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Original Article

Improved green coffee oil antioxidant activity for cosmetical purpose by spray drying microencapsulation

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ABSTRACT

The oil extracted by cold pressing unroasted coffee beans, known as green coffee oil, has been widely used for cosmetic purposes. The objective of this work was to prepare and characterize microcapsules containing green coffee oil and to verify its antioxidant activity under the effect of light, heat and oxygen. The encapsulating material was arabic gum and the microcapsules were obtained by spray drying an oil-in-water emulsion containing green coffee oil. The characterization of the microcapsules was performed by laser diffraction, scanning electron microscopy, differential scanning calorimetry and the antioxidant activity. The antioxidant activity was determined by a modified active oxygen method with light irradiation, heating and oxygen flux. The microparticles were effectively produced by the proposed spray drying method, which resulted in green coffee oil loads of 10 and 30%. The morphological evaluation of microcapsules showed spherical shape with smooth and non-porous surfaces, demonstrating the adequacy of arabic gum as encapsulating material. Calorimetric analysis of individual components and microcapsules with 10 and 30% green coffee oil showed diminished degradation temperatures and enthalpy, suggesting a possible interaction between arabic gum and green coffee oil. The antioxidant activities for pure green coffee oil and its microcapsules with loads of 10 and 30% showed high activity when compared to the reference antioxidant alfa-tocopherol. Microcapsules containing 10 and 30% of oil showed 7-fold and 3-fold increase in antioxidant activity when compared to pure green coffee oil. The new method for antioxidant activity determination proposed here, which applies heat, light and oxygen simultaneously, suggests a high improvement in encapsulated green coffee oil when compared to this active alone. The results showed herein indicate a promising industrial application of this microencapsulated green coffee oil.

Introduction

Products derived from coffee (*Coffea arabica* L., Rubiaceae) have been long used by mankind as beverages, foods and cosmetics. Most recently, the oil extracted by cold pressing the unroasted beans of coffee was introduced to the cosmetic market with great impact. This so called green coffee oil, GCO, has been studied for its activity on the skin health (Pereda, 2009; Pereda et al., 2009; Savian et al., 2011; Wagemaker et al., 2012; Chiari et al., 2014). This vegetable oil presents a unique composition and previous studies showed an expressive antioxidant activity against lipid peroxidation (Kroyer et al., 1989).

The GCO showed a dose dependent stimulation of collagen, elastin and glycosamineglycans synthesis by fibroblasts *in vitro* (Pereda et al., 2009) besides an increased release of growth factors, TGF-b1 and GM-CSF. Pereda et al. (2009) also found AQP-3mRNA expression 6.6 fold higher in the presence of GCO, indicating a protective effect of this oil on physiological balance of the skin. Pereda et al. (2009) also concluded that the GCO is effective against cellulitis. Although cosmetic formulations containing the GCO showed low antioxidant and antimicrobial activities *in vitro* (Wagemaker et al., 2012) there was also observed lack of toxicity *in vitro* and in clinical evaluation (Wagemaker et al., 2013). Those effects of GCO on the skin health may probably be related to its lipid fraction rich in triacylglycerols, sterols and tocopherols, as well as diterpenes of the kaurene family (Speer and Kolling-Speer, 2006), which have been previously connected to benefic actions to the skin (Nakayama et al., 2003). However, the most studied dermatological application of GCO is certainly as a photoprotection aid (Savian et al.,

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2011; Chiari et al., 2014). A non-ionic O/W emulsion containing 3% (w/w) GCO was proposed as a topic formulation for photoprotection (Savian et al., 2011). Recently, a study of GCO as an additive to sunscreen formulation containing ethylhexylmethoxycinnamate showed a synergistic effect of this oil by increasing the sun protection factor, SPF, by 20% as compared to synthetic sunscreen alone (Chiari et al., 2014).

One of the drawbacks for the cosmetic application of vegetable oils or fats is their lipid oxidative stability (Ramalho and Jorge, 2006) since the unsaturated waxy acids may undergo photo oxidation, thermal oxidation, auto oxidation and enzymatic oxidation. GCO photo oxidation may be a limiting factor especially for sunscreen applications and the use of synthetic antioxidants are subject to many formulation and regulatory aspects of topical administration. Microencapsulation is an effective way to protect these materials, as well as other components, like the diterpenes, against lipid oxidation (Pu et al., 2011; Jimenez et al., 2006) and other environmental factors. According to Costa et al. (2007) many studies have demonstrated the use of microparticles to reduce toxicity and increase the efficiency of active substances.

There are many techniques that can be applied for the production of microparticles, including the spray drying, spray cooling and fluidized bed (Pu et al., 2011). Among the many techniques, the spray drying has caught attention for plant extracts and oils (Jafari et al., 2008; Couto et al., 2013a,b; Peixoto and Freitas, 2013; Porto et al., 2013) due to its many advantages (Oliveira and Petrovick, 2010). There are also many materials that can be used as encapsulating agents such as gums, waxes and polymers (Oliveira and Petrovick, 2010; Couto et al., 2013a,b; Peixoto and Freitas, 2013; Porto et al., 2013). Arabic gum is noted for presenting excellent emulsifying properties and is widely used for the retention and protection of oil (Jimenez et al., 2006; Jafari et al., 2008). They are widely used for controlled release of active and have good stability in variations of pH and moisture levels in addition to being biocompatible (Jafari et al., 2008; Ranjha et al., 2010)

Thus, the objective of this study was to prepare microparticles by the technique of spray drying containing GCO and using AG as the wall forming material. In addition important characteristics of microparticles such as the morphology, thermal behavior and photocatalytic activity were studied. Possible interactions between the GCO and AG were evaluated by differential scanning calorimetry, DSC.

Material and methods

GCO microcapsules preparation

Materials

Arabic gum powder analytical grade batch number 144617 was supplied by Labsynth Ltda (São Paulo, Brazil). The green coffee oil brand name 'Melscreen Coffee' (Chemyunion Química Ltda, Sorocaba, Brazil) batch number CN102-0811 was purchased from Distriol Comércio de Insumos Ltda (São Paulo, SP). The DL-α-tocopherol acetate cosmetic grade (Zhejiang Medicine Co, China) batch number 20120615 with 99.6% purity was supplied by Viafarma Ltda (São Paulo, Brazil). The castor oil fatty acid Acros Organics BVBA containing 85% ricinoleic acid (12-hydroxy-oleic acid) was purchased from Janssen Pharmaceuticalaan (Geel, Belgium).

Microencapsulation

AG was dissolved in Milli-Q (EMD Millipore, Billerica, MA, USA) water (1:2 w/w) under magnetic stirring at 250 rpm and at room temperature (25 ± 2 °C) 24 h before the preparation of the emulsion for drying. The emulsions were prepared from the aqueous solution of AG by incorporating GCO in concentrations of 10 and 30% (w/w) relative to AG. The emulsion was then prepared using a high shear homogenizer Turratec Te-102 (Tecnal Ltda, Piracicaba, Brazil) was used at 14,000 rpm for 5 min at room temperature. After the preparation the enulsions were readily spray dried.

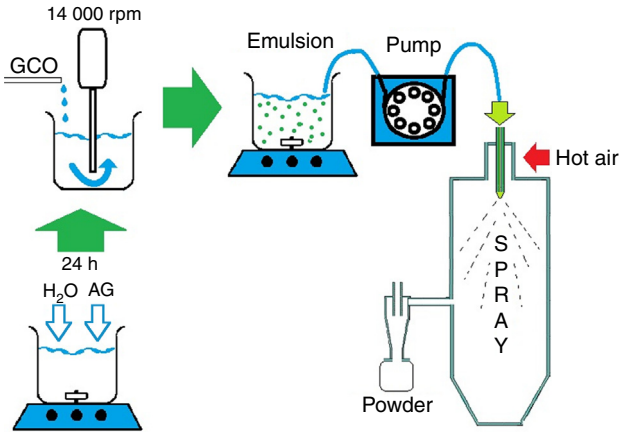

Fig. 1. Sequence of steps during GCO microcapsules preparation.

The drying process of the emulsion was performed using a laboratory scale spray dryer model MSD 0.5 (Labmaq Ltda, Ribeirão Preto, Brazil). The emulsion was atomized by a pneumatic spray nozzle in the drying chamber and the microparticles were separated by a cyclone and collected in a flask. The following drying conditions were kept constant during the experiments: emulsion feed rate 6 ml/min; drying air flow rate 1.25 m^3/min; atomization pressure 6 bar, atomizing air flow rate 50 ml/min; inlet and outlet drying air temperature 140 and 100 °C, respectively. Fig. 1 depicts the sequence of steps during GCO microcapsules preparation.

Morfology

The microcapsules were poured on a stub and coated with gold in a Bal-Tec sputter coater. Microparticles morphology was observed by Scanning Electron Microscopy, SEM, using a microscope XL30-TMP NO and FEG XL 30 (Phillips Co., Netherland).

Thermal analysis

Samples (5 mg) were placed in aluminum pans and heated to 420 °C at a rate of 10 °C/min under a nitrogen flux of 10 ml/min. DSC measurements were performed using a DSC-50 (Shimadzu Corp., Kyoto, Japan).

Photocatalytic activity

The samples were analyzed by an Active Oxygen Method, AOM, adapted from the conductometric technique Rancimat® (Lima et al., 2009; Nosari, 2012; Lima and Serra, 2013). The experimental assembly is shown in Fig. 2. Castor oil (3 ml) was placed in the flask 2A (Fig. 2) and 40 mg sample of alpha-tocopherol, GCO or microcapsules was added. This mixture was submitted to constant stirring at 120 °C and irradiation of light by a xenon lamp Xenarc D-H4R of 35W (Osram GmbH, Munchen, Germany). The volatile degradation compounds formed during photo-oxidation are dragged by a controlled flux of air to the flask 2B (Figure 2) containing 17 ml of Milli-Q (EMD Millipore, Billerica, MA, USA) water, where the conductivity is measured by a conductimeter model C708 (Analion Ltda, Ribeirão Preto, SP, Brazil). In this method the generation of volatiles species by oxidation under light, airflow and heat was evaluated for castor oil, CsOil, employed as reference, and also for CsOil containing GCO, Vitamin E and microcapsules prepared by spray drying with GCO concentration of 10 and 30% GCO.

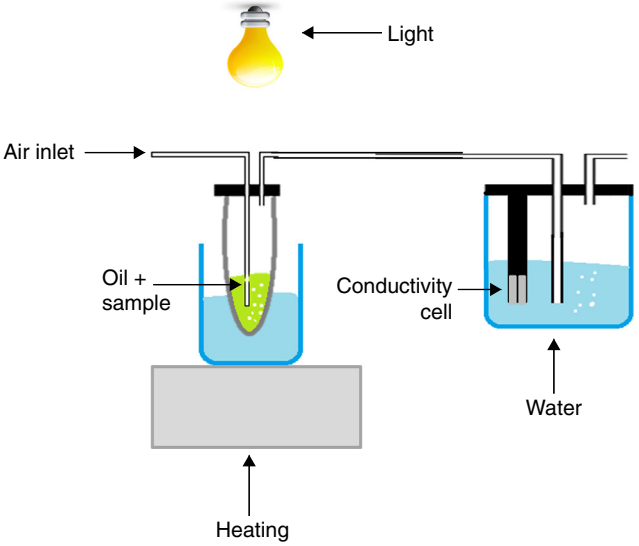

Fig. 2. The experimental assembly for photoxidation assay.

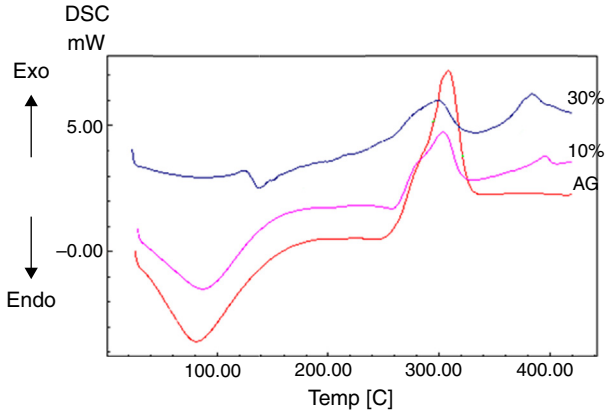

Fig. 4. Differential scanning calorimetry of spray dried microparticles.

Results and discussion

Physical characterization

The preparation of microparticles by spray dryer was successful, resulting in a powder with sizes ranging from 4 to 11 μm. The yield after drying was about 60%, which may be considered good for a lab scale spray dryer. Fig. 3 shows SEM photomicrographs. They indicate that GCO was encapsulated by the AG within a typical morphology for spray dried microparticles, with a spherical shape, smooth solid surface, displaying no cracks, fissures or pores, which allows for greater protection of oil (Santos et al., 2005; Trindade and Grosso, 2000; Bertolini et al., 2001). Some flat or concave surfaces observed are probably caused by shrinkage of the liquid drop due to rapidly moisture loss during the early stages of spray drying (Santos et al., 2005). The morphology of the microparticles shown in Fig. 3 was similar for different concentrations of GCO (10% Fig. 3a; 30% Fig. 3b).

Thermal analysis

Thermal analysis of spray drying microparticles (Fig. 4) showed endothermic and exothermic effects, where the first endothermic peak is likely associated with the water loss from functional groups of the polymer (Zohuriaan and Shokrolahi, 2004). The second peak is characterized by an exothermic event featuring the degradation of AG, which showed a shift in peak temperature when comparing AG to the microparticles. The peak degradation of AG occurs at 308 °C while the thermogram of the microparticles showed degradation peaks at 303 and 297 °C for GCO contents of 10 and 30%, respectively. These shifts in degradation temperatures are possibly due to changes in molecular weight and polarity of the polysaccharide groups (Loss et al., 2006).

Oxidative activity

The oxidative activity was evaluated by the changes in water solution conductivity in the presence of the volatile compounds which are formed during the degradation of castor oil subjected to air, heating and light irradiation (Lima et al., 2009; Lima and Serra, 2013). The high degradation of castor oil increases under this oxidative environment and consequently increases the conductivity of water in collection vessel. Thus, the oxidative activity is directly proportional to the measured conductivity (Lima et al., 2009; Nosari, 2012; Lima and Serra, 2013). Fig. 5 shows the results obtained for the water conductivity in the collection flask *versus* time for the oxidative reference CsOil and for the CsOil together with the GCO, microparticles containing 10 and 30% of GCO (SD10 and SD30) and also with α-tocopherol, α-TOH. As can be seen in Fig. 5, the highest conductivity values are found for the CsOil alone, showing values as high as 117 $\mu S\,cm^{-1}$ after 7 h of irradiation. The lower conductivities found for the runs of CsOil with the samples of GCO, α-TOH and the microencapsulated GCO proves that all samples show some antioxidant activity. The α-TOH is a common reference as antioxidant for many food and pharmaceutical products, especially for oils and waxes and as shown in Fig. 5 it decreased substantially the water conductivity measured in the collection flask. This means that the formation of CsOil degradation products was inhibited by the presence of α-TOH. In the increasing order of inhibitory capacity for CsOil degradation there are the α-TOH, SD10, GCO and SD30. In general, it was observed a constant conductivity increase for all samples studied, starting with a conductivity very close to 100 $\mu S\,cm^{-1}$, which is usually reported for

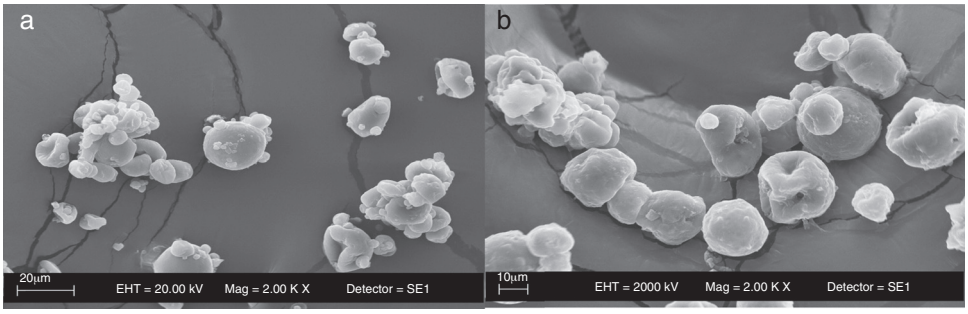

Fig. 3. SEM photomicrographs of microparticles produced by spray drying for GCO concentrations of: (a) – 10% and (b) – 30%.

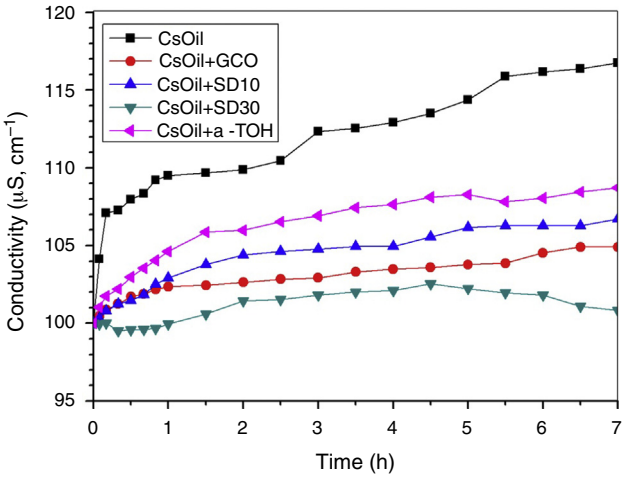

Fig. 5. Conductivity in aqueous solution as a function of time for samples of castor oil alone and added with green coffee oil, vitamin E and microencapsulated green coffee oil.

fresh water. For the CsOil sample, there is a significant burst in the conductivity in the initial curve and then a constant increase. For CsOil added with α-TOH, GCO and SD10 there is a minor burst during the first hour and then the conductivity reaches a plateau, with little or insignificant increase until the end of the experiment. However, the behavior of the SD30 sample is quite different from the others, since there is no increase in the conductivity during the first hour but an increase in conductivity in the period between 1 and 2 h, followed by a plateau from 2.5 to 4.5 h and constant decrease in conductivity until the end of the experiment. This is probably explained by the faster release of GCO from SD30 as compared to SD10 due to lower amount of encapsulating material. The effect of longer release times for higher ratios of encapsulating agent to active has been largely reported in literature (Martins et al., 2014).

Further analysis of this result could be attained by measuring the global reduction of the CsOil degradation, or by integrating the conductivity as a function of time to give the area under the curves, AUC, and comparing the areas for the several experiments. The AUC is given by equation 1. The antioxidant activity can be calculated by the reduction in the AUC for each experiment using the AUC of CsOil as the reference. The percent antioxidant activity, AOA, is defined by Eq. (2).

$$\mathrm{AUC} = \int_{t=0}^{t=t_f} C(t)dt \qquad (1)$$

$$\mathrm{AOA}(\%) = \frac{\mathrm{AUC_{CsOil}} - \mathrm{AUC_{Sample}}}{\mathrm{AUC_{CsOil}}} \times 100 \qquad (2)$$

where, $C(t)$ is the conductivity as a function of time, t_f = 7 h, and the areas under de curves are given in μS cm^{-1} s.

The AOA results for the samples studied are shown in Fig. 6A. The percent reduction of the areas under the curve are 5.02%, 6.77%, 8.10% and 9.77% for the α-TOH, SD10, GCO and SD30 respectively. The lowest reduction in the degradation of castor oil was observed for the α-TOH, which is well known for its antioxidant properties and also recommended for this application in foods and pharmaceuticals. Considering that α-TOH is a lipid-soluble antioxidant widely studied and used in cosmetics, the results herein demonstrates that the green coffee oil has a strong antioxidant activity since its AOA in this photocatalytic assay is considerably higher than the vitamin E. This result is important because there are contradictory conclusions in the literature, since a previous work (Wagemaker et al., 2012) reported a poor antioxidant activity for GCO when the DPPH method was used while a good antioxidant activity against lipid peroxidation was also reported (Kroyer et al., 1989). According to Fig. 6A, all samples containing green coffee oil had a higher antioxidative activity than vitamine E, suggesting that under these conditions, the studied samples showed a better protection against castor oil degradation. Fig. 6A shows gross values of AOA% when using 40 mg of each sample studied. It is worth remembering that the amount of green coffee oil varied in the samples from 10% (SD10), 30% (SD30) and 100% (GCO). At least for the comparison among the samples that containing GCO, which is believed to be the most important antioxidant compound, it is interesting to correct the AOA% values taking into consideration the concentration of GCO. The resulting corrected AOA% are shown in Fig. 6B and demonstrate an expressive increase in antioxidant activity of SD10 sample. Using this base, GCO maintains its 8.10% in AUC reduction, but SD10 now has 67.7% and SD30 presents now 29.3% of AUC reduction. This base of calculation represents better the increase in AOA% after GCO encapsulation, since is measured taking the mass of GCO in each sample, and demonstrates the great protection that the matrix of GA gives to green coffee oil when it is microencapsulated. SD10 and SD30 represent a 7-fold and a 3-fold increase in AOA%, respectively. One might expect a higher AOA% for SD30 then SD10, based in their GCO contents. However, the explanation is the faster release from SD30, as observed and discussed in Fig. 5, where SD30 sample showed no burst in the conductivity during the first hours of experiment. This shows that SD10 has a slower release of GCO and then a longer lasting effect which reflects in its AOA%.

The results stimulates further studies to evaluate other important parameters of spray drying, such as drying temperature and flow rate of dispersion atomization, as well as detailed analysis to verify the encapsulation efficiency and GCO stability.

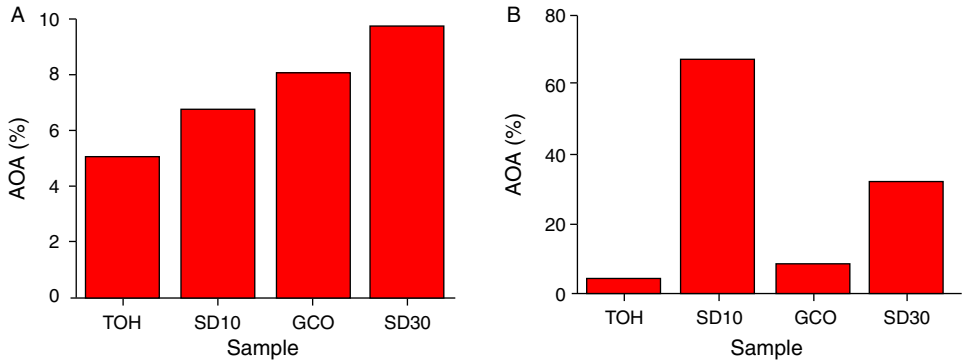

Fig. 6. Antioxidant activity measured based on the areas under the curves: (A) nominal AOA%, (B) AOA% corrected based on GCO content in each sample.

Conclusion

The results herein confirm the high antioxidant activity of green coffee oil, which is worldwide accepted in the cosmetic industry. This shows that the antioxidant test proposed here, which combines heat, light and oxygen, should be adopted for other studies for AOA% of natural oils and sunscreen products. Another important result is that GCO antioxidant activity in this work was superior to alfa-tocopherol, a widely used product as antioxidant in cosmetic industry. Furthermore, the microencapsulated systems developed herein provided better stability and long release of GCO, thus giving higher protection to CsOil than the non-encapsulated GCO. The increase in antioxidant activity for microcapsules containing 10 and 30% GCO were very expressive, indicating future industrial application in cosmetic market.

Authors contribution

ABFLN prepared the microcapsules and carried out the acquisition and analysis of photo oxidation data together with JFL. OAS and LAPF contributed for the analysis and interpretation of data. All authors participated in drafting the article and revising it critically.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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