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Research Article

Solid Lipid Microparticles As Carriers of *Vaccinium Myrtillus* And *Schinus Molle Linn* Additives for Food Application

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Abstract

The diet carried throughout our lives is closely influenced by the presence of free radicals that damage our bodies and develop diseases. Antioxidants are organic molecules capable of preventing cell damage. They are found in food of natural origins, such as blueberries and Aguaribay. A lipid-based transport system is formulated as solid lipid microparticles. This formulation helps counteract the adverse effects of directly applying the ethanolic antioxidant extracts and increases the oil matrix's useful lifespan. Characterization of the solid lipid microparticle formulations by DSLS, Zeta Potential, Rheology, and TEM confirmed that it protects the compounds encapsulated and stabilizes the active ingredient. Application of the formulation in different commercial oils allowed to establish a reduction in grape oil rancidity in stress conditions. This work indicates that the obtained solid lipid microparticle systems with natural antioxidant compounds can be used as a food additive.

Keywords: Lipid oxidation; Natural antioxidants; Oil additives; Solid lipid microparticles

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Introduction

The food sector's nano/microtechnology application is one of the fastest-growing emerging industries in recent years. Within the claims, the development of food improvements in terms of flavor, color, texture, and consistency. Also worth mentioning is the design of new packaging that increases the shelf life of food, the increased antimicrobial barrier, and sensors that help better monitor food conditions, among other [1]. The generation of the so-called "functional foods" collaborates with improving food nutrient absorption and provides new bio-nutrients or health supplements.

Solid lipid nanoparticles (SLN) or microparticles (SLM) are a solid core widely used carrier system due to their advantages over other colloidal systems [2]. They mainly comprise lipids with high physical and chemical stability, possess biocompatibility with a wide range of hydrophobic compounds, have controlled release, and protect the molecules incorporated into their hydrophobic nucleus from environmental factors. Applications in generating functional foods are a novelty when introducing bioactive but chemically sensitive compounds [3-5], and microencapsulation of bioactive compounds is an emerging concept [6]. For these reasons, to highlight SLM applications as a future potential focus of research in the food area, not only for its versatility but also for its numerous properties [7,8]. There are many SLM obtention; the simplest is generating the central lipid core and the bioactive compound. The microemulsion technique replaces the emulsions' liquid lipid (o / w) with a solid fat or solid lipids mixture at room temperature [9,10].

SLMs are a stable system; however, stability depends on the type of lipids and surfactants used to make up their structure and the production method [11,12]. In instability, the formulations can be creamated or form gels due to destabilization because of various triggers that involve an increase in energy in the dispersion generated by heat, light, and shear [13,14]. An increase in the system's energy that involves the particles causes more frequent collisions, thus inducing particle aggregation, flocculation, and cremation, culminating in the loss of the formulation's shelf life. That is why the components' selection is essential, and their posterior characterization of the SLM biophysically (rheology, particle size, zeta potential, etc.) to find the most stable formulation.

The production of fried foods when frying forms compounds harmful to health. On the other hand, frying has been widely accepted by consumers; thus, this paper describes the chemical composition of edible oils used in the preparation of these foods and some modifications they present during the thermal process, which may have effects on human health [15]. Oxidation is the most critical lipid reaction that affects the properties and storage of food. Besides, oxidation generates products that can become toxic on time and increment toxicity if ingested daily [16]. Moreover, when local communities depend on regional oil producers, this research becomes essential for preserving oil products, especially in developing countries where national entities are responsible for protecting public health by ensuring the safety, efficacy, and security of biological products to approve the consumer's goods.

The main aim of this work is to incorporate bioactive natural compounds (Due to its antioxidant, antimicrobial, antibacterial property and ease of obtention from the oleoresin of Aguaribay berries (Schinus molle Linn) are due to its antioxidant, antimicrobial, and antibacterial property by solid-liquid extraction [17-20]. Moreover, those from the blueberry extract (Vaccinium myrtillus) for its composition in terms of anthocyanins, flavonoids, and phenolic acids that provide beneficial properties for health [21-23]. Both SLM and bioactive compounds would be functional food additives in grape and coconut oils, maintaining the oil's organoleptic properties and improving their shelf life and nutritional quality.

Materials and Methods

Materials

Tween 20 from Promega Corporation, cocoa butter (CB) from Parafarm, soy lecithin (SPC) from Cargill S.A.C.I., milliQ water (mQ); Whatman No. 4 OneLab filter paper, blueberries (*Vaccinium myrtillus*) from San Pedro Bs. As berries from Aguaribay (*Schinus molle Linn*) from Berazategui Bs. As., Argentina; butylhydroxytoluene (BHT) from Biopack, Showing results for 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich.

Natural extracts obtention

A solid-liquid extraction technique obtained Aguaribay berry oleoresin. Briefly, 20 g of ground berries were placed in 100 ml of ethanol 96%. For extraction of blueberries, 25 g of the defrosted fruit were broken up with 150 ml of ethanol 96% acidified at 1% with HCl. Both preparations were shaken with 100 ml of ethanol 96% at 40°C for 48 h (80 cycles per minute). The insoluble material was filtered, and the solution evaporated to dry at 40 °C under reduced pressure (Rotavap, Heidolph Laborata 400) [24].

Once the extraction yield was determined, a fraction of the extracts was kept at 4 °C, and the other was solubilized using 96% ethanol (aguaribay) and 48% ethanol (blueberries).

SLM preparation

SLM suspensions were obtained based on methods described by [25]. Cocoa butter was dissolved with soy lecithin (1:1 mass ratio) at 75 °C. The lipid phase was dispersed in a solution of the surfactant (50 ml of water and 100 µl of Tween 20) preheated at 75 °C under stirring with an IKA Ultra-Turrax T18 kit, with S18N-19G dispersing tip (Janhke & Kunkel GmbH and Co KG, Staufen, Germany) at 16,000 rpm during 5 min, and maintaining a constant temperature. Subsequently, each microemulsion was cooled overnight at 4°C to allow the lipid phase to recrystallize and, hence, the SLM formation.

Encapsulation process

Blueberry ethanolic extract (Bb), previously obtained in the aqueous phase, was added to the preparation. In the case of oleoresin (Ol), it was added in the lipid phase. The formulations produced were labeled SPC-CB, SPC-CB-Bb, and SPC-CB-Ol, and their ratio SPC:CB: extract was maintained at 1:1:0, 1:1:0.3, and 1:1:0,02, respectively.

Characterization

Particle size

Particle size distribution was determined at 7 days intervals, at 1, 8, 15, 22- and 28-days post-production (dpp), 0.1 to 1000 µm range using a Dynamic-Static Light Scattering particle laser analyzer (DSLS) with Malvern Mastersizer 2000E equipment, Malvern Instruments Ltd, UK. The determination was made at 2000 rpm, with a degree of darkening in a range of 11-14 % and laser intensity of not less than 73 %.

Rheology

An AR-G2 controlled tension rheometer (TA Instrument) with temperature control and a 40 mm diameter cone geometry (2° cone truncated with a 55 µm center) on the Peltier plate was used. The shear strain rate was maintained between 0.5 to 180 s-1. An aliquot of 0.7 ml of sample was placed in all the tests, and the following temperatures were evaluated: 15 °C, 23 °C, and 30 °C (reached within 2 minutes until system stabilization). After that 30 seconds, the test for each one was started.

Surface charge determination

The samples were diluted 1/100 in distilled mQ water, and dilution was found adequate for the assays in 1 ml cuvettes. Each measurement was performed in triplicate using a Zetasizer Nano ZS (Malvern Instruments, UK) at 15 dpp for SPC-CB, SPC-CB-Bb, and SPC-CB-Ol.

Transmission electron microscopy (TEM)

Each SLM formulation was diluted 1/10, and 35 µl were placed on carbon cells 300 meshes. It was left to stand for 2 minutes, and uranyl acetate was added as contrast staining. Then, samples were observed with an EM10A/B high-resolution electron microscope. All the images captured were processed with AnaliSIS software, and two times of the stage-process were selected: 1 dpp and 29 dpp.

Gravitational separation

A fraction (1.5 ml) was taken from each sample and stored vertically in 2 ml microtubes in the refrigerator (4°C). Visual monitoring was performed at 1, 8, 15, and 29 dpp regarding phase separation of the different formulations. Photographs of each period were taken.

Antioxidant capacity

The colorimetric method with DPPH was compared with BHT taking positive control. Then, to 1.29 ml of a 0.003 % solution of DPPH, 21.45 µl of each sample was added. Then, samples were incubated for 30 min at 21 °C and absorbance was measured at 517 nm in a Cytation 5 Cell Imaging Reader Multi-Mode equipment from BioteK. Afterward, a graph of the percent of inhibition vs. sample concentration was obtained. Linear regression calculated antioxidant capacity expressed as IC50 (concentration at which 50% of free radicals in the sample are inhibited).

Food model system application

The effectiveness of the formulation of SPC-CB-Ol and SPC-CB-Bb was evaluated. The procedure was as follows: 5 ml of grape and coconut oil were placed in separate 15 ml tubes, and 200 ppm of the active ingredient (extract) of the mentioned formulations was added to each oil type. Then, it was subsequently incubated at 40 °C with

exposure to light for 25 days. Grape and coconut oil alone were used as references to compare the results.

Rancidity determination

The Kreiss reaction was used to determine the rancidity of the oils. For this purpose, 1 ml of each oil to be tested was separated, and 1 ml of concentrated HCl was added, stirring for 20 seconds. Then, 1 ml of phloroglucin solution was added and stirred again for 20 seconds. The sample was then incubated for 10 minutes until color developed. If the oil is rancid, the lower layer turns pink, purple, or red. When turned yellow or orange, colors discard the solution, and in this case, the test is completed with the Kerr modification. This modification involves two dilutions: 1/10 (A) and 1/20 (B), from which 1 ml of each was taken, and the reaction proceeded as previously mentioned. Possible results expected:

- Negative without dilution and negative in A and B: without rancidity
- Positive without dilution and negative in A and B: insufficient rancidity
- Positive without dilution and in A, negative in B: incipient rancidity
- Positive in all cases: defined rancidity

Statistical analysis

Data are presented as mean ± standard deviation (SD) or mean ± standard error of the mean (SEM) and analyzed by one-way ANOVA and Multiple Comparison post-test using GraphPad Prism v5.0. Only values with $p < 0.05$ were accepted as significant.

Results and Discussion

Particle size

Dynamic Static-Light Scattering (DSLS) allowed us to determine the particle size distribution profile of a suspension or polymers in solution [27,28]. This technique is widely used to track at different periods, which allowed us to obtain an association between particle size distribution and colloidal suspensions' stability. The size distribution for each formulation over time is represented in Figure 1. Then, the parameter D4.3 (Broken Mean Diameter) was selected for the analysis. This parameter becomes sensitive when a destabilization phenomenon is developed. Furthermore, this property is the main character in the study of oil rancidity. Heterogeneous populations of particles represented by the area under the curve of the Gaussian peaks were found in all formulations tested. At 1 dpp, the three formulations presented a majority population of 200 nm in size. SPC-CB formulation begins with a monomodal curve at 1 dpp. As the days go by, the presence of a second population is visualized, acquiring a bimodal character (the sample size of 79.43 µm is the majority). In the case of SPC-CB-Bb, a bimodal behavior, presenting the second population of 1.65 µm at 1 dpp, is seen, and an increment in particle size is reached at 45.70 µm. SPC-CB-Ol maintained a monomodal particle size during 29 dpp. After this day, the destabilization phenomena started to show a significant influence (Table 1).

Rheology

Rheology is the scientific discipline that studies and describes liquids and soft solids' flow and deformation when subjected to external

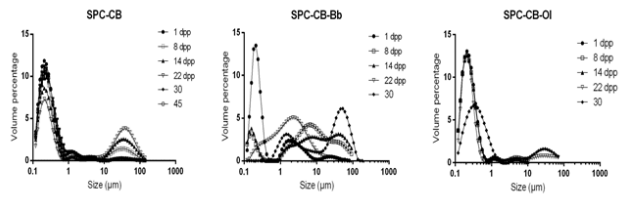

Figure 1: Size of selected formulations over time.

	Size (µm)				
dpp	**1**	**8**	**15**	**22**	**29**
SPC-CB	0,21±0,21	0,21±0,14	0,21±0,60	0,21±0,84	0,316±0,07
	1,25±0,09	5,01±0,05	1,09±0,01	1,26±0,06	79,43±0,05
	3,80±0,05	30,20±0,02	30,19±0,18	39,81±0,03	
SPC-CB-Bb	0,21±0,81	0,16±1,04	0,16±0,03	19,95±0,14	26,30±0,01
	1,67±0,23	7,58±0,65	7,58±0,35	45,70±0,55	45,70±0,04
	22,91±0,24	34,67±0,29	39,81±0,13		
SPC-CB-Ol	0,21±0,02	0,21±0,05	0,21±0,10	0,21±0,01	0,27±0,04
			1,25±0,09	1,25±0,02	8,70±0,03

Table 1: Particle size over time. Results are shown as mean ± SD of five independent measurements.

stresses. Therefore, it refers to the study of the elasticity and viscosity of matter [29,30]. The magnitude of the force determines the speed with which the fluid is deformed. The relationship between the force on the applied area and the strain rate defines another property, viscosity, which indicates how fast a liquid will flow each time a force is used. This rate will remain constant until the force is removed [31].

The non-Newtonian flow behavior model with pseudoplastic characteristics was adopted [29]. According to the above, the data were adjusted by the Power law or Ostwald's law model, according to equation 1:

$$\tau = K.\gamma^{n} \quad (1)$$

Equation 1: Power law. Where τ is shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency index ($Pa.s^{n}$), and n is the fluidity index (adimensional).

The rheological behavior responsible for the formulations' adequate flow is crucial for applying food products. Thus, SPC-CB, SPC-CB-Ol, and SPC-CB-Bb were studied at 15 °C, 23 °C and 30 °C at different deformation speeds per shear. These analyses were performed 1, 15, and 29 days post-production (dpp). The experimental data of each sample is presented in Figure 2.

The non-proportionality of the shear rate and shear stress is typical of non-Newtonian behavior. There is a decrease in viscosity with time under constant shear (shear thinning), characteristic of pseudoplastic fluids [32]. All formulations showed pseudoplastic behavior. The same increment is observed when temperature decreases due to the greater entanglement of the chains that increase the fluid's resistance to deformation, as inferred by the growing consistency index (K) in Table 2.

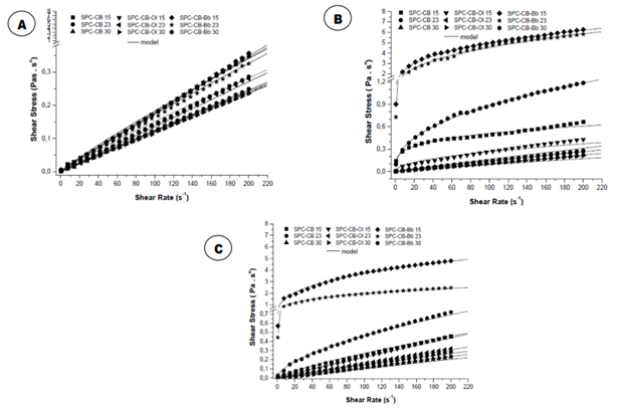

Figure 2: Experimental and Power Law model at 15 °C, 23 °C and 30 ºC for SPC-CB, SPC-CB-Ol and SPC-CB-Bb at A) 1 dpp, B) 15 dpp, C) 29 dpp.

SLM	Day 1		Day 15		Day 29	
	K (Pa.sn)	N	K (Pa.sn)	N	K (Pa.sn)	n
SPC-CB 15	0,0293 ± 0,0071	0,973 ± 0,012	0,1598 ± 0,0122	0,252 ± 0,006	0,0724 ± 0,0008	0,773 ± 0,004
SPC-CB 23	0,0242 ± 0,0094	0,898 ± 0,094	0,0028 ± 0,0005	0,904 ± 0,005	0,0311 ± 0,0009	0,915 ± 0,009
SPC-CB 30	0,0034 ± 0,0007	0,967 ± 0,013	0,0018 ± 0,0009	0,882 ± 0,006	0,0012 ± 0,0007	0,996 ± 0,017
SPC-CB-Ol 15	0,0236 ± 0,0062	0,939 ± 0,004	0,0294 ± 0,0013	0,378 ± 0,022	0,0048 ± 0,0005	0,957 ± 0,009
SPC-CB-Ol 23	0,0130 ± 0,0087	0,997 ± 0,009	0,0022 ± 0,0007	0,932 ± 0,006	0,0013 ± 0,0009	0,993 ± 0,018
SPC-CB-Ol 30	0,0012 ± 0,0085	0,996 ± 0,027	0,0012 ± 0,0004	0,984 ± 0,004	0,0010 ± 0,0006	0,996 ± 0,016
SPC-CB-Bb 15	0,0029 ± 0,0005	0,978 ± 0,008	1,1638 ± 0,0274	0,316 ± 0,005	0,6963 ± 0,0221	0,363 ± 0,005
SPC-CB-Bb 23	0,0022 ± 0,0004	0,913 ± 0,074	0,8654 ± 0,0511	0,361 ± 0,009	0,4891 ± 0,0117	0,302 ± 0,005
SPC-CB-Bb 30	0,0011 ± 0,0006	0,978 ± 0,006	0,1261 ± 0,0095	0,423 ± 0,003	0,0313 ± 0,0059	0,584 ± 0,010

Table 2: Power Law model parameters for all formulations at 15 °C, 23 °C and 30 °C for 1, 15 and 29 dpp. Results are shown as mean ± SD.

SPC-CB-Bb showed a higher consistency increment as the study days passed. The results corroborate those obtained by DSLS since an increase in the particle size increases the probability of interaction between the microparticles. Therefore, a gain of consistency results from the destabilization phenomena of the colloidal suspension. In the case of SPC-CB and SPC-CB-Ol, the consistency growth is insignificant for the blueberry formulation. It is endorsed by the coincident results of light scattering, reflecting very few particle size changes, as seen above.

Zeta Potential (ZP)

The ZP is defined as the potential difference between the particle's surface and the electroneutral region of the solution [33]. The results obtained for tested formulations are shown below (Figure 3).

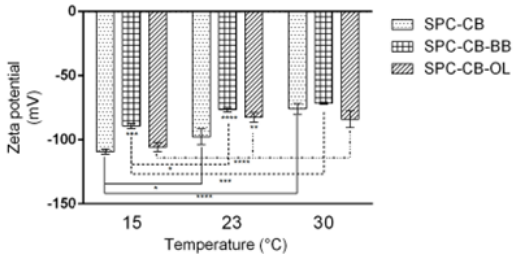

Figure 3: Measurement zeta potential of formulations. Results are shown as mean ± SEM of three independent measurements. Statistical analysis was performed by one-way ANOVA and Tukey's test (*p < 0.05, **p < 0.001, ***p < 0.001, ****p < 0.0001).

The ZP values obtained are below -10 mVm, which indicates the population's existence stability; this shows that there are populations of negatively charged nanoparticles. Soy lecithin's composition includes lipid anions like phosphatidic acid, phosphatidylglycerol, and phosphatidylinositol. These lipids are responsible for the nanoparticle's negative potential surface [34,35]. The SPC-CB-Bb formulation shows a decrease in particle charge value with increasing temperature. This effect could be attributed to anthocyanins' low stability under temperature changes [36-38].

Regarding SPC-CB-Ol, its ZP values coincide with the stability parameters, being sufficiently electronegative to keep particles individually and avoid cremation and aggregation. The increase in temperature produces a decrease in surface charge and an increase in module electronegativity. The latter could imply the stabilization of the oleoresin since, at 23 °C and 30 °C SPC-CB, a decrement of charge is observed.

Microscopy

According to [3], the shape of lipid microparticles may differ from a sphere based on the conformation acquired by the lipids in the oil composition when the SLM is formed. This result is also confirmed by TEM data, where the shape and particle size can be observed. There may be a difference if the whole range of particles is considered between the size determined by DSLS and TEM. The difference in results is attributed to the Mastersizer data being based on the laser diffraction principle. Due to the particles ' Brownian movement, this technique does not detect particles below 100 nm [39]. It will become necessary if the particle density of these particles (<100 nm) is statistically relevant. The micrographs below show morphologies characteristics of the systems under study at 1 dpp and 29 dpp (Figure 4).

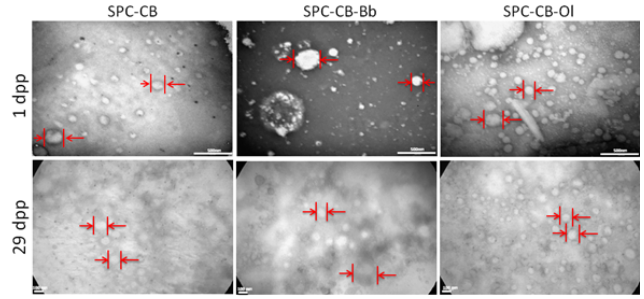

Figure 4: obtained by Transmission electron microscopy (TEM).

We can infer by TEM that microparticles are spherical bodies with defined edges for all three sample cases studied at 1 and 29 dpp

(Figure 4). For better visualization of the system, a dilution of the dispersion (1/10 in water) confirms the presence of individual microparticles below system saturation.

Formulations analyzed at selected periods show an increase in size, but mainly for SPC-CB and SPC-CB-Bb. There is also an increment in consistency attributed to destabilization like cremation and flocculation. On the other hand, the SPC-CB-Ol formulation showed individual particles of the samples even after 29 dpp.

The study with the SLMs detected aggregation processes in the formulations corroborated by DSLS and rheology results. In all, both formulations showed size polydispersity and circular particle morphology. For SPC-CB-Ol, the particle size obtained is 0.20 µm, a size maintained on the elapsed days studied and corresponds to those measured by DSLS (0.21 µm). Rheology studies and gravitational separation indicate that oleoresin generates the most stable system in terms of its characteristics.

The case of SPC-CB and SPC-CB-Bb is different; at 1 dpp, the particles have a size of 0.25 and 0.31 µm, respectively, which coincide with those obtained by DSLS. However, at 29 dpp, the particles in the micrographs maintained their size, even though other techniques increased their size 100 times, and the consistency increased due to the cremation and flocculation process confirmed by the gravitational separation. Thus, these are the least stable of the formulations. According to [40] an increase in the particle size results from the destabilization phenomena. This effect should result from their lipid character, permitting the lipids to be relocated near the suspension's surface. Only those that are smaller in size could be distributed homogeneously in the rest of the aqueous suspension. The sampling is performed by extracting from the middle center of the solution of the different formulations. A direct consequence of the sample dilution is that the flocs present are disarmed, which allows a better resolution of the TEM images.

Gravitational separation

At 1 dpp, the cremation phenomenon was not evident in the surface area of the sample tubes. In the case of SPC-CB-Bb, a violet precipitate is observed, corresponding to the Bb extract color. This precipitate can be attributed to excess extract or partially to the SPC components' interaction. A visualization of the formulation's aspect, after 8 dpp, did not register changes.

At 15 dpp, a slight color change was evident in SPC-CB-Bb, indicating anthocyanin's destabilization. The formulation's solution turned brown when destabilizing due to degradation [41,42]. Additionally, notable physical change was observed regarding the consistency increment of SPC-CB-Bb (increased viscosity), consistent with previous rheology studies. The rest of the formulation's visual aspects remained unchanged during the analysis (Figure 5).

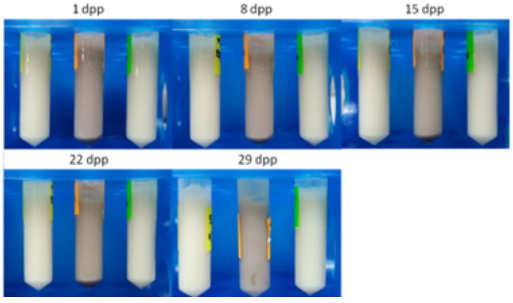

Figure 5: gravitational separation.

Antioxidant capacity

The colorimetric method's antioxidant capacity was determined using the radical chromogen DPPH (2-diphenyl-1-picrylhydrazyl) [43]. The blueberry extract and Aguaribay oleoresin contain natural antioxidants formed by different components that act synergic until reaching this activity [44,45]. Antioxidant capacity was calculated as the inhibitory concentration 50 (IC 50). According to Figure 1, the oleoresin concentration of 0.276 mg/ml inhibits 50 % of DPPH radicals. Compared to Blueberry extract (12.35 mg/ml), this concentration of oleoresin extract is low, and 6.91 mg/ml of commercial antioxidant BHT is needed to reach IC50 activity (Figure 6).

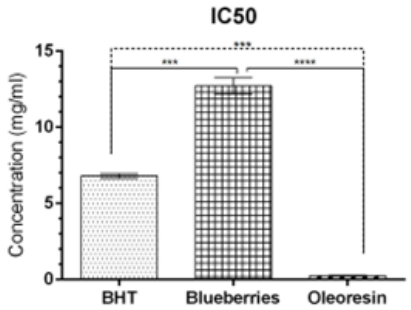

Figure 6: IC50 Results are shown as mean ± SEM of two independent measurements. Statistical analysis was performed by ANOVA and Tukey's test (*p < 0.05, **p < 0.001, ***p < 0.001, ****p < 0.0001).

Food Incorporation

Lipid oxidation is a process carried out slowly at room temperature [46], so parameters such as temperature, light, and oxygen exposure are varied to determine the oil's or fats' stability and susceptibility [47] (Figure 7). Oil samples were incubated at 40 °C in a colorless container with light exposure. At 1 and 15 days of incubation, Kreiss's reaction was performed, and the following results were obtained (Table 3).

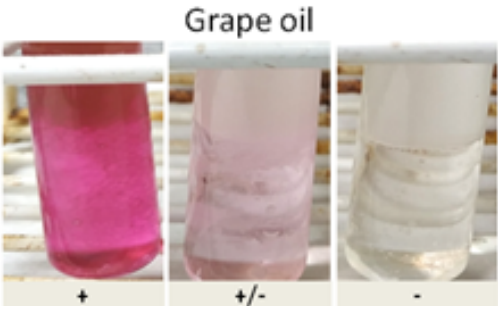

Figure 7: Possible results of Kreiss and Kerr reactions.

	1 dpp			15 dpp		
Grape Oil	+	+	+	+	+	+
SPC-CB-Bb	+/-	-	-	+	+	+/-
SPC-CB-Ol	-	-	+	-	-	-

Table 3: Results Kreiss assay and Kerr adaptation.

We can observe that storage conditions affect grape oil's oxidation speed, giving positive results (pink coloration) for both the Kreiss reaction and successive dilutions. This fact is related to the sample

rancidity, which is already well-defined in the matrix, inducing changes in color and flavor. In the case of the sample of SPC-CB-Ol, the result was negative for the original sample and the dilutions at 1 dpp, seeing incipient rancidity only at 15 dpp for the concentrated condition. Microparticles and antioxidants may help decrease lipid oxidation and improve grape oil's shelf life.

With the addition of SPC-CB-Bb, samples showed rancidity in concentrated conditions and 1/10 dilution, indicating that, to a lesser extent, the food is protected from oxidative degradation. The latter can be corroborated by what was previously mentioned. In the case of cereals, incorporating blueberries contributes to an increase in the matrix's acidity.

An increase in acidity would imply an increase in oil's free fatty acid concentration. Free fatty acids are derived from the hydrolytic rancidity of triglycerides [48], explaining why blueberry extract, even though its antioxidant properties, would not be protecting the oily matrix, on the contrary, inducing its destabilization in all the days tested.

In coconut oil, the reaction was adverse in all cases, indicating no deterioration of the oil characteristic of the sample rancidity. The coconut oil is stable under the tested storage conditions. This type of oil is more durable than sunflower oil, a feature given by the presence of lauric acid, a short-chain fatty acid with the property of being in a solid state at room temperature [49].

Grape oil, together with olive oil, is one of the most delicate oils. As previously mentioned, grape oil has a high linoleic acid content, which is susceptible to oxidation due to its double bonds [50,51]. Besides, it should be mentioned that it is one of the least processed oils without adding preservatives to maintain its natural characteristics.

Conclusion

It could be shown that the extracts obtained are healthy alternatives instead of those of synthetic origin in the food industry, not only because of their origin but also because it is easy to get and even possibly included in secondary processes where waste is recycled for further product separation within the same production line industry (for example, blueberry peel).

The fruits selected for this work gave us extracts with different characteristics regarding hydrophobicity. These conditions could have influenced the behavior of the SLM after being encapsulated. Aguaribay oleoresin has a highly hydrophobic character. When the particles are encapsulated, they will interact with the SLM's hydrophobic nucleus and, hence, decrease the surface of contact with water. This decrement could cause the high stability of the SPC-CB-Ol formulation.

On the other hand, the blueberry extract has very soluble components in water, like anthocyanins, which are natural ampholytes and interact partly with the SLM's hydrophobic interior and partly with the SLM surface protruding into the continuous water phase. This double interaction could cause SPC-CB-Bb's instability since there is a strong tendency for its components to remain in the aqueous phase, rendering destabilizing effects. As a result, this formulation will have a short shelf life.

The SPC-CB-Bb system presented unstable behavior regarding sample stability when performing the gravitational separation at 15 dpp (phase separation). When monitoring instability, the focus should be on the particle size if it gradually increases. Another property to follow is the increment in the consistency that reflects the particles' cremation and flocculation as the days elapsed.

The compound incorporation tests highlight the hydrophobic tendency of the Aguaribay extract that generates a positive effect in increasing the oils' stability under the study. The opposite case is the SPC-CB-Bb SLM, where the protective effect was shallow due to the encapsulated extract's amphipathic nature. We can also confirm the low favorable impact of this system for providing a decrement in the pH of oils, generating an unfavorable environment.

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