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EFFECTS OF SURFACTANT TYPE ON RETENTION OF BIOACTIVE COMPOUNDS OF *SYZYGIUM AROMATICUM* IN SPRAY DRIED LIPID BASED SYSTEMS

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ABSTRACT: This work investigates the effects of the surfactant system used and of spray drying temperature in physicochemical properties and retention of bioactive compounds of lipid based systems loaded with bioactive compounds of *Syzygium aromaticum*. Seven formulations were prepared, containing Compritol 888 ato and buriti oil as lipids and maltodextrin DE10 as wall material. The surfactants Poloxamer 188, Gelucire 50/13 and Gelucire 50/2 were employed and classified by their hydrophilic-lipophilic balance (HLB). The emulsions were homogenized by means of an ultrasonic probe and spray dried. The eugenol and eugenyl acetate content of each formulation was quantified by HPLC before and after the spray drying process. Higher eugenol retention was obtained for the composition using the amphiphilic surfactant Gelucire 50/13® (HLB of 13). Retention of bioactive marker compounds was affected by spray drying temperatures. A higher spray drying temperature (140 °C) increased eugenol loss. The particle morphology was affected by the formulation composition and the drying temperature.

INTRODUCTION: Aromatic plants are important sources of bioactive compounds beneficial to human health. Clove (*Syzygium aromaticum*) is a spice used as a medicinal plant due to its proven pharmacological properties including pain relief and antitumor, antimicrobial and antioxidant activities [1]. These biological effects have been attributed to the major compounds present, such as eugenol, gallic acid, and β-caryophyllene. To enhance the potential benefits of this plant in human health and in other applications, limitations associated with the low oral absorption, stability, and aqueous solubility of the bioactive compounds need to be overcome.

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Most of the phenolic compounds derived from vegetal sources present low bioavailability [2]; and the development of emulsions and other lipid formulations offers a worthwhile delivery strategy. Encapsulation in lipid carriers has attracted attention in the food and pharmaceutical sectors, due to advantages over conventional encapsulation systems that include low toxicity, low production costs, and the ability to encapsulate hydrophobic and hydrophilic substances.

Emulsions and other lipid dispersion systems have proven to be effective for the encapsulation and delivery of compounds that present low solubility in water. Lipid dispersion systems are commonly produced by dispersion of a non-water soluble (lipophilic) compound in a melted lipid or a blend of solid and liquid lipids, followed by mixing with an aqueous phase containing surfactants using different homogenization methods [3]. However, lipid systems in liquid form are more prone to physical, chemical, and microbiological in stability,

compared to dehydrated formulations. Spray drying has been successfully employed to produce dried powders from lipid-based formulations [4]. This technique increases stability and therefore extends the product shelf life. In addition, the powdered form can be easily re-dispersed in contact with aqueous solutions, when needed [5, 6]. The main factors that influence the retention of volatile compounds during spray drying are the operating conditions (such as the inlet and outlet drying temperatures), the composition of the feed and the solids concentration [7].

In general, is assumed that high spray drying temperatures favor the rapid formation of a superficial layer on the atomized droplet, which increases the retention of volatile substances and, therefore, the encapsulation efficiency. The heat transfer is also favored, accelerating the drying process [8]. On the other hand, depending on the composition, higher drying temperatures can cause degradation of thermo sensitive compounds [9]. The effect of spray drying temperature has been shown to be highly dependent on feed composition, and it is for this reason that studies are required concerning the effects of composition parameters on drying performance and product quality.

Surfactants are important in hetero-dispersed systems because they reduce the surface tension between the lipid and aqueous phases, hence influencing the stability of the feed composition. Surfactants can be classified by the hydrophilic-lipophilic balance (HLB), a measure of its degree of lipophilicity or hydrophilicity, which is dependent of the structure of the surfactant molecule. A surfactant with an HLB value higher than 11 is considered hydrophilic and form soil-in-water emulsions; whereas surfactants with HLB lower than 9 are considered lipophilic and form water-in-oil emulsions.

The HLB concept enables elucidation of the surfactant or mixture of surfactants required for the stabilization of an emulsion or a hetero-dispersed lipid system; although others factor may also impact emulsion stability. Non-ionic surfactants including poloxamers and Gelucires are commonly employed for stabilization of lipid-based formulations. Poloxamers are block copolymers of ethylene oxide and propylene oxide, while Gelucires are derived from vegetable oils and consist of mixtures of mono-, di-, and triglycerides with polyethylene glycol, resulting in stearoyl polyoxylglycerides [10]. The mechanisms proposed to explain the increased drug release and therapeutic effects of Gelucires include the formation of hydrogen bonds with the active substance [11], micelle solubilization, and decreased activity coefficient of the drug due to reduced hydrophobic interactions [12]. Gelucires and poloxamers can increase the oral bioavailability of drugs by mechanisms such as the inhibition of efflux pumps or the opening of tight junctions [11]. The physical aspects, melting points, and HLB values of the compounds depend on the characteristics of the fatty acids present and the degree of esterification.

The aim of this work was to evaluate the influence of the surfactant system spray and drying temperature on eugenol retention during encapsulation of bioactive compounds from *Syzygium aromaticum* in lipid-based micro-particles. The lipid phase of the formulations was a blend of solid and liquid lipids. The solid lipid employed was Compritol® 888 ATO, which is composed of a mixture of mono-, di-, and triglycerides of behenic acid, with a melting point in the range 65-77 °C [13]. The liquid lipid used was Buriti oil, extracted from the palm tree *Mauritia flexuosa*, which is native to the Amazon region in Brazil. This oil contains a high concentration of carotenoids (1706 ± 54µg of total carotenoids/g) [14].

MATERIALS AND METHODS:

Materials: Dried clove buds were acquired in the region of Valença (Bahia State, Brazil), in collaboration with the CEPLAC agronomic institute. Buriti oil was acquired from Amazon Oil (Ananindeua, Pará State, Brazil). Compritol® 888 ATO was acquired from Gattefossé (France), Poloxamer 188 (Kolliphor P) was a gift from BASF (Brazil), and Gelucire 50/2 and 50/13were kindly donated by Gattefossé (France). Maltodextrin DE-10 (MOR-REX 1910®) was donated by Corn Products (Brazil). Eugenol and eugenyl acetate, with purity of 99.0% and 98.0%, respectively, were obtained from Sigma-Aldrich.

Clove Extract: The clove extract was prepared from milled dried clove buds by dynamic maceration, using optimized extraction conditions

[15]. Ethanol (70% v/v) was employed as the extraction solvent, using a plant: solvent ratio of 1:10 (w/v). The extraction temperature and time were 50 °C and 30 min, respectively. The extract was vacuum filtered through filter paper (grade 14µm) and then concentrated by rotary evaporation at 55 °C and 80 Pa to reach a solids content of 7.75 ± 0.46%, determined using a moisture analyzer (Model MA35,Sartorius, Göttingen, Germany).

Preparation of Lipid Formulations: Seven lipid formulations were prepared (**Table 1**), which were classified by the HLB value of the surfactant system used; employing three different surfactants: Poloxamer 188 (HLB 29), Gelucire 50/2 (HLB 2), and Gelucire 50/13 (HLB 13). The proportions of the surfactants were calculated using Equations 1 and 2 [16].

$$HLB_{req} = (HLB_A)*(\%_A) + (HLB_B)*(\%_B) \quad \ldots\ldots (1)$$

$$100\% = \%_A + \%_B \quad \ldots\ldots (2)$$

Where HLB_{req} is the final HLB value, HLB_A and HLB_B are the HLB values of surfactants A and B, respectively, and $\%_A$ and $\%_B$ are the percentages of surfactants A and B, respectively.

The proportions of solid lipid (Compritol 888 ATO), liquid lipid (Buriti oil), and the drying carrier (maltodextrin DE10) were maintained constant in all the formulations (**Table 1**). MilliQ water was employed for preparation of the formulation and hydration of the drying carrier. Note that the total extractable content in the concentrated clove extract is 7.75%, being the remainder mainly water.

The lipids (solid and liquid) were weighed in a beaker and melted at 70 °C in a water bath. The lipophilic surfactant was added to the melted lipid phase. The clove extract was heated to the same temperature as the lipid phase, followed by dissolution of the hydrophilic surfactant. The two phases were mixed using an Ultra Turrax T18, (IKA, Wilmington, NC, USA) at 18000 rpm. The drying carrier (maltodextrin DE10) was pre-dissolved in Milli-Q water, using the same proportion for all the formulations (**Table 1**), and then was added over a period of 5 min during the pre-homogenization process. The resulting system was homogenized for 3 min with a 13mm ultrasonic probe operated at 20 kHz and 70% amplitude (Vibra-Cell, Sonics, New town, USA).

TABLE 1: COMPOSITION (%) OF LIPID FORMULATIONS PREPARED USING SURFACTANT SYSTEMS CLASSIFIED BY HLB VALUES

	HLB 0*	HLB 2	HLB 7.5	HLB 13	HLB 18	HLB 23	HLB 29
Clove extract+	53.8	53.3	53.3	53.4	53.4	53.4	53.4
Compritol®	6.9	6.9	6.9	6.9	6.9	6.9	6.9
Buriti oil	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Poloxamer 188	0.0	0.0	0.0	0.0	0.2	0.5	0.8
Gelucire 50/13	0.0	0.0	0.4	0.8	0.5	0.3	0.0
Gelucire 50/2	0.0	0.8	0.4	0.0	0.0	0.0	0.0
Maltodextrin DE10	15.4	15.3	15.3	15.2	15.3	15.3	15.3
Milli-Q Water	23.1	22.9	22.9	22.9	22.9	22.8	22.8

* HLB 0 refers to a formulation without surfactant.
+ 7.75% of which is the extractable content and the remainder mostly water.

Spray Drying: The spray dryer used was a Lab Plant Model SD05 (Lab-Plant UK Ltd., Hudders field, UK) with a drying chamber diameter of 215 mm and height of 500mm, operated in concurrent flow mode. The lipid-based formulations, initially at room temperature (25 °C), were spray dried at a feed rate of 4g/min (provided by a peristaltic pump). A two-fluid atomizer (1.0mm orifice diameter) connected to an air compressor was used. Two different inlet drying temperatures (Tge) were studied: 90 and 140 °C. The atomization pressure and air flow rate were set at 2 bar and 15 L/min, respectively. Average room conditions were relative humidity of 54.1 ± 3.9% at a temperature of 21.4 ± 0.7 °C. Outlet temperatures of 65.4 ± 0.4 °C and 100.6 ± 0.6 °C were reached at inlet temperatures of 90 °C and 140 °C, respectively.

Quantification of Eugenol and Eugenyl Acetate in the Lipid Formulations: The concentrations of eugenol and eugenyl acetate in the liquid and spray dried samples were determined by a validated high performance liquid chromatographic procedure. The analytical conditions were based on a method

previously reported [17], and the quantifications were done in achromatograph LC-20A Prominence (Shimadzu Corporation, Kyoto, Japan), equipped with an LC-6A double pump, a diode array detector (SPD-M20A), and a column oven (CTO-20A) set at 30 °C. A C-18 column was employed (Shimadzu Shim-Pack CLC (M), 4.6 mm x 25 cm, 5 µm, 100 Å), using an isocratic elution mode with methanol: water (60:40) mobile phase, with detection at 280 nm. The samples were prepared by precisely weighing 40mg portions of the powders (in duplicate) on an analytical balance (Model AG204, Mettler Toledo, Switzerland), followed by dissolution in 8mL of methanol (60% v/v) using a magnetic stirrer (Model RT15, IKA) for 15 min at 45 °C.

These mixtures were transferred to volumetric flasks, being the volumes completed to 10mL with methanol (60% v/v). The samples were centrifuged at 3500 rpm for 5 min, after which the supernatants were filtered through 0.45µm pore size membranes and 20µL volumes were injected into the chromatograph. Analytical curves for eugenol and eugenyl acetate were constructed by diluting the standards in methanol (60% v/v). The results were expressed in terms of percentage retention by comparing the concentrations (dry basis) of eugenol and eugenyl acetate in the clove extract before and after spray drying.

Water Activity: The water activities of the spray dried powders were determined (in triplicate) using an Aqua Lab 4Tev® water activity meter (Decagon Devices, Pullman, WA, USA) fitted with a capacitance electrode.

Loss on Drying: In order to determine the contents of moisture and total volatile matter, samples (approximately 2g) of the dried product were placed in the Sartorius MA35 moisture analyzer and heated to 105 °C until constant weight [18]. The analyses were performed in triplicate. Loss on drying is a measure commonly used in herbal medicinal processing, and encompasses the product moisture plus total volatile matter.

Particle Size: The particle size of the dried samples was determined by light scattering, using a Beckman Coulter LS 13320 (Beckman Coulter, Brea, CA, USA) equipped with a universal liquid module capable of analyzing particles in the size range from 0.017µm to 2000µm. The samples were prepared by dispersing approximately 40mg of powder in ethanol that had been filtered (0.45µm pore size membrane) to avoid interferences from the solvent.

Particle Morphology: The morphology of the particles was analyzed using an Inspect F-50 scanning electron microscope (FEI, The Netherlands) equipped with a field emission gun (SEM-FEG). Small quantities of powder were mounted on specimen stubs using double-sided adhesive carbon tape, then coated with gold and analyzed at 5 kV.

RESULTS AND DISCUSSION:

Retention of Eugenol and Eugenyl Acetate: The retention of eugenol and eugenyl acetate after spray drying is shown in **Fig. 1**. This parameter was determined by comparing the eugenol concentrations in the liquid and in the dried formulation. It was evident that spray drying at 140 °C caused higher losses of eugenol (73.2-80.6%) and eugenyl acetate (79.0-90.5%). The losses were significantly lower for drying at 90 °C, ranging from 44.0 to 60.5% (eugenol) and 39.5-55.1% (eugenyl acetate).

In some situations, higher drying temperatures can favor the retention of volatile compounds, due to the rapid formation of a superficial dry outer crust that acts as a semi-permeable membrane, permitting water evaporation but restricting the loss of volatile material. When water evaporates from the droplet surface, the thickness of the crust increases and its structure becomes denser [19]. This phenomenon is also strongly influenced by the composition of the drying formulation, especially the physical properties of the wall material, the solids concentration, and the oil to water ratio [9]. However, when the internal pressure in the particles is too high, the particles can explode, resulting in the loss of volatile compounds and decreased retention efficiency. For lipid based formulations, however, higher drying temperatures can cause softening of the lipids, increasing adhesion of particles to the drying chamber, which would increase the residence time of the product inside the dryer, contributing to increase the losses of bioactive marker compounds.

For these reasons, the drying temperature should be optimized for each drying composition. Depending on the physicochemical properties of the encapsulating formulation, better results may be obtained using lower spray drying temperatures [8, 9].

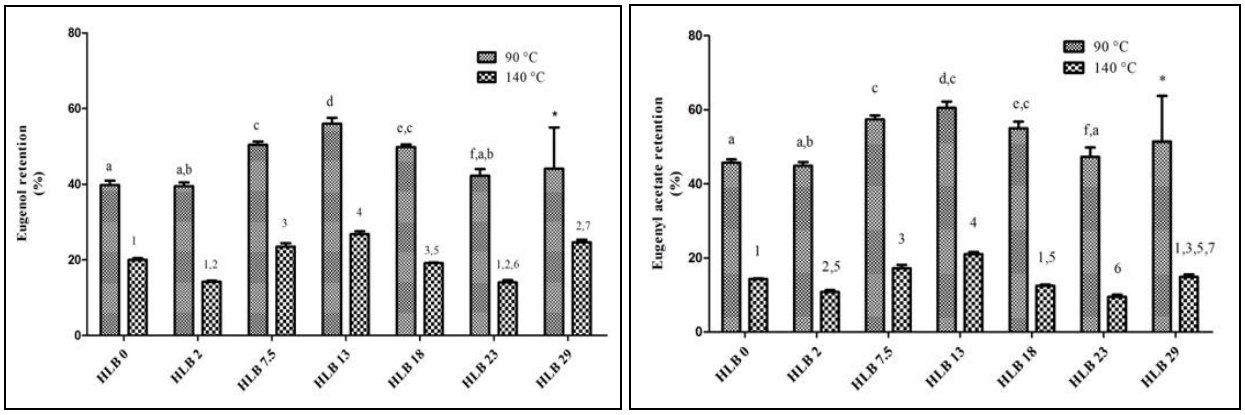

FIG. 1: EUGENOL AND EUGENYL ACETATE RETENTION AFTER SPRAY DRYING OF LIPID COMPOSITIONS OBTAINED USING SURFACTANT SYSTEMS CLASSIFIED PER THEIR HLB VALUES. SAME LETTER OR NUMBER IN THE COLUMN INDICATES NO STATISTICALLY SIGNIFICANT DIFFERENCE (TUKEY'S POST-TEST, P ≤ 0.05). * SAMPLE EXCLUDED FROM THE ANOVA TEST

Comparison of the retentions of eugenol and eugenyl acetate showed that the behaviors were similar for the two drying temperatures tested. The molecular structures of the compounds can influence their encapsulation efficiency, because larger molecules diffuse more slowly in the semi-permeable membrane formed during the initial drying stages [7]. However, in the present work the experimental data showed no strong evidence of this effect.

The results (**Fig. 1**) showed that the type of surfactant system (classified by the HLB value), influenced the retention of eugenol and eugenyl acetate. The best retentions of the compounds were found for the composition using Gelucire 50/13® as surfactant system (HLB of 13). Compositions prepared containing surfactant systems of higher (18, 23 and 29) or lower (0, 2 and 7.5) HLB values resulted in greater losses at both spray drying temperatures (90 and 140 °C). The surfactant plays a crucial role in the spray drying encapsulation of bioactive compounds in lipid-based compositions, because it influences the surface tension of the droplet and consequently the formation and characteristics of the outer crust.

The effects of surfactants in atomized droplets are linked to the hydrodynamics and the existence of oscillations, turbulence, and internal circulation in the droplets [20]. Greater retention of eugenol in the spray dried particles would be related to better bubble stability promoted by the surfactant system employed [21].

Loss on Drying and Water Activity: The results of loss on drying of the spray dried powders are shown in **Fig. 2**. The values obtained were high, compared to values reported elsewhere for spray dried powders, which are usually lower than 6% [8]. This could be explained by the high concentration of volatile compounds retained in the spray dried samples. Although formulation HLB 13 showed greater retention of eugenol and eugenyl acetate, the loss on drying was lower than for other formulations that presented lower retention. A possible explanation for this behavior was the achievement of higher encapsulation efficiency, with the particles retaining more efficiently the volatile compounds, compared to the other formulations, which was reflected in a low loss on drying (possible due to a lower product moisture content).

Fig. 1 also indicates greater losses of eugenol and eugenyl acetate when higher drying temperatures were used. This type of behavior could be extended to other volatile compounds in the sample, such as β-caryophyllene and α-humulene, among others [1]. Moreover, the amount of moisture evaporated increases with the drying temperature. Due to these factors, the loss on drying was significantly reduced at a spray drying temperature of 140 °C, compared to 90 °C.

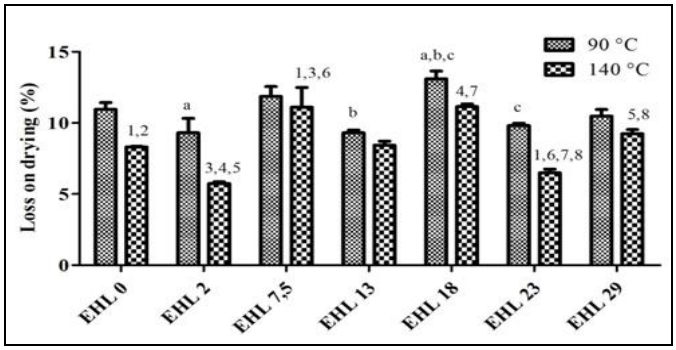

FIG. 2: LOSS ON DRYING OF SPRAY DRIED LIPID FORMULATIONS AS A FUNCTION OF THE SURFACTANT SYSTEMS, CLASSIFIED PER THEIR HLB VALUES. STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN SAMPLES (TUKEY'S POST-TEST, P ≤ 0.05) ARE INDICATED BY THE SAME LETTER OR NUMBER IN THE COLUMN

The water activity can be linked to the amount of water available for microbial growth and other degradation processes. Although factors such as storage temperature and pH can affect the growth of spoilage microorganisms and food-borne pathogens, the water activity is generally recognized as being most important [22]. **Table 2** presents the experimental values of water activity of the spray dried powders. In all cases, the results were lower than 0.5, which is generally considered a safe limit to inhibit microorganism growth [23].

TABLE 2: WATER ACTIVITY OF SPRAY DRIED LIPID FORMULATIONS AS A FUNCTION OF INLET AIR TEMPERATURE

Formulation	Inlet air temperature (Tge)	
	90 °C	140 °C
HLB 0	0.290 ± 0.007	0.382 ± 0.014
HLB 2	0.316 ± 0.008	0.319 ± 0.005
HLB 7.5	0.315 ± 0.006	0.483 ± 0.005
HLB 13	0.298 ± 0.008	0.312 ± 0.003
HLB 18	0.292 ± 0.002	0.438 ± 0.005
HLB 23	0.297 ± 0.005	0.371 ± 0.012
HLB 29	0.257 ± 0.005	0.373 ± 0.003

Particle Size: The particle sizes of the powders obtained from the lipid formulations prepared using distinct surfactant systems classified by the HLB values were measured by light scattering in the liquid mode. The results (**Fig. 3**) showed that the average particle sizes (expressed as d_{50}) obtained at 140 °C were higher than for particles obtained at a drying temperature of 90 °C. This could have been due to softening of the lipids at the higher drying temperature (140 °C), which increased the tendency of the particles to stick together and form agglomerates.

Comparison of the particle sizes of the different compositions revealed no significant differences or behavior patterns related to the surfactant systems, classified by the HLB values. The particle sizes of most of the samples were in the range from10 to 20µm, which are typical values for bench-top spray dryers [24-26].

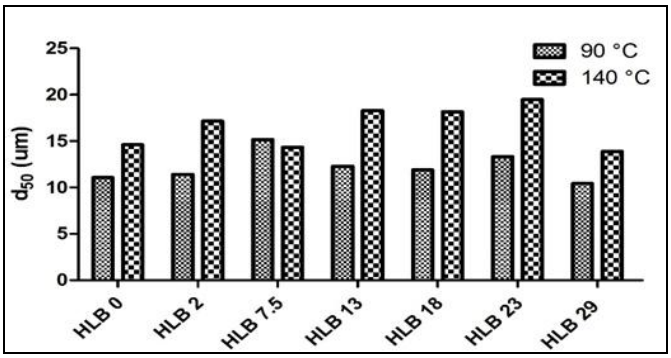

FIG. 3: MEAN PARTICLE SIZES OF POWDERED LIPIDS SPRAY DRIED AT 90 AND 140 °C, AS A FUNCTION OF THE SURFACTANT SYSTEMS, CLASSIFIED PER THEIR HLB VALUES

Particle Morphology: The particle morphology changed with the surfactant system used (**Fig. 4**). When no surfactant was used (HLB 0) and the drying temperature was set at 90 °C, spherical particles of non-uniform size were formed. The particles of the formulation prepared using Gelucire 50/2 (HLB 2) and dried at 90 °C were spherical with wrinkled surfaces (**Fig. 4b**).

The effect of temperature on particle shape can be seen for the HLB 13 formulation (**Fig. 4c** and **4d**). At the higher drying temperature (140 °C), the particles were rounded, with smooth surfaces, and the average wall thickness was 798.3nm (**Fig. 4e**). In comparison, the particles produced composition using the poloxamer (HLB 29) and an inlet spray drying temperature of 90 °C were rounded, with wrinkled surfaces.

The morphology of spray dried particles is affected by the drying kinetics during the particle formation process. In the initial drying stage, the outer layer of the atomized droplet starts to dry, forming a semi-permeable crust, after which bubbles nucleation occurs, with the bubbles rising and bursting outward through the surface during recurring inflation-deflation cycles [28]. Higher drying temperatures promote faster crust formation, which could explain the smoother surfaces observed for the particles obtained at 140 °C.

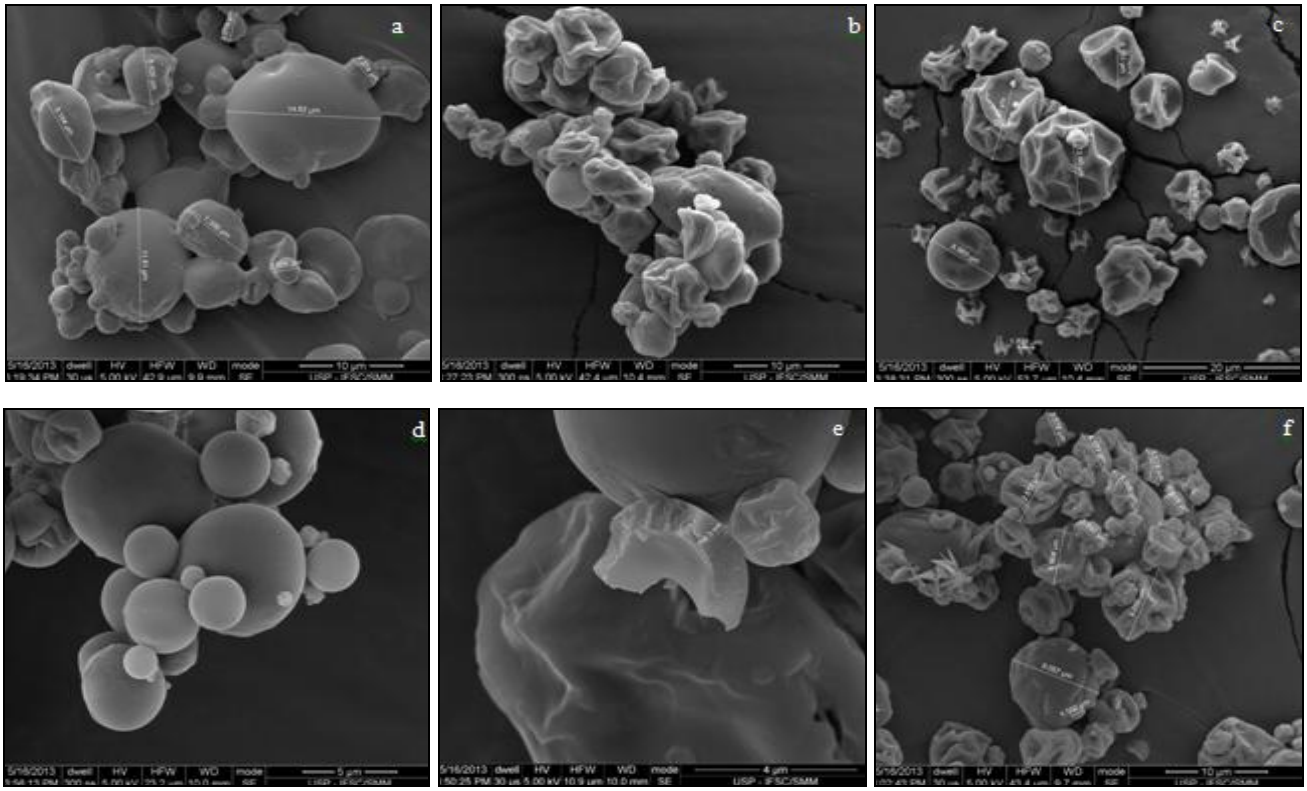

FIG. 4: SEM MICROGRAPHS OF THE SPRAY DRIED LIPID-BASED COMPOSITIONS PREPARED USING DIFFERENT PROCESSING CONDITIONS:(A) HLB 0 (90 °C); (B) HLB 2 (90 °C); (C) HLB 13 (90 °C); (D) HLB 13 (140 °C); (E) PARTICLE WALL FRAGMENT FROM FORMULATION HLB 13 (140 °C); (F) HLB 29 (90 °C)

CONCLUSION: The results obtained showed that the drying temperature and surfactant system (characterized by the HLB value) had significant effects on the retention of eugenol and eugenyl acetate during spray drying of lipid-based encapsulating formulations loaded with volatile compounds. A higher drying temperature increased losses of the compounds by between 25% and 30%. Maximum retention of the of marker compounds was achieved for the composition using Gelucire 50/13® as surfactant system (HLB of 13). The particle size was greater when particles were spray dried at 140 °C, probably due to micro-particle agglomeration caused by lipid softening. The spray drying temperature and surfactant system used had also significant effects on particle morphology.

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