An investigation into the stability of an oregano essential oil emulsion for the preparation of microparticles using spray drying.

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ABSTRACT

The objective of this study was to evaluate the viscosity, zeta potential and soluble and total solids of the emulsion and microparticles of oregano essential oil (*Origanum vulgare*) using gum arabic as a coating agent and to analyze the morphology of the microparticles produced by the spray drying method. The rheological analysis showed a viscosity for emulsion of 36.23 ± 0.59 mPas and for microparticles of 20.13 ± 0.15 mPa.s. The zeta potential of the emulsion was - 6.67 ± 0.32 mV and of the oregano oil microparticles -16.47 ± 0.70 mV. Soluble solids showed Brix° of 8.44 ± 0.13 and 0.98 ± 0.36 and total solids 8.95 ± 0.42 and 1.03 ± 0.02 % for emulsion and microparticles, respectively. The morphology of the microparticles presented irregular spherical shapes and agglomeration.

KEY WORDS: Emulsion, microencapsulation, spray drying, *Origanum vulgare,* stability, morphology.

INTRODUCTION

Essential oils extracted from plants such as spices are composed of a complex mixture of volatile compounds such as hydrocarbons, aldehydes, ketones, simple alcohols, phenols, terpenes, esters and organic acids in a wide range of concentrations (1). The essential oil of oregano (*Origanum vulgare L.*) is rich in the nutraceutical compounds thymol and carvacrol making it stand out when compared with other extracted essential oils from other biomasses (2). These beneficial compounds are responsible for the characteristic taste and odor, as well as, established antimicrobial (3, 4) and antioxidant activity (5).

However, the industrial application of essential oils in general and, in particular for oregano, is limited due to its intrinsic physicochemical characteristics, such as high volatility (6) and low solubility in water (7). Microencapsulation of oregano oil could be a useful method to improve the retention of its compounds, to enhance its solubility in water and to enable a controlled release formulation (8, 9, 10) in order to maximize its biological action.

Microencapsulation consists of coating a solid, liquid or gaseous active ingredient by adding coating agents

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to form small particles categorized as macro (> 5000 μm), micro (0.2 to 5000 μm), and nano (< 5000μm) (8). Microencapsulation using coating agents protect the active ingredient promoting its release at a controlled rate under specific conditions (10).

Several physical, chemical and physicochemical methods of microencapsulation are available for the retention of hydrophobic compounds. Among these methods the most suitable for use in the food industry, particularly if the bioactive compounds to be encapsulated are liquids, are spray-chilling, spray-cooling or spray drying (11).

In the food industry spray drying is one of the most used methods for the microencapsulation of essential oils (12, 13, 14) since it provides a rapid evaporation of water and maintains a low temperature within the particles, providing a greater retention of color, flavor and nutrients. The process consists of pumping the fluid material into the drying chamber through an atomization system, removing the moisture from the material, breaking it into small droplets and obtaining a dry powder, whose quality depends on the characteristics of the feed fluid (i.e., viscosity and flow rate), drying air (i.e., temperature, pressure, air flow), the contact between hot air and droplets in the drying chamber (either concurrent or countercurrent), and the type of equipment used (15).

The application of microencapsulation in the food industry is highly desirable for improving the stability of compounds, protecting against adverse environmental factors such as light, heat, moisture and oxygen (16, 17, 18), which increases the useful shelf life of such compounds in addition to creating differentiated and attractive products to the consumer. Currently the process is already widely used at industrial scale in the form of the encapsulation of dyes, sweeteners, microorganisms and essential oils (10). In the preparation of emulsions to be microencapsulated the gum Arabic is widely used, and its application in the food industry occurs due to the advantages conferred by the emulsion, such as viscosity and stability (19, 20).

In this context, the objective of this work was to evaluate the stability, viscosity and morphology of the emulsion and microparticles of oregano essential oil and gum arabic produced by spray drying.

MATERIAL AND METHODS

Materials

The essential oil of oregano (*Origanum vulgare L.*) was obtained from Laszlo Aromatherapy Ltda. (Belo Horizonte, Brazil) and was used as the active ingredient for the spray drying, and gum arabic in powder format was added as a coating agent (Contemporary Chemistry Dynamics LTDA).

Production of microparticles from the oregano essential oil

Initially gum arabic was hydrated in deionized water for approximately 12 hours in a refrigerator (10 to 12°C). Then the curated coating agent was dissolved in 900 mL deionized water at 60-70°C using a Turratec homogenizer (Tecnal, TE 102, Brazil) at a rotation speed of 20000 RPM for 30 minutes.

The proportion of the coating agent and the process parameters used in the production of the oregano essential oil microparticles were optimized to obtain the desirable characteristics of the microparticles that would retain as much of the essential oil as possible and exhibit low water activity (21).

The coating agent was used at a concentration of 100% gum arabic. After a complete dissolution of the coating agent at a temperature close to 10°C, 10 mL of the oregano essential oil was added until a completely homogeneous emulsion was obtained at a rotation of 20000 RPM for 5 minutes, forming the oregano essential oil emulsion.

The emulsion formed was spray dried using a LABMAQ Brazil, model MSD 0.5 (Ribeirão Preto, São Paulo, Brazil), fitted with a dual-fluid nozzle spraying system and an opening of 1.0 mm. The operating conditions of the equipment were: air inlet temperature at 120 ± 3°C, drying air flow rate was maintained at 2.0 m^3/min, flow rate ± 30 L/min, feed flow rate was adjusted to 0.50 Lh^{-1} and the compressed air pressure to the spray flow was 2-4 bar.

After processing, the powder obtained was stored under refrigeration (4 to 7ºC) in glass flasks protected from light, water vapor and gas permeation, to minimize possible changes in the material, such as agglomeration caused by the absorption of water, and oxidation. The analyzes were performed later.

Properties and stability of emulsion and microparticles of oregano essential oil

The stability and viscosity of the emulsion and oregano essential oil microparticles were evaluated, respectively, through the zeta potential and rotational rheometer. Soluble solids, total solids and morphology of the emulsion and microparticles were also analyzed.

Rheology

The rheological behavior of the samples was determined using a rotational rheometer (LamyRheology, model RM 200, France), with a concentric tube 1 and spindle 2 (DIN system). The samples were analyzed at a temperature of ± 19.5°C and subjected to a strain rate of 531 s-1 for 20 seconds.

Zeta potential

Zeta potential was analyzed using the Zetasizer Nano-ZS equipment (Malvern Instruments, UK). Analyzes occurred at a temperature of approximately 25°C. The emulsion was analyzed in concentrated form and the microparticles were rehydrated at a ratio of 1:100 mL of deionized water.

Soluble solids and total solids

The determination of soluble solids was carried out by refractometry according to the method 932.12, from the Association of Official Analytical Chemists - AOAC (22) using the analog refractometer (Edutec model EEQ9030). The total solids were determined by the gravimetric method, by drying the sample in a vacuum oven at 55°C until constant weight (23).

Morphology

The morphology of the emulsion was analyzed using a optical microscopy on a Nikon microscope (Eclipse E200MV, Tokyo, Japan). Two drops of the emulsion were placed on the blades, covered with coverslips and viewed at 40X magnification. The images were captured by the TSView7 program. In relation to microparticles, the morphology was assessed using scanning electron microscopy TESCAM (VEGA LMH model) available in the multiuser laboratory of advanced microscopy of the Federal University of the Valley Jequitinhonha and Mucuri, Brazil. The powder was placed under a double-sided adhesive tape fixated on stub and coated with gold. The samples were observed in the microscope operated at 30 kV with magnification of 1000X and 2000X.

Statistical analysis

The results were analyzed by analysis of variance with software Statistica® 8.0 (Statsoft Inc., Tulsa, USA). The mean analysis was performed using Tukey's test at significance level $p < 0.05$.

RESULTS AND DISCUSSION

Rheology

The determination of the viscosity of the essential oil as part of the rheological study is important, since this parameter affects directly the microencapsulation efficiency. A lower viscosity (10.33 mPa.s) causes the diffusion of essential oil droplets towards the surface of the particle, reducing the efficiency of the microencapsulation (24), on the other hand, high viscosity can generate particles with high moisture content (25, 24, 26). As stated, viscosity can directly impact the encapsulation efficiency, resulting as an end-result as a greater agglomeration of the microparticles. Thus, for fine-tuning of the atomization step it is desirable for the essential oil to have a viscosity of less than 300 mPa.s (27).

The emulsion of the essential oregano oil in this study had an average viscosity of 36.23 ± 0.59 mPa.s (Table 1), considered within an acceptable range for an efficient microencapsulation process. As was shown by Flores *et al.,* the viscosity of chitosan emulsion with carvacrol was 0.067 to 0.101 Pa.s. The authors also measured viscosity to check if this parameter was in the suitable range to proceed with microencapsulation process (28). Battista *et al.,* also determined viscosity for a phytosterol spray drying microencapsulation with gum Arabic, maltodextrin and a surfactant (employed in different concentrations) (27). The viscosity in these suspensions ranged from 16.83 ± 0.22 to 41.30 ± 1.44 mPa.s. The rehydrated microparticles had an average viscosity of 20.13 ± 0.15 mPa.s (Table 1) also considered as an appropriate viscosity.

Table 1 Properties of the emulsion and microparticles of oregano essential oil and gum arabic.

PROPERTIES	EMULSION	MICROPARTICLES
Rheology (mPa.s)	36.23 ± 0.59	20.13 ± 0.15
Potential Zeta (mV)	-6.67 ± 0.32	-16.47 ± 0.70
Solubles solids (°Brix)	8.44 ± 0.13	0.98 ± 0.36
Total solids (%)	8.95 ± 0.42	1.03 ± 0.02

^{a} and ^{b} indicates a significant difference between the mean values of the Tukey's test results ($p < 0.05$).

The viscosity value is consistent with that referenced by Battista *et al.,* indicating, therefore, that the emulsion and hydrated oregano essential oil microparticles had a proper viscosity for microencapsulation (27).

Zeta potential

Zeta potential is related to the surface charge of the particle commonly used to ensure the stability of the colloidal system (29). Such charge stabilization can be achieved at zeta potential values around ±30 mV (29, 30) and is influenced by the chemical properties of coating materials, stabilizing agents and pH (31).

The zeta potential value for the emulsion of essential oregano oil analyzed was - 6.67 ± 0.32 mV (Table 1), considered an unstable emulsion. Sankar *et al.,* analyzed nanoparticles of essential oregano oil and found a zeta potential of -26 ± 0,77 mV (32), meanwhile Millan-Sango *et al.,* obtained zeta potential value in nanoemulsions of essential oregano oil ranging from -25.93 ± 4.88 to -35.6 ± 3.73 mV, resulting in particles with enhanced stability (33). Regarding the oregano oil microparticles in this study, the zeta potential value was -16.47 ± 0.70 mV (Table 1), indicating a better stability compared with the non-encapsulated oil. Stability of nutraceutical oil microparticles was also analyzed by other studies in the literature. Cazado and Pinho evaluated babassu oil microparticles obtaining a zeta potential of -42.4 ± 3.02 mV (34) and Lai *et al.,* had a value of -25.49 mV (± 2.02 mV), -21.51 mV (±2.02 mV), and -22.02 mV (±6.27 mV) for alginate, casein and milk microparticles, respectively (35).

It was observed that even with an unstable emulsion, characterized by the low zeta potential value, there was an improvement in stability after microencapsulation which is important to promote greater conservation of the volatile compounds of the oregano essential oil, envisioning long-term stability for oregano essential oil. The low value of the zeta potential for the emulsion can be attributed to gum arabic, the coating agent used. This material promotes the generation of microparticles with a low zeta potential value. In turn, the improvement in stability of the microparticles in comparison to the emulsion may have been influenced by the change of viscosity after microencapsulation (17).

Soluble and total solids

Determination of soluble and total solids content, is needed to evaluate the efficiency of microencapsulation (36). Soluble solids of the emulsion and essential oregano oil microparticles had a mean of 8.44 ± 0.13 and 0.98 ± 0.36 °Brix (Table 1), respectively. For a similar emulsion Jacomino *et al.,* obtained a value of 8.82 °Brix, which is close to that found in this study. Regarding total solids, the emulsion and microparticles presented the respective mean values of 8.95 ± 0.42 and 1.03 ± 0.02 (Table 1).

Azeredo explained that the greater the solids content in an emulsion, the shorter the time required to form the microparticles, which favors the retention of the volatile compounds (38). In addition, Jafari *et al.,* confirmed that the increase in solids concentration is relevant for obtaining an optimum viscosity attribute (39).

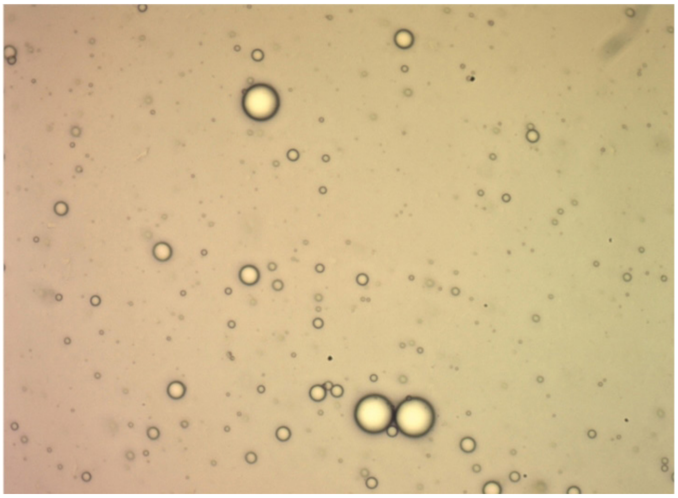

Figure 1 Microscopy optical of microparticles of oregano essential oil and gum arabic.

Powder morphology

Figure 1 shows the morphology of the drops of the oregano essential oil emulsion which are characterized by rounded, dispersed and droplets of various sizes. According to Silva *et al.,* and McClements emulsion droplets influence rheological properties (viscosity), stability (gravitational separation, flocculation and coalescence) and optical properties (lightness and color) (10, 40).

Figure 2 shows the scanning electron microscopy morphology of the powders produced with essential oregano oil and gum arabic. The microparticles shows a spherical shape and a concave and rough surface, characteristics of spray drying, which according to Frascareli *et al.,* results from the rapid evaporation of water during drying (41).

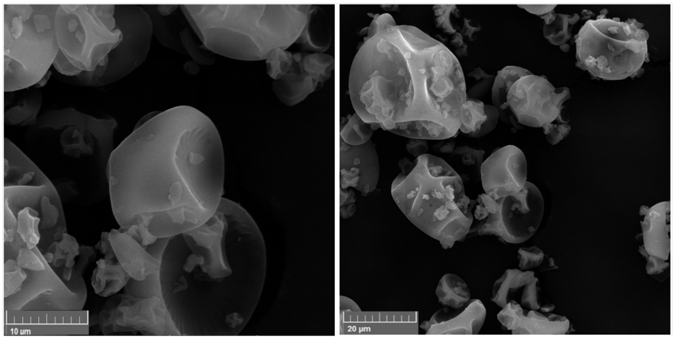

Figure 2 SEM morphology of microparticles of oregano essential oil and gum arabic.

It is possible to visualize that most of the microparticles presented an irregular spherical shape, absence of cracks, varied sizes and agglomeration. Li *et al.,* explained that the concave surface is an advantage of the spray drying because it contributes to reducing the permeability to gases and promotes a better protection of the active ingredient (36).

Similar morphology to that of this study was observed in fish oil microparticles (36), coffee oil (41), green coffee oil (10), jussara pulp and anthocyanin (42). All these studies of microencapsulation used gum arabic as a coating material alone or in combination with other agents.

CONCLUSIONS

The viscosity of the rehydrated microparticles was lower compared to the viscosity of the emulsion. The emulsion presented less stability compared to the microparticles. The solids content of the emulsion was decisive for obtaining a suitable viscosity in the spray drying. In addition, the morphological evidenced microparticles of irregular shape, varied sizes and agglomerated characteristics of spray drying.

The microencapsulation of the oregano essential oil as food ingredient was satisfactory, giving relevance to the work and with possibilities of future applications in the design of novel food products with enhanced stability and industrial relevance.

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CONFLICT OF INTEREST

The authors report no declarations of interest

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