immiscible ingredients, such as oils, fats, water and many biologically and therapeutically active compounds. Adding cosmetic and pharmaceutical raw materials and their properties as hydro- or lipophilicity and the HLB parameter value, allow for obtaining diverse formulations with wide applications in the cosmetics and pharmaceutical industry (Nemichand Kale and Laxman Deore, 2017). Thanks to this, it is possible to introduce into one system both fat-soluble non-polar compounds and mostly water-soluble biologically active polar raw materials. Studies show that despite many raw materials, including those of natural, plant origin, containing the desired biologically active compounds, the development of stable physicochemical emulsion is sometimes problematic, and the need for new, better systems is still current. Moreover, emulsions are thermodynamically unstable and they may decompose by different mechanisms, including gravitational separation, flocculation, coalescence, Ostwald ripening, and finally phases separation (Tafuro et al., 2022). To stabilize emulsion forms interfacial active molecules are used. These compounds are adsorbed on the oil droplet interface.

Interesting systems, with promising properties and applications in formulation emulsion systems and in the cosmetic and pharmaceutical industries are forms containing oleosomes, fat-storing structures known from plant cells. The high physical and chemical stability of oleosomes has recently raised the interest to utilize them as natural oil droplets in emulsions for cosmetics and pharmaceuticals (Nikiforidis, 2019; Weiss and Zhang, 2020). The advantages of using oleosomes are that: they are natural and derived in simple steps from plant sources, no additional emulsifiers are needed, and their specified membrane opens new paths for advanced applications. In the past years, the development of oleosome research has been slow, and the understanding of oleosome behaviour in formulations in emulsion type systems, their composition, stability, and interfacial behaviour has not been enough. Therefore, there is a need for research of oleosomes. They can be added to a cosmetics cream, lotion and dermatological ointment, paste, gel etc. They can even be used for hand sanitizers, where there is a high percentage of

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monohydric alcohol. They exhibit unique properties due to the high interfacial activity of molecules on their surface and are capable of stabilizing foams and multiphase emulsions (Ntone et al., 2023). However, little is known about their properties and applications in emulsifying process and their behaviour on interfaces. Oleosomes are natural oil, lipid droplets abundant in plants. They have a lipophilic core and a hydrophilic surface stabilized by a monolayer of phospholipids membrane. The core of the oil droplets is comprised by vegetable oil triacylglycerols. In stabilization participate phospholipids and various interfacial proteins with long hydrophobic parts that are anchored into the oil phase, while amphiphilic parts rest on the hydrophilic oleosome surface (Huang, 2018).

The mechanism behind the stability of oleosomes is still not fully known. Oleosomes in isolated preparations, have displayed good physical and chemical stabilities due to the presence of a surface membrane composed of proteins and phospholipids, preventing their aggregation or coalescence (Nikiforidis et al., 2014). The triacylglyceride core of the oleosomes is surrounded by a phospholipid monolayer containing densely packed proteins called oleosins (up to 70-80% of the oleosome membrane protein content, molecular weight ranging from 15-30 kDa) which consist of both hydrophilic and hydrophobic domains. Oleosins are suggested to play a key role in the stability emulsion system, but little is known on the exact function of oleosins for the physical and oxidative stability of oleosomes. Because of their spherical orientation, exhibit good emulsification efficiency (in the scope hydrophilic-lipophilic balance parameter HLB from 5 to 15), even at low concentrations. This unique architecture reminds the structure of surfactants with a polar head and a lipophilic chain, providing oleosins with a significant interfacial activity. Moreover, this surface-active ingredients are biobased and may be alternatives for synthetic surfactants (Le Moigne et al., 2022; Plankensteiner et al., 2023). Oleosomes have promising features, such as a high physical and chemical stability against lipid oxidation and droplet coalescence. It is possible as a results of protective properties protein-phospholipid membrane and hydrophobic and electrostatic forces (Ding et al., 2020). Oleosomes were isolated from some plants as sunflower seeds and used as an emulsifier for the stabilization of W/O and O/W and interfaces. In both cases, they showed high interfacial and emulsifying activity (Karefyllakis et al., 2019).

Emulsion systems with Safflower (Carthamus tinctorius) oleosome can be used for their antioxidant properties. Tocopherol (vitamin E) is naturally present in the oil core, and can prevent oxidation of the unsaturated triglycerides. On the other hand, fatty acids (eg. oleic and linoleic fatty acids) are an important component for skin health and can be used in cosmetic formulations and dermatological products. Studies have shown that astaxanthin, a very sensitive molecule to oxidation, could be entrapped and protected in the oleosome core. Encapsulated astaxanthin retained its antioxidant activity (Acevedo et al., 2014). Safflower seeds are of great interest in the cosmetic industry, as they contain a large number of oil bodies, have lower surface tension, and higher ability to act as an emulsifier. Moreover, after isolating the oleosomes, both oleosomes and their isolated interfacial molecules exhibited a similar behaviour on the oil-water interfaces, forming predominantly elastic interfacial films, and also showed a similar emulsifying ability. The high interfacial activity of the molecules at oleosome membrane could be exploited to stabilize the interfaces of foams or emulsions.

It should be investigated how the structure of the oleosomes changes during the emulsification process and how the membrane behaves and participates in the formation of the newly available interface. An important aspect is to examine dispersed drops. There is a need to study the interfacial activity and emulsifying ability of the oleosomes. To investigate the role of the oleosomes in stabilizing emulsions in this work was studied their emulsifying and interfacial properties. In this research Safflower (Carthamus tinctorius) oleosomes was used. There is recent growing interest in safflower oleosomes due to their potential applications in dermatology, especially as a carrier technology to improve drug penetration through the skin (Cheng et al., 2024). The high oil content of safflower oleosomes also creates low surface tension, increasing the oleosome's ability to act as an emulsifier (Patel et al., 2023).

The study in this work focuses on developing the exemplary composition natural cosmetic formulations with oleosomes and evaluating their stability. Moreover, the objective of this research is to investigate the potential of oleosomes as carriers of highly unsaturated, sensitive and liphophilobic compounds, including Coenzyme Q10, Omega-3, and Linoleic acid. By encapsulating these compounds in oleosomes, we ensure their stability in the cosmetic formulation.

2. Materials and methods

Cosmetic raw materials and chemicals were obtained from those commercially available on the market: from Alchem, Surfachem and Alfa Sagittarius. Oleosomes were extracted from safflower seeds (Carthamus tinctorius) and oil-in-water emulsions were created after homogenization with oleosomes. The extracted oil body suspension was homogenized (and coagulated by pH manipulation). The first step to prepare the cosmetic formulations consists in the encapsulation of the active ingredients in the oleosomes, to be used later in the formulation of the cream. The resulting emulsion-cream, consisting of intact oil bodies, was studied with respect to particle size distribution, ζ potential and interfacial tension at the O/W interfaces.

2.1. Oleosome emulsion systems preparation

An aqueous extraction cold process was implemented according modified procedures described in earlier literature (grinding the seeds with aqueous medium, filtration, centrifugation, Iwanaga et al., 2007; White et al., 2008; Nikiforidis and Kiosseoglu 2009; Ntone et al., 2020). The purified oleosome cream was then stored at 4°C for further analysis. This procedure keeps the oleosomes intact, maintaining the oil droplets with their full qualities and properties. To prepare the cosmetic formulations where the oleosomes encapsulated the active ingredients (Omega-3 oil, Coenzyme Q-10, and Linoleic acid) formulation described below was used.

Oleosome+Coenzyme Q10 preparation: Coenzyme Q10 in sunflower seed oil (1:9 ratio) was dissolved in 40°C, system was cooled until 25°C and add it slowly to the oleosomes while mixing at 400 rpm, mix for 30 min. Oleosome+Omega-3 preparation: Omega-3 oil to 50°C was heated and slowly add to oleosome preparation while mixing at 400 rpm with a propeller, in a 1:3 ratio, mix for 30 min. Oleosome+Linoleic acid preparation: linoleic acid to oleosomes was added while mixing at 400 rpm with a propeller, in a 1:3 ratio, mix for 30 min.

After the encapsulation of the active agents in the oleosomes, the formulation takes place using the ingredients and composition shown in the table below (Table 1). The formulation the emulsions is performed following the next steps: add all components of phase A.1 and mix, mix phase A.2 and add to phase A.1, mix and heat components of phase B.2 until 60°C, cool down phase B.2 (<50°C) and add slowly to the Oleosome+active agent preparation, mix for 30 minutes, heat phase A and phase B to 50°C, add phase B to phase A while mixing with a propeller. Next homogenize emulsion for 5 minutes (Benchmark Scientific Homogenizator D1000-E), and adjust pH with L-arginine (pH 4.5-5.5). The creation of smaller oil droplets through homogenization enhances the physical stability of the emulsion,

Table 1. Creams composition.

Phases	Ingredients	Mass [g]	Compositions [%]
PHASE A.1	Water Propanediol Sodium benzoate	80.43	57.37
PHASE A.2	Glycerin	3.75	2.50
	Xanthan gum	1.20	0.80
PHASE B.1	Oleosome+active agent	20.00	13.33
PHASE B.2	Glyceryl Stearate Citrate, Cetearyl Alcohol, Glyceryl Caprylate	4.50	3.00
	Cetearyl alcohol	3.00	2.00
	Parkii Shea butter	6.00	4.00
	Caprylic/Capric Triglyceride	19.50	13.00
	Cetearyl alcohol, Cetearyl Glucoside	6.00	4.00

preventing separation. The results indicate that homogenization effectively prevents phase separation and preserves the initial particle size of oleosomes (Qin et al., 2014).

The particle size distribution of the oleosomes emulsion was determined by laser diffraction (Malvern Instruments, Ltd. UK) after dilution of the mixture with water at a ratio of 1:100. The used refractive index was 1.47. The differential particle size distribution of the oleosomes is given as function of the volume density. To measure the charge of the proteins, present in mixture under different pH conditions, titration was performed by using a ZS Nanosizer (Malvern Instruments, Ltd, Worcestershire, U.K.). The sample was prepared by dispersing the mixture in deionized water. The equilibration time was set to 120 s and the temperature to 25°C. The ζ-potential of the sample was measured at a pH ranging from 3 to 12, by adjusting it with HCl (0.5 M) or NaOH (0.1 or 0.5 M). The interfacial tension measurements at the O/W interfaces were recorded using an automated drop tensiometer (ADT) (Tracker, Teclis-IT Concept, France). The viscosity of creams was measured by an IKA Rotavisc Me-Vi viscometer (IKA-Werke, Germany), pH was determined using pH-meter CPO-505 with ERH-111 electrode (Electron, Poland). All the measurements and extractions were performed at least in triplicates. One-way analysis of variance (ANOVA) test was applied to detect differences analyses were performed with the IBM SPSS statistics 23 software. Differences were considered to be significant at $p < 0.05$.

3. Results and discussion

Oleosomes are extracted from safflower seeds (Carthamus tinctorius) using various methods, primarily aqueous extraction processes designed to maintain their natural structure and properties for applications in cosmetics. Due to hydrophilic character of the surface and the electrokinetic potential, oleosomes can be extracted at pH above 7.0 by alkaline aqueous solvents. For obtain a pure oleosome, we need washing steps, pH adjustment, and centrifugation process. A purified oleosome system is relatively stable, but we should be careful and don't provoke physical or chemical changes as intense heating, prolonged storage, or adding to formulations other reactive chemical agents.

The oleosome preparation can be loaded with different lipophilic compounds, which are sensitive to oxidation. Thanks to the monolayer of phospholipids, oleosomes are capable of protecting oil molecules with a controlled release process. By encapsulating these compounds in oleosomes, we ensure their stability in the cosmetic formulation. The described safflower oleosomes can emulsify oil. In this work studies were performed reviewing the stability, reology of the simplest emulsions O/W type using oleosomes and Coenzyme Q10, Omega-3, and Linoleic acid. The objective was to obtain a cosmetic formulation – a cream. This is because when low viscosities are obtained, low number of particles are present, and therefore a larger amount of emulsifier is needed to stabilize the preparations. Therefore, in the formulation a larger amount of the thickening agent was used and as well as the addition of Caprylic/Capric Triglyceride, which is known to help with the consistency of the cosmetic formulations. Due to the membrane, oleosomes can emulsify additional oils and to stabilize the larger structure. Oleosomes stabilization is related to intact oleosins, which are anchored in the phospholipid monolayer reaching the triglyceride core. The hydrophilic chain provides steric stabilization, preventing coalescence, and maintains the emulsification properties. Oleosomes surface charge and aggregation behavior are dependent on the protein interactions. Emulsions are stabilized by the steric and electrostatic repulsions of oleosins, along with the interactions with different zwitterionic phospholipids.

3.1. Physicochemical characterization of oleosomes emulsions

The size distribution oleosomes droplets was analyzed. Fig. 1 shows that the oleosomes with linoleic acid and coenzyme-Q10 have a bimodal size distribution. Shape and distribution peaks may be due to intermolecular forces like hydrophobic interactions. Oleosomes emulsions were spherical droplets that appeared in various diameters. The presence of large oleosomes could be attributed to partial coalescence during the extraction procedure. Mixing and extensive hydrophobic forces between neighbouring oleosomes is probably forcing them to aggregate and to a certain extent coalesce. Large particles were observed, with a size range between 15 and 20 μm, which are not oleosomes and could be emulsion droplets formed in the homogenization step. In the preparations containing coenzyme Q10

and linoleic acid, observations indicate that some of the large particles are degraded, probably also due to the high shear mixing, showing less physical stability than oleosome droplets, which remain intact. Partial destabilization of the bigger oleosomes could have positive impact on their behaviour as emulsifiers, as the oleosome membrane fractions contain highly interfacially active molecules.

Electrokinetic zeta potential is the electric potential on the interface of colloid particles as they move through an electric. It is a key indicator of the stability of oleosomes in dispersions, with higher absolute zeta potential values indicating stronger electrostatic repulsion, leading to more stable emulsions. The stability of oleosome emulsions are in uenced by electrostatic interactions between the associated proteins (among these proteins, oleosin is the major protein). Knowledge of the zeta potential allows us to understand many properties of dispersed systems (Fig. 2).

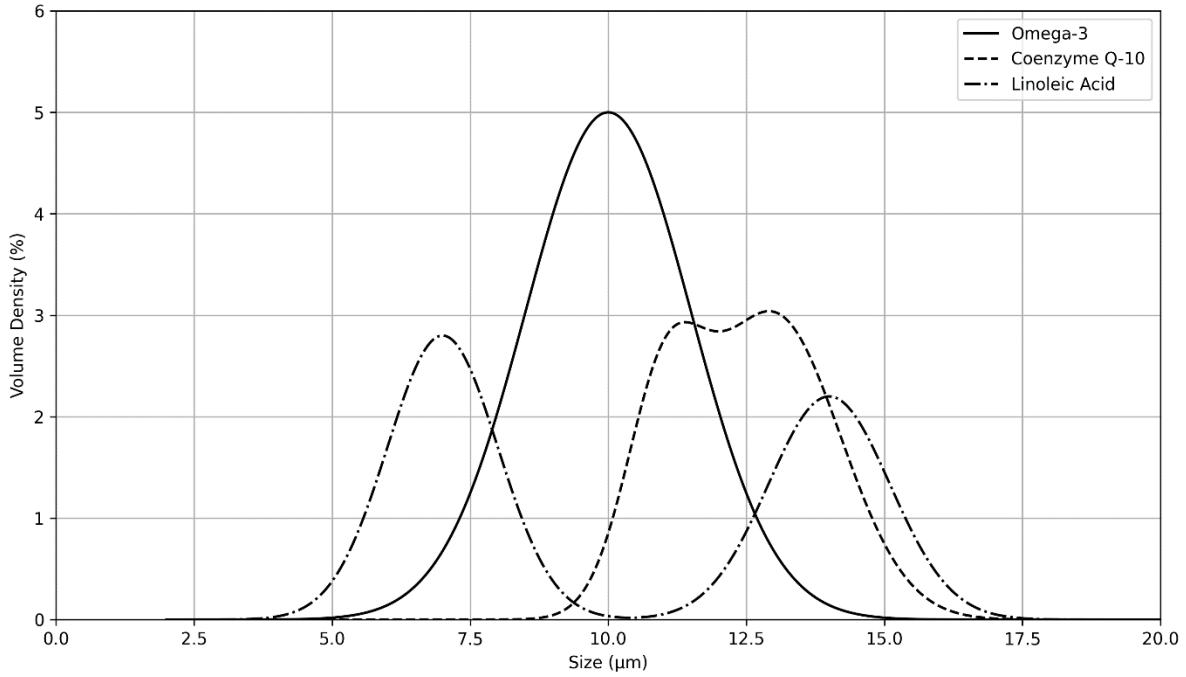

Fig. 1. Particle size distribution as a percentage of volume density of oleosomes preparates

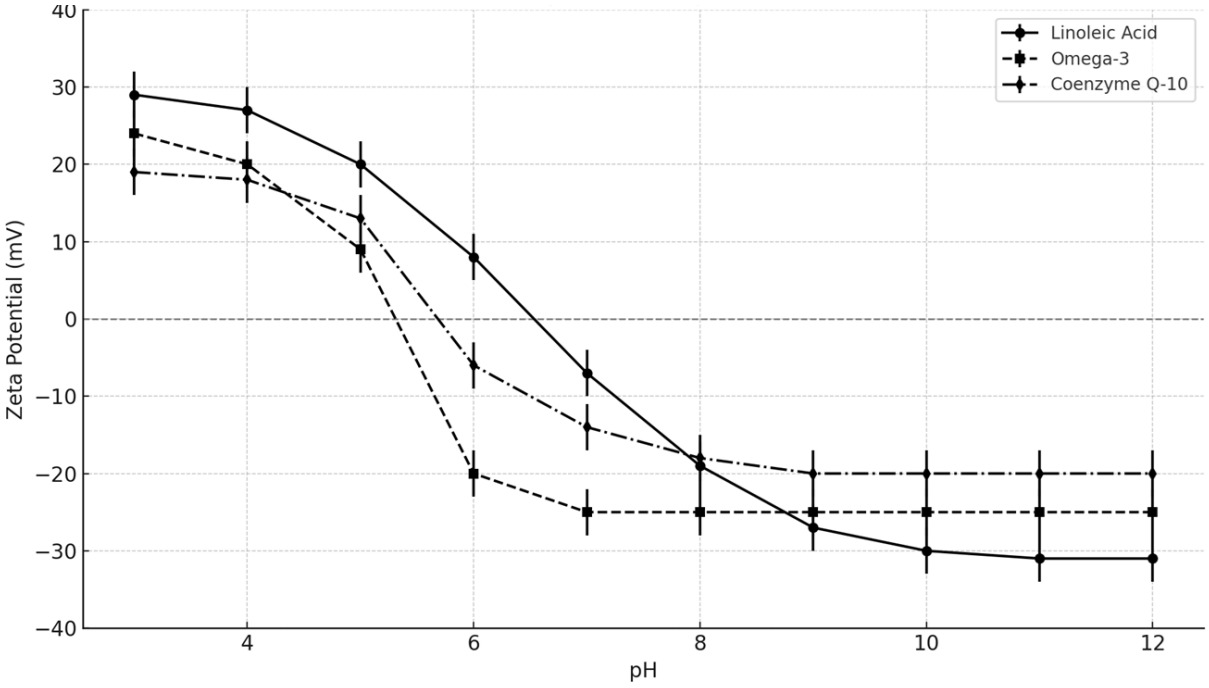

Fig. 2. Influence of pH on ζ-potential

At lower pH, proteins associated with the oleosome surface undergo protonation, resulting in a positive surface charge, while at higher pH, deprotonation induces a negative charge. As the pH deviated from the isoelectric point, the absolute zeta potential values increased, reflecting enhanced stability due to stronger electrostatic repulsion. In order to investigate the interfacial activity of the safflower oleosomes pendant droplet tensiometry analysis was performed.

The example emulsion with oleosome-omega-3 complex showed some interfacial activity (Fig. 3). The absorption profile of the dispersion indicates that besides storage proteins that are present in system, oleosomes and also material from their membrane are absorbed at the interface. The low value of interfacial tension achieved by oleosomes could be explained from the partial coalescence of oleosomes droplets during extraction that resulted in bigger and less stable oleosomes. The membrane of those oleosomes can rupture and its fragments with other proteins can adsorb on the interface, because they show interfacial activity.

The stability was evaluated by store in an incubator at the following temperatures: 4 and 40°C. The properties of the emulsions were observed after 8 weeks. Experiments showed that oleosome emulsion system demonstrate a good stability. No coalescence, sedimentation, flocculation, clarification or separation occurred. Results show physical and chemical stability in all the samples stored under different conditions, as no changes in pH or colour can be appreciated (Tab. 2). It can be concluded that oleosomes provide the emulsion systems with beneficial sensory properties. This is proven by the viscosity values.

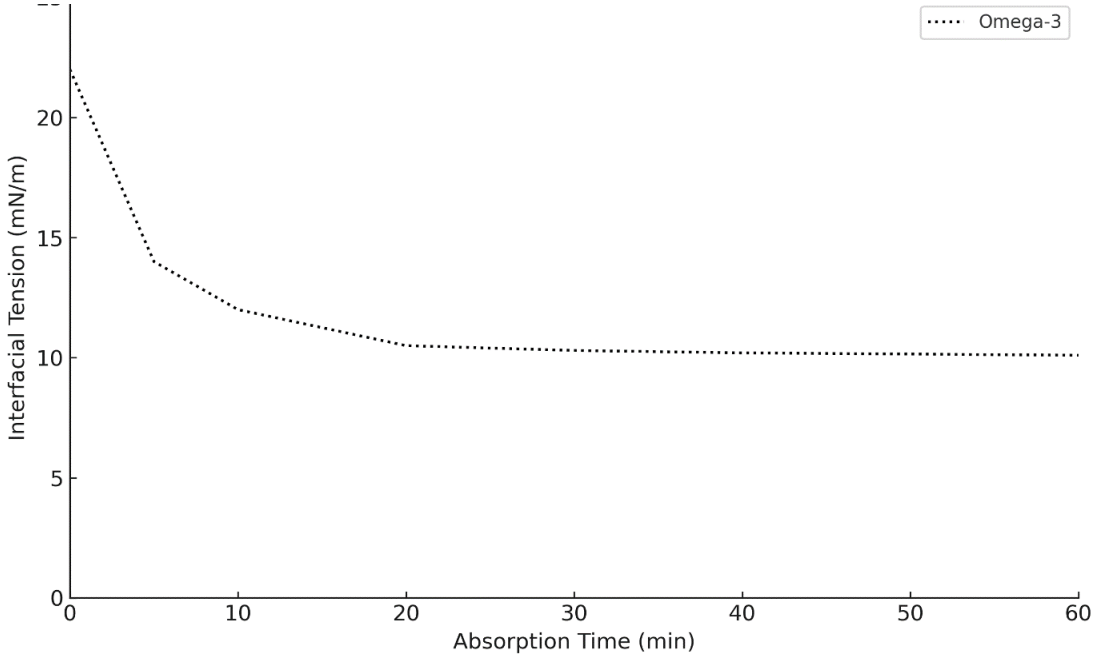

Fig. 3. Interfacial tension at the O/W interfaces as a function of absorption time in the presence of oleosome+Omega-3 complex

Table 2. pH, viscosity and color oleosome creams after preparation and at storage

Oleosome cream sample with:	pH	Viscosity [mPa s]	Color
Linoleic acid	4.95	17.123	white
4°C	4.85	12.829	no change
40°C	4.71	13.169	no change
Coenzyme Q10	5.21	12.342	yellow
4°C	5.07	11.687	no change
40°C	4.99	10.840	no change
Omega-3	4.93	23.985	white
4°C	4.89	21.847	no change
40°C	4.77	19.765	no change

When to system was added oleosomes, interfacial tension between two immiscible phases reduces to minimum, causes spontaneous formation of micro emulsions and formed negative free energy helps to make emulsion thermodynamically stable. Oleosins in oleosomes are amphiphilic in nature with long hydrophobic domains that are anchored into the oil phase, while their amphiphilic domains rest on the hydrophilic oleosome surface and plays key roles on integrity and stability the system by electrostatic repulsion and steric hindrance. The membrane lipids and proteins are anchored on the oleosome interface due to their amphiphilic character; the interfacial network is probably supported through electrostatic interactions. The high stability of system was associated with the negative charge of the oleosome droplet surface in combination with steric repulsive forces originating from the protruding hydrophilic parts of the oleosin molecule. The stability against aggregation and creaming of oil bodies, heavily depended upon the pH indicating that the surface charge is a crucial parameter in determining the oil body stability against aggregation and creaming. On the other hand, their remarkable stability against coalescence could be partly connected to the properties of the mixed phosholipid-oleosin.

4. Conclusions

Oleosomes emulsion systems represent a promising application for cosmetic active ingredients due to their advantages over the conventional formulations. Innovative emulsions are widely investigated for preparing personal care products with attractive sensorical properties and rheological characteristic as appearance or stability. These systems may be attractive for the preparation of various pharmaceuticals and cosmetic products - for use as moisturizing and soothing agents, as body cleansing agents, also as sun care cosmetics with sunscreens, as antiperspirants. These oleosomes emulsion systems have very good properties and may be products which allows rapid cutaneous penetration biological active compounds. Oleosome particles stabilize the emulsion against coalescence and their membrane components, are highly interfacially active.The understanding of the behavior of oleosomes as emulsifiers, opens many possibilities to use oleosomes as alternative to synthetic emulsifiers in food and pharma applications. Oleosomes are a great choice to replace synthetic lipid droplets, as they are abundantly present in natural sources and are environmentally friendly. They can be easily extracted and incorporated in cosmetic formulations, enhancing the properties of the preparations, and allowing the incorporation of sensitive active ingredients. The preparation of cosmetic emulsion systems employing oleosomes is simple, and in the studies carried out, oleosomes have shown the ability to act as carriers of different active and unsaturated compounds, like coenzyme Q10, linoleic acid and omega-3 acids.

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