Current Trends in Polymer Science
Vol. 17, 2016

Original Communication

Preparation and characterization of ultrafine fibers made from pulps of cherry (*Malpighia emarginata DC.*) and Surinam cherry (*Eugenia uniflora L.*), and a polymeric matrix of Eudragit L100 using electrospinning technique

Lidiane Martins Mendes Gomes[1,*], Raquel Pires Gonçalves[2], Marcos Lopes Dias[2], Renata Valeriano Tonon[3], Anna Paola Trindade Rocha Pierucci[4], Kátia Gomes de Lima Araújo[5] and Maria Helena Miguez da Rocha-Leão[6]

[1]Instituto de Química (IQ), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro; [2]Instituto de Macromoléculas Professora Eloísa Mano (IMA), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro; [3]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agroindústria de Alimentos, Guaratiba - Rio de Janeiro; [4]Instituto de Nutrição Josué de Castro (INJC), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro; [5]Faculdade de Farmácia, Universidade Federal Fluminense (UFF), Niterói -RJ; [6]Escola de Química (EQ), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.

ABSTRACT

The preparation and characterization of bioactive fibers made from pulps of cherry and Surinam cherry, and a polymeric matrix of Eudragit® L100 using electrospinning technique was investigated. The chemical characterization of the pulps obtained from cherry and Surinam cherry revealed that they are good sources of vitamin C (721.34 mg/100 g and 25.23 mg/100 g), carotenoids (3,138.5 µg/100 g and 14,015.5 µg/100 g) and phenolic compounds (784.89 GAE/100 g and 285.84 GAE/100 g. Their encapsulation in ultrafine fibers of Eudragit® L100 by electrospinning was successfully achieved. The morphology of the fibers was observed using scanning electron microscopy (SEM). The fibers obtained had dimensions in the microscale range. The images showed that the best preparation conditions to generate uniform and homogenous fibers involved Eudragit® L100 at a concentration of 15% together with the addition of 3% pulp while applying 15 kV and 20 kV voltages.

*Corresponding author: lidianemendes7@hotmail.com

KEYWORDS: fruits, bioactive compounds, fibers, encapsulation, morphology

INTRODUCTION

Packaging films containing bioactive antioxidant compounds are considered important food packaging materials, especially for the purpose of avoiding oxidation reactions that occur in food products and limit their preservation [1]. Therefore, films developed using fruits may take advantage of the antioxidant activity of these fruits used as additives/ fillers. Since the early 1990s, the technique of producing ultrafine fibers based on the use of high voltage and low current electric field, such as electrospinning, have been used as an encapsulation technique. Electrospinning has been used for the production of fibers, with diameters in the micrometer range down to nanometer range [2, 3], that have good applicability in food and nutraceutical formulations, coatings, and bioactive food packaging as well as in the food processing industry [4]. The use of electrospinning technique to encapsulate fruit pulps in polymer nanofibers may also provide an interesting method of stabilizing and preserving their functional

components, due to the barrier imposed by the polymer layer over the encapsulated particle [5]. Essentially, an electrospinning system is composed of a high voltage power supply, a metallic collector and a syringe pump, as depicted in figure 1. In this process, a high electric field is generated between the surface of a polymer solution and a collection target. As the intensity of the electric field increases, the surface of the solution at the tip of the syringe elongates and a charged jet of the solution is consequently ejected from the tip of the cone. As the polymer solution jet travels, the solvent evaporates, and a charged polymer fiber is finally accumulated on the surface of the collector, resulting in a nonwoven web of oriented fibers with diameters in the nanometer or micrometer scale [6]. Regarding matrix encapsulation, various commercially available polymers can be used, including chemically modified natural and synthetic polymers.

In this work, Eudragit® was used as the matrix for encapsulation. To the best of our knowledge, there are still only a few reports on the electrospinning of fibers from the Eudragit series of polymers. Among the different types of this series of commercial polymers, Eudragit L100 (methacrylic acid) is an enteric and pH-dependent polymer (soluble above pH 6) [7]. Due to this behavior, Eudragit® L100 is insoluble in the mouth and in the stomach, and it becomes soluble in the duodenum (pH of approximately 6). Considering this phenomenon, the aim of this study is to produce and characterize fibers using the electrospinning technique, incorporating a blend of pulps from Brazilian tropical fruits, i.e., cherry (*Malpighia emarginata DC.*) and Surinam cherry (*Eugenia uniflora L.*) in an Eudragit® L100 polymer matrix.

MATERIALS AND METHODS

Materials

Cherry (*Malpighia emarginata DC.*) and Surinam cherry (*Eugenia uniflora L.*) pulps were purchased from a small farm located in São Paulo, Brazil. Eudragit® L100, which is an acrylate-methacrylate copolymer with a molecular weight of 150,000 Da, was obtained from Röhm Company (Germany). The solvents used were ethyl alcohol (95%, reagent grade), purchased from Sigma-Aldrich, and distilled water.

Pulp preparation

The fruits were subjected to the following preparation steps: washed under running water, sanitized with sodium hypochlorite solution (30 ppm) for 20 minutes, rinsed under running water for chlorine removal, pulped with a depulper (Bonina 0.25 dF) composed of a sieve with a 0.6 mm diameter and centrifuged.

To minimize the loss of bioactive compounds, the centrifuged pulps were stored in a freezing chamber at -18 °C for further analysis. Only the amount required for chemical analysis was thawed slowly to preserve the nutritional and sensory properties of the original product.

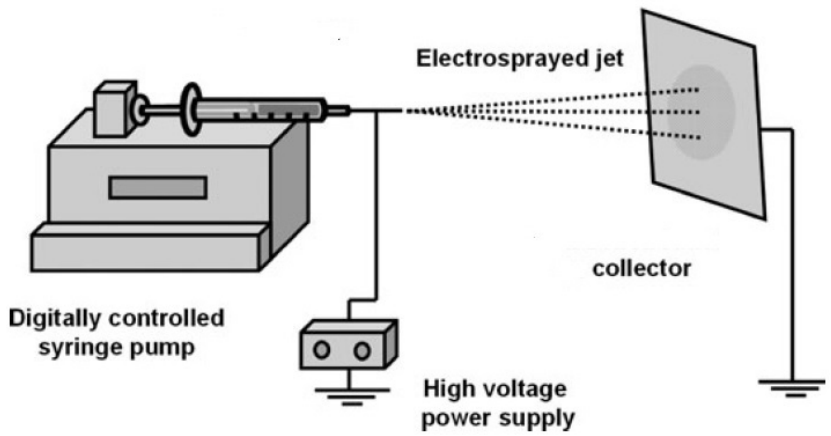

Figure 1. Electrospinning device.

Cherry and Surinam cherry pulp analysis

Bioactive compounds were determined using the following methodologies: vitamin C by the 2,6-dichlorophenolindophenol assay established by the AOAC (Association of Analytical Communities) [8], total carotenoids (μg/100 g) by spectrophotometry according to Rodriguez-Amaya [9] and Pacheco [10], and total phenolics according to the Folin-Ciocalteu method [11]. The results were expressed as gallic acid equivalents (GAE)/100 g. All these analyses were carried out in triplicate.

Spinning solutions

Spinning solutions were prepared in a mixed solvent system at room temperature. Eudragit® L100 was dissolved in a mixture of ethanol-water (4:1) (v/v) at concentrations of 15 and 20% (w/v). The blend from the cherry and Surinam cherry pulps (1:1) (w/w) was freeze-dried before the electrospinning procedure and dispersed in ethanol and distilled water at concentrations of 1 and 3% (w/v). All solutions were magnetically stirred overnight at room temperature. To the solutions containing the fruit blend at concentrations of 1 and 3% Eudragit® L100 solution at a concentration of 15 or 20% (w/v) was added.

Electrospinning

Homogenous solution of Eudragit® L100 and dispersions of Eudragit® L100 with cherry or Surinam cherry pulps were placed in a 5 ml syringe and the solution/dispersion was pumped with a syringe pump (KDS 100 series) at a rate of 0.25 ml/h. The metallic needle (0.80 mm x 30 mm) was connected to a high voltage supply (Glassman High Voltage, PS/FC 60p02.0-11), and a collector was placed 10 cm from the tip of the needle horizontally to collect the ultrafine fibers. All electrospinning was performed at room temperature and at an applied voltage of 15 and 20 kV.

Scanning electron microscopy

The surface morphology of the electrospun ultrafine fibers was assessed using a Quanta 250 FEG scanning electron microscope (FEI, Hillsboro, Oregon, USA). Prior to examination, the samples were fixed in stubs with a 10 mm diameter using carbon adhesive tape and coated with platinum under an argon atmosphere using a high vacuum coater apparatus (Leica EM ACE 600®, Germany). Pictures were then taken at an excitation voltage of 30 kV. The average fiber diameter was determined using size metric software (National Institutes of Health, USA).

RESULTS AND DISCUSSION

Pulp characteristics

Table 1 shows the chemical characteristics of the pulps from cherry and Surinam cherry. Ascorbic acid analysis showed that cherry is not considered to be a rich source of vitamin C (25.23 mg/100 g). Oliveira *et al*. (2006) [12] determined an ascorbic acid content of 13.42 mg/100 g in whole Surinam cherry pulp, while Melo, Arroxelas and Birth [13] determined an ascorbic acid content of 42.9 mg/100 g in mature Surinam cherries. Cavalcante [14], in a study of Surinam cherries, found an ascorbic acid content of 16 mg/100 g. For cherries, the ascorbic acid content was found to be quite high (721.34 mg/100 g) compared to that of Surinam cherries. Compared to other fruits rich in ascorbic acid, cherries stand out for being considered a main source of vitamin C, followed by cashew with its yellow and red varieties, guava, mango and citrus fruits [12]. Gorgatti *et al.* [15] reviewed the content of vitamin C in cherry at different stages of maturation, and the contents ranged from 560 mg/100 g to 1,822 mg/100 g. Researchers claim that the more mature a cherry is, the lower the content of this nutrient will be. The total phenolic content of cherry was higher than that of Surinam

Table 1. Chemical contents in cherry and Surinam cherry pulps.

Compound(s)	Cherry	Surinam cherry
Vitamin C (mg/100 g)	721.34 ± 0.08	25.23 ± 0.06
Total phenolics (GAE/100 g)	784.89 ± 1.40	285.84 ± 2.73
Total carotenoids (μg/100 g)	3,138.5 ± 1.2	14,015.5 ± 1.6

Values are the mean ± SD of three determinations.

cherries. Cherry contained 784.89 GAE/100 g, while Surinam cherry contained 285.84 GAE/100 g. In a study on the antioxidant activity of cherries and their derivatives, Mezadri *et al.* [16] found total phenolic contents that ranged from 805 to 1,150 GAE/100 g. In relation to Surinam cherry, a similar content (210 GAE/100 g) was described by Bagetti [17]. By contrast, in terms of bioactive compounds, the total carotenoids in surinam cherry was higher than that in cherry: 14,015.5 µg/100 g and 3,138.5 µg/100 g, respectively. A similar content (3090 µg/100 g) was found by Almeida *et al.* [18] in the initial stage of ripeness of cherries from temperate and tropical regions in Brazil. Using a spectrophotometer, Lima *et al.* [19] quantified the carotenoid content in red Surinam cherry and observed lower levels than those in the present study (10,400 µg/100 g). The chemical composition of fruits can be affected by several factors such as the food source, weather conditions, harvesting and handling conditions, ripeness degree, transportation and storage. Thus, these factors could also explain the difference in values between the same types of foods [20]. The high content of bioactive compounds in both pulps demonstrates that these fruits are a great source of antioxidants and can be considered as functional ingredients. Therefore, the present study will report the incorporation of these fruits, which contain bioactive compounds with known beneficial properties for human health, into fine polymer fibers by means of electrospinning to generate a novel technology for the encapsulation of value-added food ingredients.

Fiber morphology

The morphology of the obtained ultrafine fibers was studied using scanning electron microscopy (SEM). SEM images of the Eudragit® L100 fibers are shown in figures 2 and 3. All the Eudragit® L100 solutions were clear and transparent. It is apparent that the electrospinning of the polymer solution at concentrations of 15 and 20% exhibited ribbon-like morphologies (Figure 2), probably due to the low surface tension and low boiling point of the solvent. As demonstrated in figure 2 (a) and (c), the two types of fibers are relatively uniform and homogeneous in structure compared to those shown in figure 2 (b) and (d), which had irregular structures.

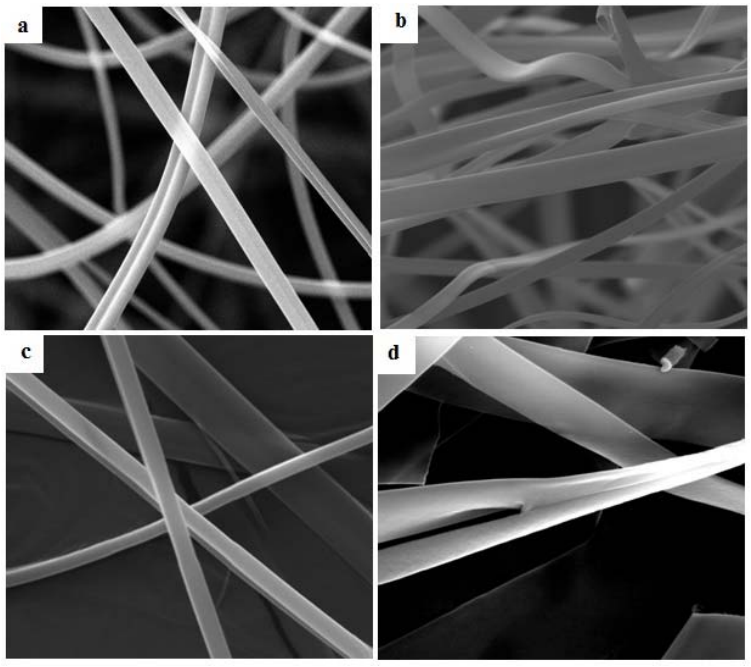

Figure 2. Selected SEM images of fibers: Eudragit at concentrations of 15% (a) and 20% (b) electrospun at 15 kV voltage and Eudragit at concentrations of 15% (c) and 20% (d) electrospun at 20 kV voltage. The magnification used was 10000X.

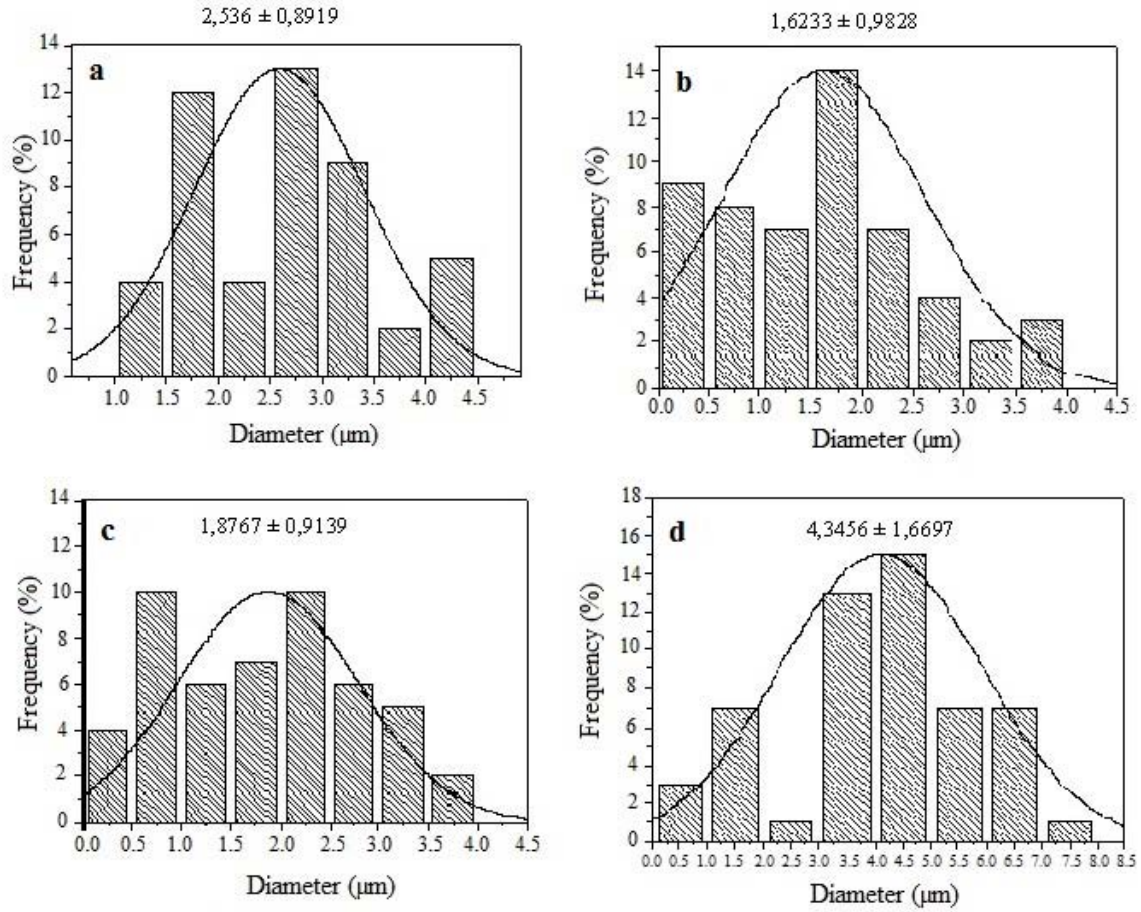

Figure 3. Diameters of the fibers: Eudragit at concentrations of 15% (a) and 20% (b) electrospun at 15 kV voltage and Eudragit at concentrations of 15% (c) and 20% (d) electrospun at 20 kV voltage.

The average diameter of the fibers of Eudragit® L100 were in the micrometer range (1.6-4.3 µm), as shown in figure 3. However, Eudragit® L100 fibers prepared at a concentration of 20% and 20 kV (Figure 3 (d)) had a higher diameter of 4.34 µm. Based on these results, it was decided to add cherry and Surinam cherry pulps at concentrations of 1 and 3% into the solution of Eudragit® L100 prepared at 15% in order to produce fibers rich in nutrients. The morphology of the Eudragit® L100 fibers prepared at a polymer concentration of 15% with the addition of 1 or 3% pulp while applying 15 or 20 kV voltages is presented in figure 4. Figure 5 shows the distribution of the fiber diameters measured from each SEM image. By comparing the images of fibers prepared with 1% and 3% pulp added to a 15% Eudragit® L100 solution, it is possible to conclude that the fibers prepared with 3% pulp had a uniform morphology, as illustrated in figure 4 (a) and (d). The addition of pulp promoted a decrease in the fiber diameter (mean ± standard deviation), except for those prepared at an Eudragit® L100 concentration of 15% with the addition of 1% pulp (Figure 5 (a)). However, there was a higher diameter distribution, with nearly 20% of the fibers with diameters up to 1 µm and some reaching 5 µm in diameter (Figure 5 (a) and (c)). This behavior was not observed in figure 5 (b) and (d), which showed average diameters of 1.43 µm and 1.51 µm, respectively. It is possible that in the fibers containing 3% pulp, the solid was better dispersed in the polymer solution, thus explaining the shapes, as discussed earlier. This better pulp distribution may have contributed to a slight increase in the viscosity, favoring the production of uniform fibers at both voltages, 15 and 20 kV. Moreover, it seems to be very likely that the same compounds contributed to the observed thickening of the fibers due to the agglomeration of bioactive molecules, as seen in figure 4 (a) and (c).

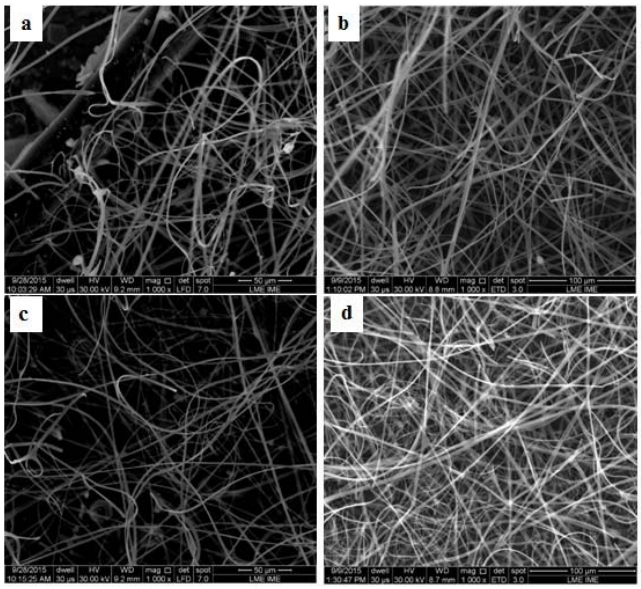

Figure 4. Selected SEM images of fibers: Eudragit at a concentration of 15% with 1% (a) and 3% pulp (b) electrospun at 15 kV voltage and Eudragit at a concentration of 15% with 1% (c) and 3% pulp (d) electrospun at 20 kV voltage. The magnification used was 10000X.

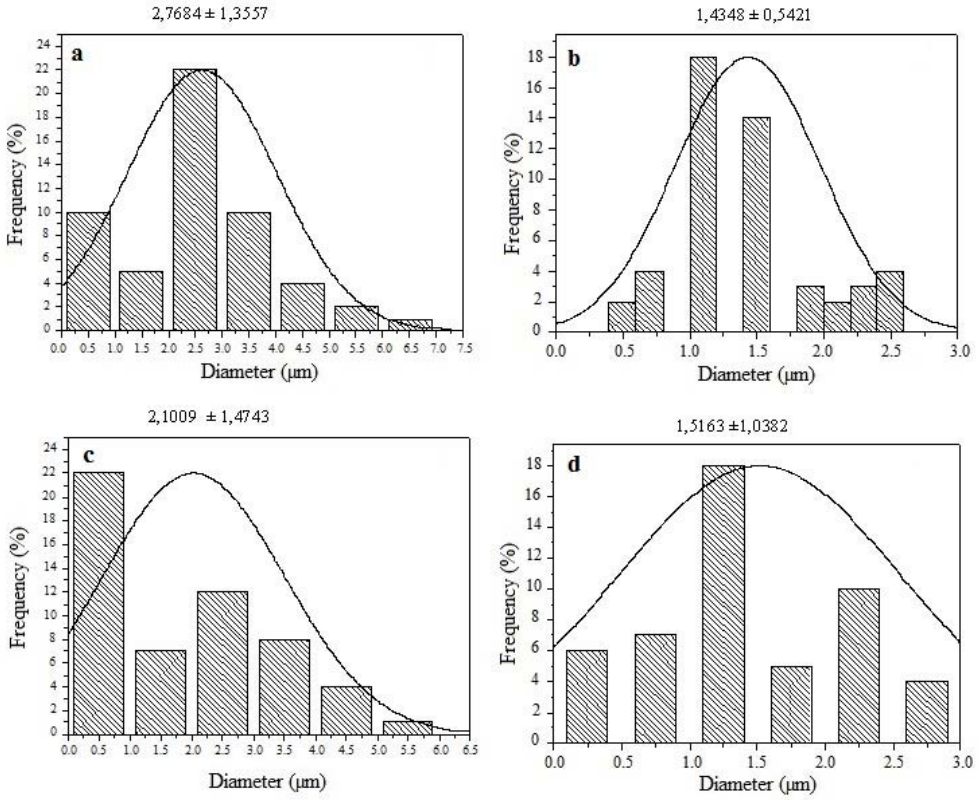

Figure 5. Diameters of the fibers: Eudragit at a concentration of 15% with 1% (a) and 3% pulp (b) electrospun at 15 kV voltage and Eudragit at a concentration of 15% with 1% (c) and 3% pulp (d) electrospun at 20 kV voltage.

CONCLUSION

The current study demonstrated that cherry and Surinam cherry are sources of bioactive compounds, such as vitamin C, carotenoids and phenolics, and that electrospinning technology is a potential new plataform to encapsulate food with antioxidant properties in fibers of Eudragit. Morphological studies confirmed that the best conditions to attain uniform and homogenous fibers included Eudragit® L100 prepared at a concentration of 15% with the addition of 3% pulp while applying voltages of either 15 or 20 kV. Electrospinning is therefore reported here as a technology that is able to produce value-added polymer microfibers that have good potential for use in food and nutraceutical formulations and coatings, bioactive food packaging and the food processing industry.

ACKNOWLEDGMENTS

The authors wish to acknowledge the financial support from CAPES, Institute Militar of Engineering (IME-RJ), for the scanning electron microscopy analyses.

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Nerín, C. 2008. J. Food Eng., 84, 313-320.
2. Fong, H., Chun, I. and Reneker, D. H. 1999, Polymer, 40, 4585-4592.
3. Qi, H., Hu, P., Xu, J. and Wang, A. 2006, Biomacromolecules, 7, 2327-2330.
4. Fernandez, A., Torres-Giner, S. and Lagaron, J. M. 2009, Food Hydrocolloid, 23, 1427-1432.
5. Li, D. and Xia, Y. 2004, Adv. Mater., 16, 1151-1170.
6. Ziabari, M., Mottaghitalab, V. and Haghi, A. K. 2009, Braz. J. Chem. Eng., 26, 52-62.
7. Weiss, G., Knoch, A., Laicher, A., Stanislaus, F. and Daniels, R. 1993, Drug Dev. Ind. Pharm., 19, 2751-2764.
8. AOAC. Official Method of Analysis of AOAC International, 1990, Association of Official Analytical Chemists, Arlington, VA, USA.
9. Rodriguez-Amaya, D. B. 2001, A guide to carotenoid analisys in food. ILSI Press, Washington DC, USA.
10. Pacheco, S. 2009, Preparation of analytical standards stability study and assay validation parameters for carotenoids by liquid chromatography. MS Thesis, Federal Rural University of Rio de Janeiro, Brazil.
11. Georgé, S., Brat, P., Alter, P. and Amiot, M. J. 2005, J. Agr.Food Chem., 53, 1370-1373.
12. Oliveira, A. L., Lopes, R. B., Cabral, F. A. and Eberlin, M. N. 2006, Food Chem., 99, 1-5.
13. Melo, E. A., Arroxelas, V. L. G. L. and Nascimento, P. P. 2000, Sci. Agri., 57, 629-634.
14. Cavalcante, M. L. 1991, Composition of carotenoids and vitamin A in surinan cherry (*Eugenia uniflora*) and cherry (*Malphighia glabra L.*). MS Thesis, University of Campinas, São Paulo, Brazil.
15. Gorgatti, N. A., Ardito, E. F. G., Garcia, E. E. C., Bleinroth, E. W., Freire, F. C. O. and Menezes, R. E. 1996, Acerola export: harvest procedure and post-harvest, EMBRAPA, Brasília-DF, Brazil.
16. Mezadri, T., Villaño, D., Fernandéz-Pachon, M. S., Garcia-Parrila, M. C. and Trancoso A. M. 2008, J. Food Compos. Anal., 21, 282-290.
17. Bagetti, M. 2009, Physicochemical characterization and antioxidant capacity of surinam cherry *(Eugenia Uniflora L.)*. MS Thesis, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.
18. Almeida, C. B., Monica-Berto, R., Franco, J. J., Pegoraro, C., Fachinello, J. C. and Silva, J. A. 2009, Comparison of the carotenoid content in native fruits of tropical and temperate regions. MS Thesis, Federal University of Pelotas, Rio Grande do Sul, Brazil.
19. Lima, V. L. A. G., Mélo, E. A. and Lima, D. E. S. 2002, Sci. Agri., 59, 447-450.
20. Aldrigue, M. L., Madruga, M. S., Fioreze, R., Lima, A. W. O. and Sousa, C. P. 2002, Aspect of science and food technology. In. UFPB, Paraíba, João Pessoa, Brazil.