Antifungal synergistic effect of paprika and cinnamon oleoresins and their coencapsulation by spray chilling technique to produce a carotenoid-cinnamaldehyde rich food powder

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Research Article

Keywords: bioactive compounds, solid lipid carriers, microencapsulation, natural colorant

Posted Date: July 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1816385/v1

Abstract

Oleoresins are natural extracts obtained from plants and spices. Cinnamaldehyde and carotenoids are the main compounds found in cinnamon and paprika oleoresins, respectively, and exhibit pronounced antimicrobial and antioxidant potential. The coencapsulation of the two oleoresins can promote greater stability and synergism between them. Microencapsulation by spray chilling generates solid lipid microparticles when the atomized matrix crystallizes in contact with the cold chamber. This technique allows greater trapping of volatile compounds as they are more soluble in lipid materials. This work's main objective was to evaluate the synergistic antifungal effect between cinnamon and paprika oleoresins and their coencapsulation by spray chilling. Cinnamon:Paprika (1:1 and 2:1) mixture showed a synergistic effect against *Penicillium paneum* and *Aspergillus niger*. The extracts also prevented the growth of microorganisms without direct contact with the agar. The microparticles showed a spherical surface and polymorphic β' characteristic. Particles containing paprika oleoresin presented orange color, while samples with only cinnamon were off white. The size distribution was multimodal with ($D_{0.5}$) ranging from 13.6 μm for samples containing only cinnamon to 1152.7 μm for samples containing only paprika oleoresin. The storage temperature (5 and 25°C) affected the release of cinnamaldehyde from the microparticles. Regarding the first 14 days, samples stored at 5°C released more cinnamaldehyde compared to that stored at 25°C. Nevertheless, at the end of the 49 days, the content of cinnamaldehyde retained in the particles was practically the same. The concentration of carotenoids in the particles remained constant throughout the 49 days of storage for both temperatures. The co-encapsulation of oleoresin from paprika and cinnamon made it possible to obtain a lipid coloring powder that may have antimicrobial properties.

Introduction

With increasing consumer concerns for healthier, natural, and synthetic-free products, the challenge of ensuring a longer shelf life for products has increased. For this reason, the search for natural compounds with antimicrobial and antioxidants properties has been intensified. Oleoresins are composed of lipophilic terpenoids, phenylpropanoids, and derivatives of low molecular weight aliphatic hydrocarbons (Shahidi & Hossain, 2018). Its use in food industry occurs mainly as flavor, aroma, and color additives to improve the sensory properties of many foodstuffs. However, they may also be sources of natural compounds with different functional properties such as antioxidant, antimicrobial, anti-inflammatory, and anticancer (Jugreet et al., 2020).

Cinnamon and paprika oleoresins are examples of natural extracts rich in active compounds with recognized antimicrobial and antioxidant properties. Cinnamaldehyde is the main compound of cinnamon oleoresin, with carotenoids being the most important in paprika oleoresin (Asbahani et al., 2015; Muhammad & Dewettinck, 2017). Cinnamaldehyde is the responsible for the characteristic flavor of cinnamon products and has been recognized as an antimicrobial, antioxidant and anti-inflammatory compound (Muhammad et al., 2021; Muhammad & Dewettinck, 2017). Paprika oleoresin is a viscous and

red-brownish extract rich in carotenoids, which makes paprika a common coloring additive in food products (Villa-Rivera & Ochoa-Alejo, 2020; Mendes & Gonçalves, 2020; Melgar-Lalanne et al., 2017).

While generally exhibiting a significant compound, oleoresins comprise a complex mixture of compounds of a distinct chemical nature, and their activity is due to that combination. As antimicrobial agent for example, each compound's acts in a different way on the microbial cell-matrix depend on many factors, such as chemical nature, concentration, and solubility (Zhang et al., 2015). Because of the varied chemical composition of spices and their extracts, there may be a synergism in mixing oleoresins from different sources. Their properties could be improved and the amount required to ensure activity decreased.

In order to ensure the stability to volatile, flavor and color compounds, as well as attenuating the sensorial effects to the product, the addition of oleoresins as microparticles could be a promising alternative. Microencapsulation is defined as the embedding of a substance in a matrix (Gaonkar, 2014). Coencapsulation can be defined when two different actives are encapsulated together in the same matrix. Another form of coencapsulation is using two combined techniques to obtain the microparticles (Comunian et al., 2018; Guo, Yin, & Chen, 2018; Olga et al., 2015). The mixture of compounds not naturally found together can promote a pronouncement of their effects. Among the physical methods, the spray chilling technique has gained considerable attention for its ease of large-scale reproduction and absence of solvent during the process (Silva et al., 2018; Arslan-Tontul & Erbas, 2017; Alvim et al., 2016; Bampi et al., 2016). The spray chilling microencapsulation technique allows the atomization of a lipid matrix within a cold chamber, giving rise to solid lipid microparticles. Due to the hydrophobic character of many chemical constituents present in oleoresins, greater solubility and stability are achieved when these compounds are added to liquid lipids (Paravisini & Guichard, 2016).

According to Katouzian et al. (2017), the solid lipid carriers present as advantages the absence of biotoxicity and the prolonged release of the active. The use of solid lipid microparticles loaded with oleoresins, in this case with a high content of carotenoids and cinnamaldehyde, are an alternative to protect these compounds and facilitate their application. Through the spray chilling technique, we obtain the transformation of a liquid extract into powder, without losing its lipophilic characteristic.

In this way, this work aimed to evaluate the synergism between cinnamon and paprika oleoresins against two common foodborne molds, to produce and characterize the solid lipids microparticles by their coencapsulation using the spray chilling techniques.

Material And Methods

Material

Cinnamon (CO) and paprika oleoresin (PO) were provided by Synthite (Valinhos/São Paulo, Brazil). Palm fat (PF) and commercial vegetable fat (VF, melting point of 40°C) used as carrier agents were kindly donated by Cargill (Mairinque/São Paulo, Brazil). Cinnamaldehyde (Cn) standard (Sigma Aldrich, São

Paulo, Brazil) was used in the gas chromatography analysis. All other used reagents were of analytical grade.

Methods

Minimal inhibitory concentration (MIC) of CO and PO individually and in a mixture

The minimum inhibitory concentration of pure and combined oleoresins was determined by the microdilution method, according to Siddiqi et al. (2010), with modifications, against *Penicillium paneum* and *Aspergillus niger*, common contaminants of bakery products. Stock solutions containing 3g of oleoresins for each 100 mL of dimethylsulfoxide (10% v/v) (DMSO, Sigma – Aldrich, USA) in the ratios 1:0, 0:1, 1:1 and 2:1 between cinnamon:paprika, respectively, were prepared. Then, dilutions were performed with the appropriate culture media for each microorganism. Using a sterile 96-well plate, a 100 µL aliquot of each microorganism tested (10^5 CFU/mL) was transferred to each well, and the plates incubated at 25°C for five days. After the incubation period, the plates were macroscopically evaluated and the MIC was determined by the lowest concentration capable of inhibiting the visible growth of the tested microorganisms.

To the synergistic effect evaluation between the extracts, the fractional inhibitory concentration (FIC) was used. FICA and FICB are defined as the A and B concentrations (expressed as a fraction of its MIC), respectively, necessary to inhibit microorganism growth, when combined with a known amount of a second antimicrobial agent. The FIC values of each oleoresin were calculated applying the following equations (Reyes-Jurado et al., 2019):

$$FICA = \frac{MICoffAinthepresenceoffB}{MICoffAindividually}$$

1

$$FICB = \frac{MICoffBinthepresenceoffA}{MICoffBindividually}$$

2

Where: A and B represent the evaluated cinnamon and paprika oleoresins, respectively.

After obtaining the FICA and FICB values, the fractional inhibitory concentration index (FIC_{Index}) for the oleoresins tested was calculated using Eq. 3.

$$FIC_{index} = FICA + FICB$$

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Since FIC_{Index} is calculated based on the MIC of each oleoresin in combination, the resulting value indicates the mixture's effect. An FIC index < 1.0, = 1.0 or > 1.0 means the synergistic, additive, or antagonistic effect of the tested combination, respectively (Burt, 2004).

The antimicrobial activity of oleoresins was also evaluated by the headspace volatilization technique (Reyes-Jurado et al., 2019). Concentrations of 1, 0.5, and 0.25 g/mL of each oleoresin or the mixtures (1:1 and 2:1) between cinnamon: paprika were evaluated. Solutions were prepared using DMSO (10% v/v). An aliquot of 20 µL of each solution was added to a 3 cm diameter disc of filter paper. The discs were fixed on the lid of a 12-well plate, using six wells spaced per plate, and hermetically closed. As controls, blank discs and discs containing 20 µL of DMSO solution were evaluated.

Production of SLM by spray chilling

The oleoresins and the carrier agents were weighed according to their respective proportion, as shown in Table 1. Spray chilling particles were obtained using a Büchi-B290 spray dryer set in the spray chiller mode (Büchi, Flawil, Switzerland), according to Oriani et al. (2018). First, the lipids were heated up to 70°C by a temperature-controlled water bath (Tecnal, TE-184, Piracicaba, Brazil) to assure complete melting. Then they were added to the oleoresins: Cinnamon and Paprika (M1), Cinnamon (M2), Paprika (M3), and the total mixture (100 g) was kept under magnetic stirring. A sample with only the carrier agents (M4) was also produced. The mixture was fed into a heated double fluid atomizer with a nozzle diameter of 0.7 mm using a peristaltic pump, at a mass flow rate of 0.7 kg/h. SLMs formation occurs within a cooling chamber where the inlet air temperature was 7°C. Atomizing air and cooling airflow rates were 831 L/h and 35,000 L/h, respectively. At the end of the process, samples were collected, stored in closed containers and kept at 5°C. Each experiment was performed in duplicate. Table 1 shows the composition of the formulations used in the SLM production.

Table 1
Solid lipid microparticles composition.

Formulations	**Ratio** *VF: PO*	**Oleoresin final concentration (g/100 g of lipid matrix)**	**Ratio C:P**
M1	80:20	3	2:1
M2	80:20	3	1:0
M3	80:20	3	0:1
M4	80:20	-	-
VF: vegetable fat; PO: palm oil; C: cinnamon oleoresin, P: paprika oleoresin.			

Color of microparticles

The color of the solid lipid microparticles was determined using an UltraScan-VIS 1043 colorimeter (Hunter Lab, Reston, USA) with CIELab scale (*L*, a*, b**) as described by Anthero et al. (2020). SLMs were

uniformly packed in the 10 mm glass optical cell before it was placed in the reflectance port for reading, using D65 as an illuminant and a 10° observer angle as a reference system. ΔE^* values determine the difference between samples containing oleoresins and the control (Eq. 4).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

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Where L* represents *lightness*, a* *redness*, b* *yellowness*, and ΔL*, Δa*, Δb* represent the difference between each parameter for the particles containing or not the oleoresins.

Crystalline structure

Samples were analyzed using the X-ray powder diffraction technique. A Philips X-ray diffractometer (Analytical, X-Ray X'Pert-MPD, Almelo, Netherlands) was used to observe the crystalline structure. X-rays of λ = 1.54056 Å were generated by a Cu Kα source. The diffraction was measured in the 2θ range, from 15 to 25°, in a rate of 0.02°/s, according to the AOCS method Cj 2–95 (AOCS, 2004).

Particle size

The size distribution and the polydispersity index (Span) of the SLM were determined by the light scattering technique, using laser diffraction in a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK), following the methodology described by Oriani et al. (2018). A dry air dispersion accessory was used (Scirocco 2000, Malvern Instruments Ltd, Malvern, UK), and the readings were done in triplicates. The analysis was conducted at room temperature. Size distribution was characterized by $D_{0.1}$, $D_{0.5,}$ and $D_{0.9}$, representing the diameter of the accumulated distribution of 10%, 50%, and 90% of total particles.

Morphology

The SLM morphology was investigated with images obtained from a scanning electron microscope (SEM) with Energy Dispersive X-ray Detection (LEO Electron Microscopy Leo 440i, Oxford — Cambridge, England). Microparticles were covered with a gold layer (200 Å) by a Sputter Coater (EMITECH, model K450, Kent, United Kingdom) before analysis, which was performed with an accelerating voltage of 10 kV and a 50 pA beam current. Micrographs were obtained with magnifications of 500× and 5000×.

Total Entrapment Efficiency

The stability of oleoresin's major compounds in solid lipid particles was evaluated for 49 days at 5 and 25°C. Samples (10 g) were placed in sealed airtight plastic containers (200 g). The formulations M1 and M2, which contained cinnamon oleoresin, were assessed for cinnamaldehyde retention. For carotenoid analyzes, the formulations M1 and M3 were evaluated.

Carotenoids retention

The total carotenoid content in the microparticles was determined according to the methodology described by Hornero & Míngues (2001), with modifications. For the analysis 0.20 g for the microparticles

were weighed and hexane (20 mL) was used for carotenoid extraction. After complete homogenization, samples were diluted at 1:10 using hexane. Absorbance reading was performed by a UV/VIS spectrophotometer (Biospectro) at 472 nm and 508 nm. The isochromatic red and yellow fractions and the total carotenoid content were calculated using the following equations:

$$C^R = \frac{A_{508}x2144.0 - A_{472}x403.3}{270.9} \quad (5)$$

$$C^Y = \frac{A_{472}x1724.3 - A_{508}x2450.1}{270.9} \quad (6)$$

$$C^T = C^R + C^Y \quad (7)$$

C^R represents the content of the isochromatic red fraction, C^Y represents the yellow isochromatic fraction, C^T the total carotenoid content, A_{472}, and A_{508} the absorbance values at 472 nm and 508 nm, respectively.

Cinnamaldehyde retention

Cinnamaldehyde quantification was made to express the total amount of cinnamon oleoresin entrapped in the microparticles. The determination of cinnamaldehyde content in cinnamon oleoresin microparticles was performed using a Headspace solid-phase micro-extraction (HS-SPME) method followed by gas chromatography analysis. First, solid lipid microparticles (0.1 g) were added to a 100 mL headspace vial and kept at 54°C for 30 min. Then, the extraction of cinnamaldehyde was conducted by exposing a Polydimethylsiloxane (PDMS) fiber (Supelco, USA) to the headspace for 10 min. After extraction, the thermal desorption was carried at 250°C for 5 min in injector from Gas Chromatography (GC) (HP7890, Agilent Technologies, USA) system equipped with a flame ionization detector (FID), DBWAX capillary column (30 m × 0.25 mm × 0.25 m - J&W Scientific, EUA) and using helium as carrier gas, at a 1.5 mL/min flow rate. The chromatographic conditions were based on an analytical method previously described by Procópio et al (2018) with modifications. The oven temperature was set initially at 80°C for 2 min, increasing at a rate of 20°C/min until 240°C, kept for 2 min. The quantification was performed by plotting AUC (area under the curve) in a cinnamaldehyde standard curve ranging from 25-9900 µg/mL with 2-octane as an internal standard. Cinnamaldehyde retention was calculated based on the ratios of initial cinnamaldehyde amount (C_I) in free added oleoresin and final cinnamaldehyde amount (C_F) remaining on microparticles, with the Eq. 8.

$$\%Cinnamaldehyderetention = \frac{C_{Iafterencapsulationprocess}}{C_{Fbeforetheencapsulationprocess}} x100$$

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Statistical analysis

Results were expressed as the mean value ± standard deviation of two or more independent experiments. Analysis of the variance (ANOVA) with a significance level of 5% followed by the Tukey test was applied, and significant differences were considered when *p*-value < 0.05. The Minitab software trial edition (Minitab 18.0, Minitab Inc., State College, PA, USA) was used for data analysis.

Results And Discussion

Minimum inhibitory concentration and synergism between CO and PO

The effect of cinnamon and paprika oleoresins combinations (1:1 and 2:1) was assessed to investigate the possible synergism between them. The minimum inhibitory concentration (MIC) results by the microplate dilution method are presented in Table 2.

Free cinnamon oleoresin presented higher efficiency against *Penicillium paneum* than paprika, with a MIC value of 1.16 mg/mL. No difference between cinnamon and paprika oleoresin against *Aspergillus Niger* was observed, being both minimum concentrations of 2.76 mg/mL. Regarding the individual effect of oleoresins, *P. paneum* was more sensitive than *A. niger*, with lower MIC values. The difference in sensitivity between fungi may be related to the sterol content of the cell membrane (Debonne et al., 2020). The absence or low amount of sterols in the lipid bilayer of the cell membrane can cause osmotic and metabolic instability (Nazzaro et al., 2017).

Polyphenols, aldehydes, and isothiocyanates have, for example, different ways of acting against bacterial cells (Vasconcelos et al., 2018; Clement et al., 2016; Dufour et al., 2015). Cinnamaldehyde acts by disturbing the microbial cell membrane and its lipid profile (Shreaz et al., 2016). Molecules that have phenolic groups are able to cross the phospholipid bilayer of the bacterial cell. The hydrophobic characteristic of volatile compounds present in essential oils and oleoresins allows them to infiltrate between the lipids constituting the bacterial cell membrane and mitochondria, disturbing the structure and making them more permeable (Calo et al., 2015). Once the cytoplasm is accessed, these compounds bind to proteins harming its metabolisms (Vasconcelos et al., 2018).

The synergistic effect between the two oleoresins was observed against both evaluated microorganisms. In the case of *A. Niger*, the MIC was 1.10 mg/mL in either 1:1 or 2:1 ratio of cinnamon: paprika. For *P. paneum*, the oleoresin mixture (2:1) was slightly more effective, with a MIC of 0.83 mg/mL. Clemente, Aznar & Nerín (2016) found a synergistic effect of cinnamon and mustard essential oils against

Aspergillus ochraceus, *Rhizopus stolonifer*, and *Fusarium oxysporum.* The confirmation of synergism is interesting because it indicates that the combination of oleoresin components makes lower concentrations of the extracts necessary to enhance their properties.

Table 2
Minimum inhibitory concentration (MIC) and antimicrobial activity by headspace of pure and mixed oleoresins.

Ratio (w/w)	MIC (mg/mL)	Findex	Effect		
Cinnamon: Paprika	P. paneum				
1:0	1.16	-	-		
1:1	1.10	0.72	Synergetic		
2:1	1.10	0.79	Synergetic		
0:1	2.20	-	-		
Cinnamon: Paprika	A. niger				
1:0	2.76	-	-		
1:1	0.91	0.33	Synergetic		
2:1	0.83	0.30	Synergetic		
0:1	2.76	-	-		
Ratio (w/w)	**Mixture final concentration (mg oleoresin/mL)**	**Mixture final concentration (mg oleoresin/cm³ of air)**		**Growth result**	
Cinnamon: Paprika				P.paneum	A. Níger
1:0	100.98	4.04		-	-
	50.15	2.01		-	-
	25.53	1.02		-	-
1:1	102.17	4.09		-	-
	50.54	2.02		-	-
	25.36	1.01		-	+
2:1	100.93	4.04		-	-
	50.21	2.01		-	-
	25.18	1.01		-	+
0:1	101.14	4.05		+	+
+: growth; -: no growth.					

Ratio (w/w)	MIC (mg/mL)	Findex	Effect	
	50.65	2.03	+	+
	26.02	1.04	+	+
DMSO control			+	+
Blank control			+	+
+: growth; -: no growth.				

DMSO: dimethylsulfoxide.

Vapor inhibitory effect

The antimicrobial potential of oleoresins through headspace volatilization was evaluated and the results are present in Table 2. According to Manso et al. (2014), as the Agar absorbs the volatile compounds, a diffusion process begins, causing different zones of inhibition in the plate. The results presented in Table 2 show that paprika oleoresin could not inhibit microbial growth at any of the evaluated concentrations. This result was expected since the main antimicrobial compounds present in this oleoresin are carotenoids that cannot be volatilized. In contrast, cinnamon oleoresin, which is mostly composed of volatiles compounds, prevented the growth of *P. paneum* and *A.niger* through the headspace generated in the microplate. The cinnamon and paprika mixtures were also effective in both 1:1 and 2:1 ratios, with the minimum required 50 mg/mL concentration. Similar results were reported by Reyes-Jurado et al. (2019) who found a synergistic effect of the vapor phase of oregano and thyme essential oils mixed in 1:1 or 1:2 proportions against *Penicillium expansum*. Different combinations of thyme, cinnamon, rosemary, and marjoram essential oils were used in the study by Nikkhah et al. (2017) to evaluate the synergistic effect against pathogenic fungi (*Botrytis cinerea* and *P. expansum*) responsible for the deterioration of some fruits. The triple combination of cinnamon, marjoram, and thyme oils had a better synergistic effect reducing from six to eight times the minimum inhibitory concentration of the extracts used alone.

Physical parameters: color and size distribution

The size distribution of the solid lipid microparticles obtained by spray chilling is shown in Table 3. The diameters values were between 2.84 and 1670.05 μm showing a significant difference between each powder's characteristics. The formulation containing 3 g of cinnamon oleoresin /100 g of lipid matrix (M2) and the control (M4) showed the smallest diameters. Since the carrier lipid matrix was the same for all formulations, the size difference between the microparticles was influenced by each oleoresin's characteristics. In addition, the cooling rate directly influences lipid crystallization and the characteristics of the solid matrix.

Sample (M3) containing the paprika oleoresin presented the highest diameters values ranging from 741.98 μm ($D_{0.1}$) to 1670.71 μm ($D_{0.9}$), with a polydispersity index (Span) of 0.82. Despite the low Span

value, the higher diameter values found for this formulation indicate the formation of large agglomerates that are outside from the micro scale. Paprika oleoresin is more viscous than cinnamon and may have negatively influenced the formation of the crystalline lipid matrix. The incomplete lipid matrix crystallization can cause a greater number of agglomerates directly affecting the flow properties and the correct reading of the particle diameter (Santos et al., 2019).

Table 3. Microparticles size distribution and color parameters.

Sample	*Size distribution*			
	M1	M2	M3	M4
D $_{0.1}$ (µm)	3.46±0.19^{b}	2.84±1.58^{b}	741.98±159.44^{a}	4.13±0.10^{b}
D $_{0.5}$ (µm)	44.15±14.30^{b}	13.59±10.56^{b}	1152.67±136.91^{a}	15.12±0.41^{b}
D $_{0.9}$ (µm)	76.28±8.97^{b}	26.88±16.07^{b}	1670.05±71.84^{a}	37.16±1.15^{b}
Span	1.79±0.67^{ab}	2.23±0.88^{a}	0.82±0.16^{b}	2.18±0.06^{a}
	Color parameters			
*a**	26.37±0.03^{b}	0.52±0.03^{c}	35.04±0.03^{a}	0.15±0.06^{d}
*b**	38.76±0.02^{b}	7.17±0.02^{c}	40.05±0.04^{a}	6.23±0.28^{d}
*L**	65.73±0.01^{c}	86.95±0.01^{b}	59.65±0.03^{d}	91.05±0.69^{a}
ΔE*	48,85±0,48	4.21±0.71	57.86±0.49	-

Different lowercase letters in each line represent a statistically significant difference ($p \leq 0,05$).

M1: (80:20) vegetable fat: palm fat+ cinnamon and paprika oleoresin (3%) (2:1);

M2: (80:20) vegetable fat: palm fat+ cinnamon oleoresin (3%);

M3: (80:20) vegetable fat: palm fat+ paprika oleoresin (3%);

M4: (80:20) vegetable fat: palm fat.

The chamber's size and the cooling rate of the spray can influence the crystallization of the lipid matrix. Crystal growth occurs when the lipid matrix is subjected to cooling, and stable nuclei are formed in the liquid part (McClements & Decker, 2010). After that, the triacylglycerol molecules clump together, forming

a three-dimensional network. The heterogeneous lipid composition of palm oil makes this process much slower when compared to other natural fats (Oliveira et al., 2015). The rate and the space covered in the spray chilling chamber may not have been sufficient for the complete crystallization and favored agglomerates' formation.

Silva et al. (2019) also found a varied size distribution for particles produced with vegetable fat (54°C melting point) and loaded with guarana seed extract. The average diameter values in this work were between 1 and 82 μm.

The use of cinnamon oleoresin in the food industry is related to its aroma and flavor. For paprika oleoresin, the interest is most on its color. Different concentrations of the extract can give different shades to the powder, as shown in Table 3. Table 3 also presents the values of the color parameters (a^*, b^*, L^*) found for all formulations, as well as the color difference (ΔE^*) found between particles containing oleoresins and the control (only lipid carriers).

The formulations influenced the color parameters a^*, b^*, and L^*. Positive values of a^* and b^* are related to the red and yellow tonality, respectively. As shown in Table 3, samples M1 and M3 are in this color range, showing less intense M1 values. Díaz et al. (2019) produced microparticles containing paprika oleoresin, canola oil, and maltodextrin by spray drying. They founded values for a^*, b^*, and L^* of 29.30, 59.71, and 66.13, respectively. These values are close to those found in our work for the M3 formulation containing only paprika oleoresin.

According to Obón, Castellar, Alacid, and López (2009), the human eye is only able to distinguish the color difference when $1.5 < \Delta E^* < 5.0$ and become evident when $\Delta E^* > 5.0$. As the oleoresins are colored, i.e., having a pigment role, an important color difference was expected for powders containing it in relation to the control powder (empty particles). Analyzing the obtained results, it is possible to observe that the formulation M2 containing only cinnamon oleoresin was the one that showed the slightest collor difference from the control but was still perceptible to the human eye, with ΔE^* equal to 4.21. All the others showed an evident difference, with ΔE^* above 40.

Crystalline structure

Figure 1 presents the diffractograms obtained in the polymorphic characterization of commercial vegetable fat, palm oil, and solid lipid microparticles. All formulations presented *short spacings* of 4.2 and 3.8 Å, characteristic of the polymorphic form β'. High levels of palmitic acid and diversified fatty acid profile can contribute to the formation of β' crystals (Oliveira et al., 2015).

All the formulations presented the same polymorphic characteristics as the carrier materials. Crystals' shape and organization are influenced by several factors, but mainly by the cooling rate to which lipids are subjected from their liquid state (McClements & Decker, 2010). These results indicate that there were no influence of the atomization process and oleoresin content on the microparticles polymorphism.

Similar results were found by Carvalho et al. (2019) for fully hydrogenated palm oil (FHPO) and palm oil (PO) microparticles loaded with ascorbic acid. Different proportions (100/0; 90/10; 80/20; 70/30 and 60/40) of FHPO and PO, respectively, were analyzed and *short spacings* characteristic of β' form were observed. In their study, the microencapsulation process also did not change the polymorphism of the lipid material.

Morphology

The morphology of the microparticles was evaluated by the scanning electron microscopy technique (Fig. 2). All lipid microparticles were spherical and without pores. Particles obtained by spray chilling are generally matrix type, where the active compound is completely dispersed in the matrix (Alvim et al., 2016). This type of geometry gives the microparticles good flow properties.

Through the images, it is possible to notice a significant level of aggregation between the microparticles. The agglomerate formation can be positive as it improves the powder's flow properties since larger particles flow better than fine particles (Bhandari Bansal et al. 2013). However, excessive agglomeration can lead to so-called caking, a defect in powder products where huge lumps are formed, resulting in loss of quality. Moreover, these clusters may negatively affect size determination, thus masking the actual reading of the samples.

The low melting point of lipids carriers explains the high index of particle agglomeration presented in the photomicrographs (Fig. 2). Higher-melting point lipids crystallize more quickly. After the spray chilling atomization process, there may be liquid lipid droplets in the particles' interstices. These droplets can settle next to crystals already formed, causing a disturbance in the crystalline network and a greater tendency to agglomerate (Santos et al., 2019).

Pelissari et al. (2016) produced solid lipid microparticles using spray chilling technique and a mixture of hydrogenated vegetable oils, lycopene, gum Arabic, and carboxymethylcellulose. The morphology found by these authors was similar to the observed in the present study, particles with spherical shape, agglomerations, variable diameters, and rough surface.

Total Entrapment Efficiency -cinnamaldehyde and carotenoids retention

The influence of the storage temperature (5 and 25°C) in the cinnamaldehyde and carotenoids entrapment was evaluated and the results are present in Fig. 3. The day zero was considered as the total retention followed by the loss of cinnamaldehyde from then on. The cinnamaldehyde content (Fig. 3a) of the particles stored at 5°C decayed faster, which may be related to delayed crystallization of liquid lipid fractions in the matrix.

Storage temperature may influence a reordering of the crystalline lipid matrix, which may lead to greater expulsion of the active compound. Oriani et al. (2018) evaluated the release of volatile compounds from

solid lipid microparticles containing ginger oleoresin stored at 25 and 40°C, during 84 days. Samples stored at room temperature and containing oleic acid in the lipid matrix guaranteed greater stability to the compounds. The temperature of 40 ºC accelerated the release of volatiles after the 28th day resulting in the lowest retention values.

In addition to promoting greater stability, the encapsulation of bioactive compounds aims to improve their bioavailability. This property is related to the number of bioactive compounds that reach the bloodstream. According to Faridi et al. (2018), lipids carriers for hydrophobic bioactive compounds can favor their small intestine absorption. This happens due to micelles' greater presence that can solubilize and transport these hydrophobic compounds, such as cinnamaldehyde and carotenoids. Despite this, changes on the cell membrane permeability may raise concerns regarding the toxicity of microparticles and their compounds, but the absence of solvent or any other additive in the spray-chilling encapsulation process can be an advantage. According to McClements (2021), emulsified systems may have greater toxicity than bulk oils as they contain ingredients, such as surfactants and alcohols, which are able to disrupt the cell membrane. Thus, solid lipid microparticles produced with vegetable fat and loaded with cinnamon and paprika oleoresins can be interesting and less toxic additives carrying compounds with bioactive properties. An investigation into the release and bioavailability of microparticles in simulated digestive fluid may be interesting for future studies.

Conclusion

According to results presented in this work, cinnamon and paprika oleoresins have a synergistic effect against *Penicillium paneum* and *Aspergillus niger*. Moreover, volatile compounds present in these extracts could generate a headspace and inhibit these microorganisms' growth, without direct contact. Oleoresins spray chilling coencapsulation's generated particles with spherical surface, β' polymorphism, and a large number of agglomerates due to the low melting point of the lipid matrix. The storage temperature influenced the release of cinnamaldehyde, probably due to the changes caused in lipid crystals' organization when stored at refrigeration temperature. The solid lipid microparticles were able to protect the carotenoids from degradation over the 49 days of storage.

Declarations

Acknowledgments

The authors gratefully acknowledge "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)" for the PhD assistantships (Fernanda Ramalho Procopio #141111/2018-6; Mariana Costa Ferraz #140054/2019-7; Leonardo do Prado-Silva #140092/2017-0), the productivity grants (Miriam Dupas Hubinger #306461/2017-0; Paulo J. A. Sobral #30.0799/2013-6; Anderson S. Sant'Ana #302763/2014-7, #305804/2017-0) and the financial support (Miriam Dupas Hubinger #428644/2018-0). Fernanda Ramalho Procopio thanks Cargill for the donation of the lipid carriers. Miriam Dupas Hubinger thanks

FAPESP for the thematic project FAPESP 2019/27354-3. The authors acknowledge the support from “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES-Brazil; Finance code 001).

Conflict of Interest

The authors declare no competing interests.

Author Contributions Statement

Fernanda Ramalho Procopio: Conceptualization, Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. Mariana Costa Ferraz: Formal analysis, Validation, Writing – review & editing. Leonardo do Prado-Silva: Formal analysis, Methodology, Validation. Bruno Nicolau Paulino: Methodology, Validation. Glaucia Maria Pastore: Resources, Supervision. Anderson S. Sant'Ana: Resources, Supervision. Paulo José do Amaral Sobral: Supervision, Writing – review & editing. Miriam Dupas Hubinger: Supervision, Writing – review & editing, Project administration, Funding acquisition.

Funding

This work was funded by **“**Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” with the PhD assistantships (Fernanda Ramalho Procopio #141111/2018-6; Mariana Costa Ferraz #140054/2019-7; Leonardo do Prado-Silva #140092/2017-0), the productivity grants (Miriam Dupas Hubinger #306461/2017-0; Paulo J. A. Sobral #30.0799/2013-6; Anderson S. Sant’Ana #302763/2014-7, #305804/2017-0) and the financial support (Miriam Dupas Hubinger #428644/2018-0). Partial financial support was received from FAPESP through the thematic project FAPESP 2019/27354-3.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

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Figures

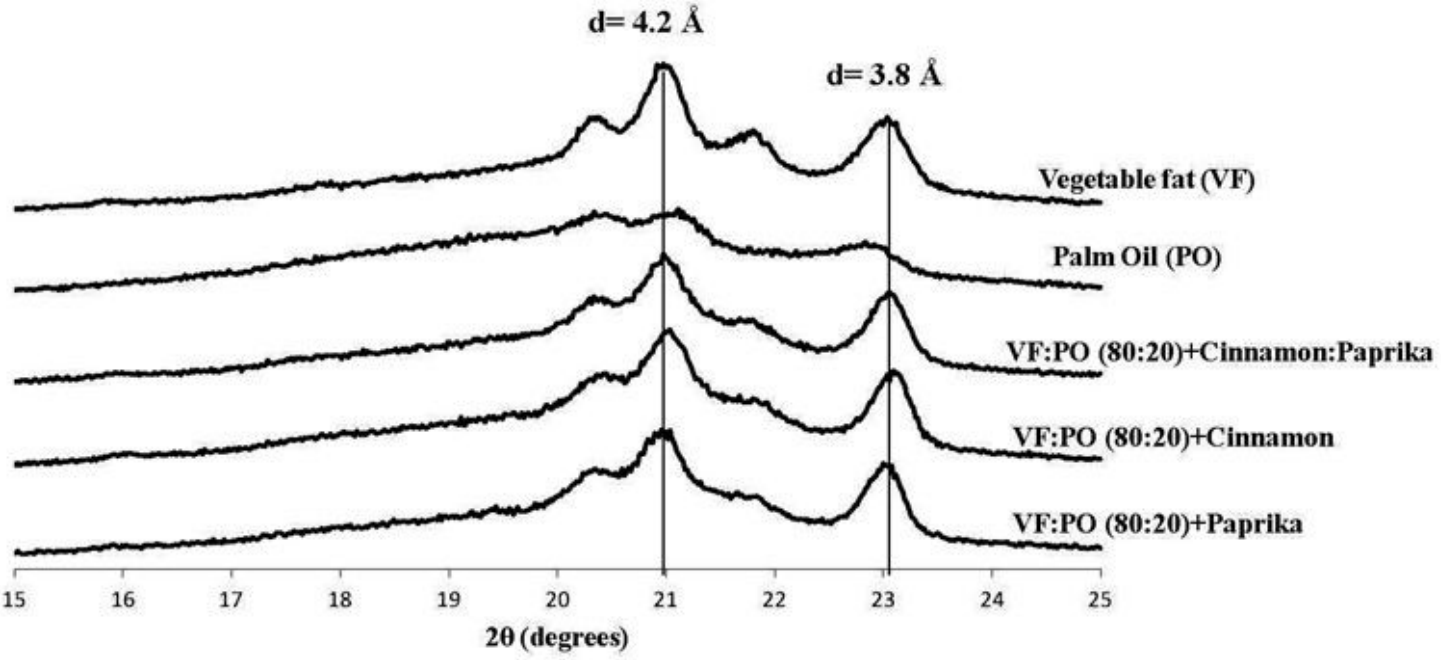

Figure 1

X-ray diffraction of solid lipid microparticles and lipid materials.

Figure 2

Scanning electron microscopy of solid lipid microparticles. M1: (80:20) vegetable fat: palm fat+ (2:1) cinnamon:paprika oleoresins (3 g/ 100 g of lipid matrix); M2: (80:20) vegetable fat: palm fat+ cinnamon oleoresin (3 g/ 100 g of lipid matrix); M3: (80:20) vegetable fat: palm fat+ paprika oleoresin (3 g/ 100 g of lipid matrix); M4: (80:20) vegetable fat: palm fat.

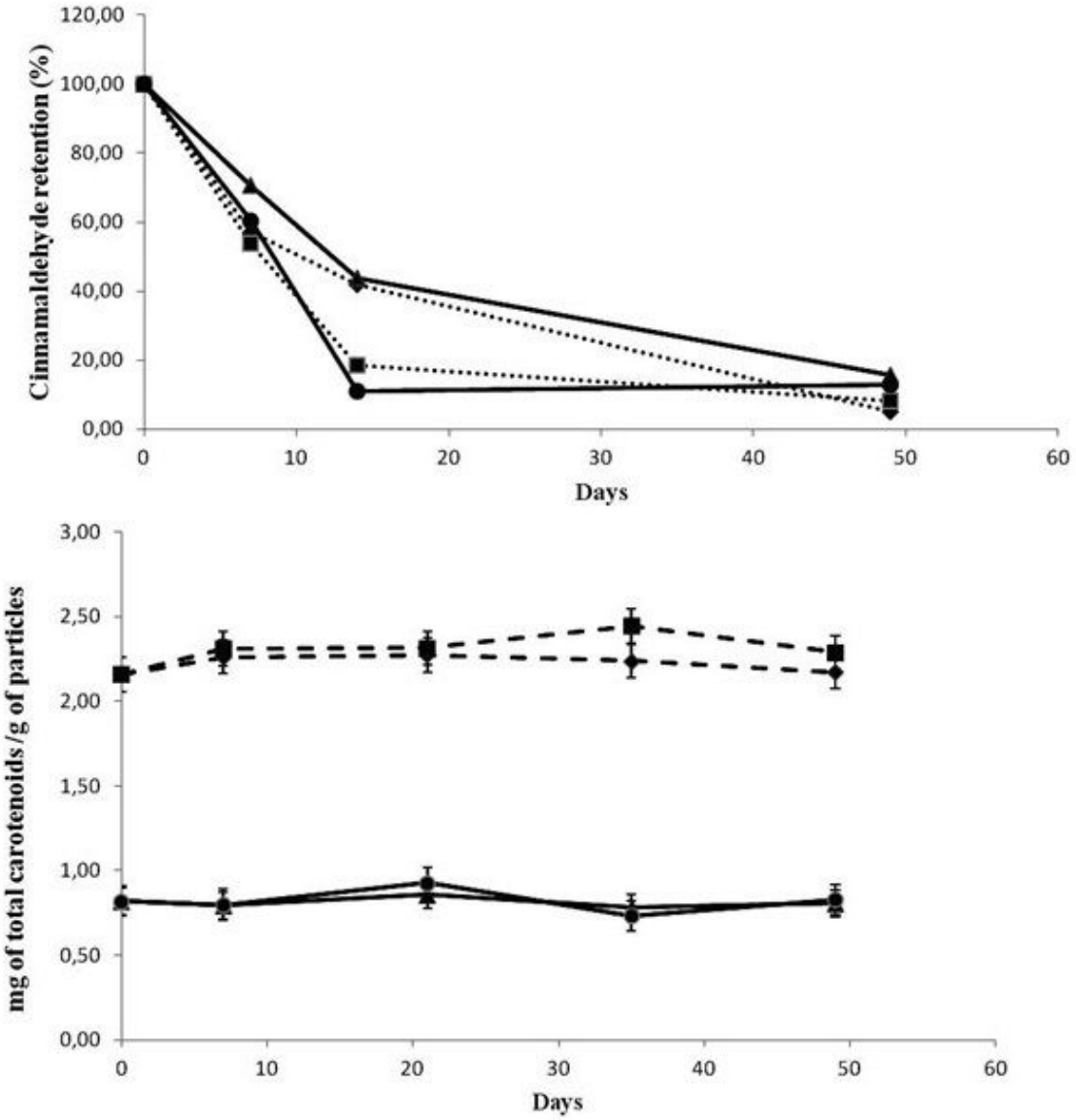

Fig. 3. Cinnamaldehyde decay curves (a). M2-25 °C (--◆--); M2-5°C (--■--); M1-25°C (—▲—); M1-5°C (—●—). Carotenoids retention (b). M3-5 °C (--◆--); M3-25°C (--■--); M1-5°C (—▲—); M1-25°C (—●—). M1: (80:20) vegetable fat: palm fat+ (2:1) cinnamon:paprika oleoresins (3 g/ 100 g of lipid matrix); M2: (80:20) vegetable fat: palm fat+ cinnamon oleoresin (3 g/ 100 g of lipid matrix); M3: (80:20) vegetable fat: palm fat+ paprika oleoresin (3 g/ 100 g of lipid matrix); M4: (80:20) vegetable fat: palm fat.

Figure 3

See image above for figure legend