EFFECT OF EXPERIMENTAL PARAMETERS ON THE ENCAPSULATION OF ROASTED COFFEE OIL BY COMPLEX COACERVATION[1]

Camila Carolina Veiga[2]; Rayssa Cristine Simoni[3]; Odinei Hess Gonçalves[4]; Marianne Ayumi Shirai[5]; Fernanda Vitória Leimann[6]

[1] Trabalho financiado pela Fundação Araucária.

[2] Departamento Acadêmico de Alimentos (DALIM), Universidade Tecnológica Federal do Paraná, Campus Campo Mourão - (UTFPR-CM), camilacarolveiga@hotmail.com.
[3] Departamento Acadêmico de Alimentos (DALIM) - UTFPR-CM, rayssa.simoni@gmail.com.
[4] Programa de Pós-Graduação em Tecnologia de Alimentos (PPGTA) - UTFPR-CM, odinei@utfpr.edu.br.
[5] Programa de Pós-Graduação em Tecnologia de Alimentos (PPGTA) - UTFPR-CM, marianneshirai@utfpr.edu.br.
[6] Programa de Pós-Graduação em Tecnologia de Alimentos (PPGTA) - UTFPR-CM, fernandaleimann@utfpr.edu.br.

ABSTRACT: Roasted coffee oil is a coffee industry byproduct composed by a great number of compounds including the volatiles responsible by coffee aroma. The main objective of the present work is the encapsulation of roasted coffee oil in enzimatically crosslinked gelatin and gum Arabic microcapsules. The microcapsules were obtained by complex coacervation. A 2^2 factorial experimental design was applied to verify the effect of the roasted coffee oil amount used and the stirring rate on the average diameter, oil recovery and encapsulation efficiency. The microcapsules presented average diameter up to 21 µm with narrow size distributions. The results varied from 80 to 100 % (oil recovery) and 33 to 68% (encapsulation efficiency).

KEYWORDS: transglutaminase, FTIR, encapsulation efficiency, oil recovery.

EFEITO DOS PARÂMETROS EXPERIMENTAIS NA ENCAPSULAÇÃO DO ÓLEO DE CAFÉ TORRADO POR COACERVAÇÃO COMPLEXA

RESUMO: O óleo de café torrado é um subproduto da indústria de café composto por um grande número de compostos incluindo os voláteis que são responsáveis pelo aroma do café. O objetivo principal deste trabalho é a encapsulação do óleo de café torrado em microcápsulas de gelatina e goma arábica reticuladas enzimaticamente. As microcápsulas foram obtidas por coacervação complexa. Um planejamento experimental 2^2 foi aplicado para verificar o efeito da quantidade de óleo de café torrado e da taxa de agitação no diâmetro médio, na recuperação percentual e na eficiência de encapsulação. As microcápsulas apresentaram diâmetro de até 21 µm com distribuição de tamanhos estreita. Os demais resultados variaram de 80a 100 % (recuperação percentual) e 33 a 68% (eficiência de encapsulação).

PALAVRAS-CHAVE: transglutaminase, FTIR, eficiência de encapsulação, recuperação percentual.

INTRODUCTION

Freshly brewed coffee is appreciated by consumers due to its pleasant aroma. However, this aroma is not stable and changes rapidly after coffee preparation (Hofmann and Schieberle 2002). Aroma is one of the most important attributes of roasted coffee (de Oliveira et al., 2005) since it contains properties that can enhance the flavor of coffee-based products. During instant coffee processing, its flavor is partially lost due to the processing conditions. Different roasting degrees, coffee bean varieties and extraction processes lead to a great variability on the aroma profile (Kobayashi and Benassi 2012). Many strategies have been applied in order to supplement the lost flavors during soluble coffee production, being this approach a technological challenge that involves the use of roasted coffee oil (Figueiredo and Miguel 2010). Complex coacervation technique offers a unique method for the encapsulation of flavours, is a simple, scalable, low cost, solvent-free and reproducible method, being a highly advantageous technique for the fabrication of microcapsules in industry (Xiao et al. 2014b). A crosslinking agent must be used to stabilize the microcapsules structure. This hardening step can be achieved with the use of aldehydes, such formaldehyde or glutaraldehyde, the most common crosslinking agents used. However they present elevated toxicity levels and thus are undesirable for food processing applications. Many researches are now exploring new security crosslinking agents such as transglutaminase (TGAse) (Xiao et al. 2014a).

In this work, complex coacervation was applied to microencapsulate roasted coffee oil. Gelatin and gum Arabic were used as wall forming material and TGAse was used as crosslinking agent of microcapsules. The effect of oil load and stirring rate were investigated on the final microcapsules size, size distribution, oil recovery and encapsulation efficiency.

EXPERIMENTAL

Materials

Gelatin and gum arabic (P.A.,Vetec Química Fina) were used as wall material for microcapsules. The crosslinking agent transglutaminase was kindly supplied by Ajinomoto Interamericana Ind. e Com. Ltda. (Activa TG-S®, São Paulo, SP, Brazil), with nominal activity of 100 U/g of powder, according to the information provided by the industry. Roasted coffe oil was kindly supplied by CIA. Iguaçú de Café Solúvel. Cloridric acid (0.25 mol/L) and sodium hydroxide (0.01 N) (P.A., Vetec Química Fina) aqueous solutions were used to modify the pH of dispersions. Dicloromethane (P.A., Vetec Química Fina) was used in the oil load and encapsulation efficiency determinations.

Microcapsules production

Table 1 presents the experiments carried out to evaluate the effect of stirring rate and the amount roasted coffee oil added at the formulation. Complex coacervation procedure adopted was described previously by Prata et al. (2008).

Table 1 – Experimental conditions used in the experiments.

Experiment	Stirring rate (RPM)	Roasted coffee oil (g)
1	350	0.8250
2	350	0.4125
3	500	0.4125
4	500	0.8250

Microcapsules characterization

The procedure adopted was described by Leimann et al. (2009). Optical observations of the microcapsules were carried out with the aid of an optical microscope (Bioval L-2000A) attached to a digital camera. The microcapsules average size was determined measuring the microcapsules diameter (D) using an image analysis software. About 300 microcapsules were measured for each experiment. The polidispersity index (PDI) was determined with the Equation 1 were “σ” (µm) represents the standard deviation of the diameter measurements and “Dp” the average diameter (µm).

$$PDI = \left(\frac{\sigma^2}{Dp^2} \right) \qquad \text{Eq. (1)}$$

For the roasted coffee oil recovery (OR) determination the final suspension of microcapsules was freeze dried (Liotop L101). The concentration of the roasted coffee oil (C_{OR}, mg/mL) was determined using a previously prepared calibration curve. The roasted coffee oil recovery (OR) was then calculated with Equation 2 (Zhao, Brown, and Jones 2010) were C_0 (mg/mL) is the concentration related to the original amount of roasted coffee oil added to the system during the microencapsulation procedure. For the roasted coffee oil encapsulation efficiency (EE) determination 25 mL of the final suspension of microcapsules was vacuum filtered and washed three times with dicloromethane (10 mL) to remove the free oil. After that the filter paper with the microcapsules was dried in an oven at 40 ºC during 1 h. Finally the same procedure of the oil recovery was adopted to determination of the roasted coffee oil concentration (C_{EE}, mg/mL) and the encapsulation efficiency was calculated with Equation 3 (Zhao et al. 2010).

$$OR(\%) = \frac{C_{OR} \cdot 100}{C_0} \qquad \text{Eq. (2)}$$

$$EE(\%) = \frac{C_{EE} \cdot 100}{C_{OR}} \qquad \text{Eq. (3)}$$

RESULTS AND DISCUSSION

Microcapsules morphology, average size and size distributions

Microcapsules morphology can be evaluated by the micrographs presented in Figure 1 as well as the respective size distributions obtained for the experimental conditions analyzed. In Table 2 are shown the values of average diameter, standard deviations and polidispersity indexes.

Table 2 – Average diameters (Dp), standard deviations (σ) and polydispersity indexes (PDI) of the produced microcapsules.

Experiment	Roasted coffee oil (g)	Stirring rate (RPM)	Dp (μm)	σ (μm)	PDI (-)
Exp. 1	0.8250	350	25.7	10.9	0.17
Exp. 2	0.4125	350	21.0	7.6	0.13
Exp. 3	0.4125	500	22.9	8.2	0.13
Exp. 4	0.8250	500	21.5	8.6	0.16

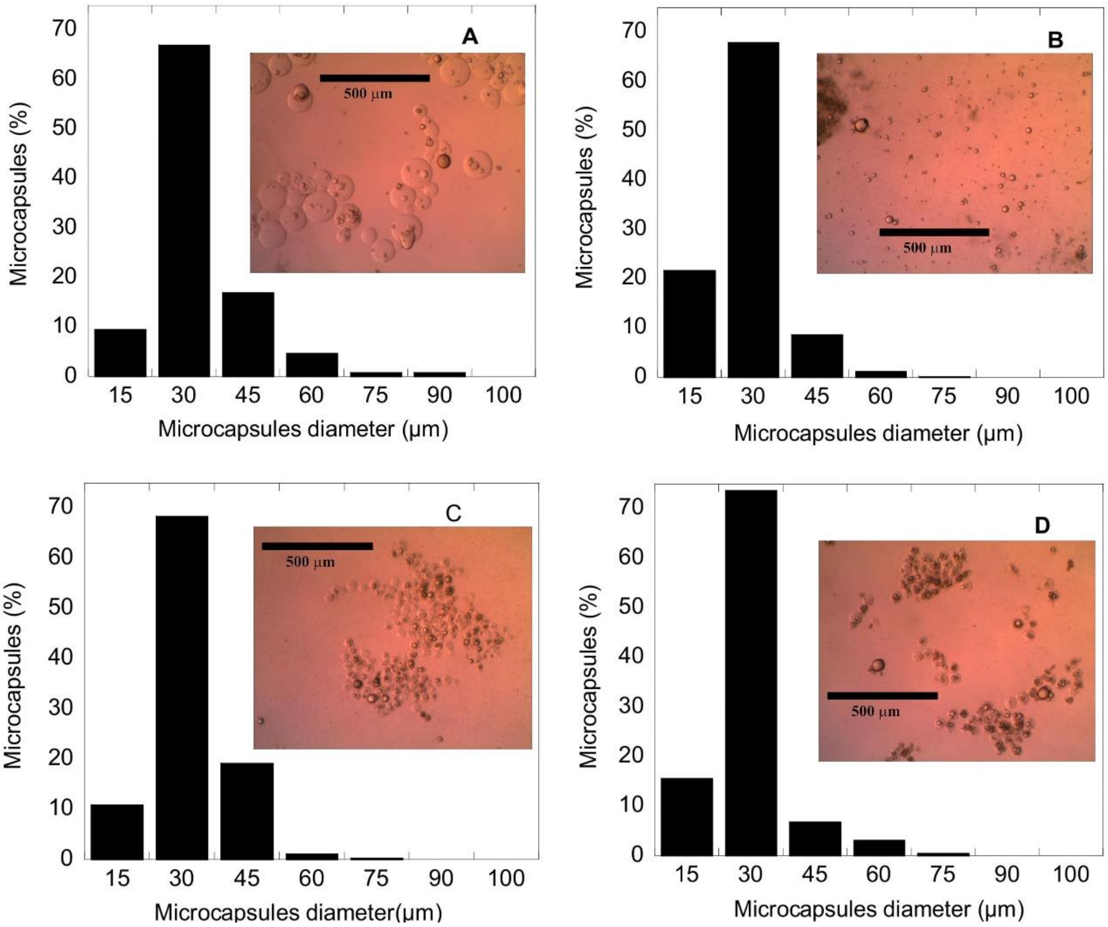

Figure 1- Microcapsules micrographs (100x magnification) and size distribution: (a) Exp. 1: 350 RPM and 0.8250 g oil; (b) Exp 2: 350 RPM and 0.4125 g oil; (c) Exp 3: 500 RPM and 0.4125 g oil; (d) Exp. 4: 500 RPM and 0.8250 g oil.

Microcapsules with well defined spherical shape were obtained in all experimental conditions as observed in the micrographs (Figure 1). Size distributions indicated that the majority of the microcapsules presented diameter between 15 and 45 μm. The average diameters presented in Table 2 are in agreement with the images. Bachtsi et al. (1996) stated that there is a limiting value for the average diameter that occurs when the stirring rate or variations in other conditions from the dispersion system no longer affects it. Probably for the experimental values studied this limiting value was reached. Another important point to be noted in Figure 1 is that all size distributions presented monomodal behaviour.

The calculated polydispesity indexes (Table 2) showed that the narrower size distribution obtained was for Experiment 3, where the smallest amount of oil was added and the stirring rate was increased. According to Bachtsi et al. (1996) and Shinnar and Church (1960), in general the average diameter of the droplets in a liquid-liquid dispersion is determined by the balance between the turbulent forces which tend to break drops and the interfacial tension and viscous forces that tend to keep the beads together, or coalesce. It is apparent that with increasing the stirring rate the size distribution shifts to smaller diameters as the turbulent kinetic energy associated with the breaking of the drops increases. In the case of Experiments 2 and 3 in which the amount of oil was kept constant (lower level) the increase in the stirring rate leaded to an increase in microcapsules with diameter in the range of 45 µm and a decrease in the range of 15 µm. However, there was no change in the size distribution. According to Xing et al. (2004) an increase in the stirring rate can destabilize the emulsion of oil in water resulting in a distortion of the microcapsules that may have occurred in this case. For Experiment 1 (350 RPM and 0.8250 g oil) a wider size distribution can be observed in Figure 1 and was confirmed by PDI data (Table 2). In this case microcapsules in the range of 90 µm were obtained. The experimental condition that led to the production of the largest amount of microcapsules with diameter smaller than 15 µm was Experiment 2 (350 RPM and 0.4125 g oil).

Roasted coffee oil recovery and encapsulation efficiency

The roasted coffee oil recovery and encapsulation efficiency results are presented in Table 3.

Table 3 – Roasted coffee oil recovery and encapsulation efficiency results.

Experiment	Roasted Coffee oil (g)	Stirring rate (RPM)	Oil Recovery (%)	Encapsulation Efficency (%)
Exp. 1	0.8250	350	104.4	33.4
Exp. 2	0.4125	350	80.2	68.0
Exp. 3	0.4125	500	87.6	46.8
Exp. 4	0.8250	500	82.2	35.0

All recovery values obtained were satisfactory and above 80%. Freiberger (2013) encapsulated roasted coffee oil in PLLA nanocapsules and also obtained recovery values above 80% using the miniemulsion/solvent evaporation technique. In the case of encapsulation efficiency the experimental condition that showed better result was 350 RPM and 0.4125 g of roasted coffee oil (Experiment 2). Maji and Hussain (2009) also applied the complex coacervation technique to encapsulate *Limonella Zanthoxylum* oil in gelatin and chitosan and obtained maximum encapsulation efficiency of 60% which is in agreement with the results obtained in the present work.

The effect of stirring rate was negative for both, roasted coffee oil recovery and encapsulation efficiency as observed by Tayade & Kale (2004). The roasted coffee oil amount presented a positive effect for the oil recovery, although for the encapsulation efficiency the effect was the opposite. The oil recovery represents the amount of oil that kept on the system after the encapsulation procedure, inside or outside the microcapsules (free). However, the encapsulation efficiency depends of the relation between the amount of polymer available to encapsulate the oil and the surface area of the oil droplets generated during the dispersion. As the surface area of the microcapsules produced in the experiments with smaller amount of oil is higher, a greater amount of polymer is necessary to cover their surface. If the gelatin and gum Arabic amount used in the experiment is maintained constant and at this condition (higher surface area) this amount is not enough to cover the surface of all droplets the encapsulation efficiency is reduced.

CONCLUSIONS

Roasted coffee oil was successfully microencapsulated by complex coacervation in gelatin/Gum Arabic crosslinked microcapsules. The oil recovery, microencapsulation efficiency, average diameter and size distributions were evaluated as a function of the production parameters, stirring rate and amount of roasted coffee oil. The parameters presented influence at the oil recovery and encapsulation efficiency, but to the average diameter and size distribution a limiting value was reached under those conditions.

ACKNOWLEDGMENTS

The authors thank Fundação Araucária for the financial support.

REFERENCES

BACHTSI, A. R., C. J. BOUTRIS, AND C. KIPARISSIDES. 1996. Production of oil-containing crosslinked poly (vinyl alcohol) microcapsules by phase separation: Effect of process parameters on the capsule size distribution. *Journal of Applied Polymer Science* 60(1):9–20.

FIGUEIREDO, A. C., AND M. G. MIGUEL. 2010. Aromatic plants , spices and volatiles in food and beverages. *Flavour and Fragrance Journal* 25:251–52.

FREIBERGER, E. B. 2013. *(Master thesis)*. Produção de nanocápsulas de poli(L-ácido lático) contendo óleo de café torrado. Universidade Tecnológica Federal do Paraná-Campus Campo Mourão.

HOFMANN, T., AND SCHIEBERLE, P. 2002. Chemical Interactions between Odor-Active Thiols and Melanoidins Involved in the Aroma Staling of Coffee Beverages. *Journal of Agricultural and Food Chemistry* 50(2):319–26.

KOBAYASHI, M. L., AND BENASSI, M. T. 2012. Caracterização sensorial de cafés solúveis comerciais por Perfil Flash. *Semina: Ciências Agrárias* 33(2):3069–74.

LEIMANN, F. V., GONÇALVES, H. G., MACHADO, R. A. F., AND BOLZAN, A. 2009. Antimicrobial activity of microencapsulated lemongrass essential oil and the effect of experimental parameters on microcapsules size and morphology. *Materials Science and Engineering: C* 29(2):430–36.

MAJI, T. K., AND HUSSAIN, R. 2009. Microencapsulation of Zanthoxylum limonella Oil (ZLO) in Genipin Crosslinked Chitosan – Gelatin Complex for Mosquito Repellent Application. *Journal of Applied Polymer Science* 111:779–85.

DE OLIVEIRA, A. L., CRUZ, P. M, EBERLIN, M. N., AND CABRAL, F. A. 2005. Brazilian roasted coffee oil obtained by mechanical expelling: compositional analysis by GC-MS. *Food Science and Technology* 25(4):677–82.

PRATA, A. S., ZANIN, M. H, RÉ, M. I., AND GROSSO, C. R. F. 2008. Release properties of chemical and enzymatic crosslinked gelatin-gum Arabic microparticles containing a fluorescent probe plus vetiver essential oil. *Colloids and surfaces. B, Biointerfaces* 67(2):171–78.

SHINNAR, R., AND CHURCH, J. M. 1960. Predicting particle size in agitated dispersions. *Industrial & Engineering Chemistry* 52(3):253–56.

TAYADE, P. T., AND RAJENDRAKUMAR, D. K. 2004. Encapsulation of water-insoluble drug by a cross-linking technique: effect of process and formulation variables on encapsulation efficiency, particle size, and in vitro dissolution rate. *The AAPS Journal* 6(1):112–19.

XIAO, ZUOBING, WANLONG LIU, GUANGYONG ZHU, RUJUN ZHOU, AND YUNWEI NIU. 2014a. Production and characterization of multinuclear microcapsules encapsulating lavender oil by complex coacervation. *Journal of the Science of Food and Agriculture* 94(8):1482–94.

XIAO, ZUOBING, WANLONG LIU, GUANGYONG ZHU, RUJUN ZHOU, AND YUNWEI NIU. 2014b. A review of the preparation and application of flavour and essential oils microcapsules based on complex coacervation technology. *Flavour and Fragrance Journal* 29(3):166–72.

XING, FUBAO, GUOXIANG CHENG, BINGXING YANG, AND LINRONG MA. 2004. Microencapsulation of Capsaicin by the Complex Coacervation of Gelatin , Acacia and Tannins. *Journal of Applied Polymer Science* 91:2669–75.

ZHAO, Y., BROWN, M. B., AND JONES, S. A. 2010. The Topical Delivery of Benzoyl Peroxide Using Elegant Dynamic Hydrofluoroalkane Foams. *Journal of Pharmaceutical Sciences* 99(3):1384–98.