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Microencapsulation of tocopherols in lipid matrix by spray chilling method

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Abstract

Microcapsules were prepared by spray chilling, using interesterified fat with no trans isomers fatty acids prepared with fully hydrogenated soybean oil and soybean oil in the ratio of 70:30% w/w respectively, and α-tocopherol as active ingredient. For this work we used a design with four trials a proportion of Lipid: Core material (%) 90:10, 80:20, 95:5 and 85:15 coded A, B, C, D respectively, which were subjected to storage for 180 days at three different temperatures (BOD at 22°C and -18°C freezer, in the absence of light and temperature at 25±3°C with light) and X-ray diffraction analysis (0, 60 , 120, 180 days) and calorimetric measurements (time zero) were performed. The results showed values of encapsulation efficiency above 90%. Thermograms obtained by DSC, in zero time, showed no differences between the samples. The diffraction patterns obtained by X-ray diffraction, were very similar among the tests and were found the presence of three major peaks in the following angles 2θ = 19.3 ° d = 4.6A, 2θ = 22.8 ° d = 3.8A and 2θ = 23.1 ° d = 3.7A, which appear to be associated with the β polymorphic form. The percentage of crystallinity of the formulations obtained over time was relatively low, below 30% with no significant difference due to storage time and very similar between the tests.

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Keywords: spray chilling, microencapsulation, interesterified fats, X-ray diffraction

1. Introduction

Vitamins are essential micronutrients that contribute to normal growth and health maintenance. Some studies highlight the role of some vitamins and antioxidants that might protect the body against free radicals, preventing degenerative diseases. Nutritionally, alpha-tocopherol is the most important representative of the group of compounds with vitamin E activity, because it prevents cell damage by inhibiting lipid peroxidation, the formation of free radicals and cardiovascular disease, improves blood circulation, regenerate tissues and is useful in treating fibrocystic breasts and premenstrual syndrome [1].

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When an industrially produced food does not have the minimum recommended amounts of certain nutritional compounds such as vitamins that can be enriched. According to Brazilian Ordinance Nº. 31, dated January 13, 1998, it is allowed to enrich foods with vitamin E, adding alpha-tocopherol or other substances with vitamin E activity, they can e used in the form of solutions or pre-mix. However, vitamin E is very unstable because it is slowly oxidized by atmospheric oxygen through a reaction catalyzed by light and heat in the presence of metals such as silver and iron. Microencapsulation is a technology for coating substances for protection and/or controlled release of them. In the case of alpha-tocopherol it becomes a possible alternative for transport, storage and use in industrial processes. Likewise, the microencapsulation particles employing as lipid matrix of active substances have been proposed as an alternative to liposomes and colloidal systems [2], due to its great flexibility on packaging materials and size of the particles. Among the lipids, it can be used, phospholipids, triacylglycerols, waxes, fatty acids or mixtures of them. This work aimed to study the stability of lipid microcapsules prepared by spray chilling, composed of interesterified fats (no trans isomers) with fully hydrogenated soybean oil and α-tocopherol as the active ingredient to be encapsulated antioxidant α-tocopherol.

2. Materials & Methods

2.1. Raw Material

Interesterified fat coming from cottonseed oil with fully hydrogenated palm oil, refined and deodorized produced at UNICAMP (Laboratory of oils and fats) Food Engineering. Fully hydrogenated soybean oil (FHSO) industrially refined and deodorized, provided by Triângulo Alimentos. Core material α-Tocopherol - Roche (E307 *).

2.2. Production of lipid microcapsules (LMP)

Four trials were prepared with different Lipid:core material (%) ratios 90:10, 80:20, 95:5 and 85:15 coded A, B, C and D respectively as coating lipid matrix composed of interesterified fat without *trans* isomers with fully hydrogenated soybean oil in the ratio of 70:30% w / w respectively, and α-tocopherol as the active ingredient to be encapsulated .Lipid matrices were melted and kept in bath temperature of 65ºC. The α-tocopherol (concentration from 5.000 to 20.000 mg tocopherol/100 g of lipid) was added and homogenization using an Ultra-Turrax for 5 min. The lipid microcapsules were sprayed using a double fluid atomizer (part of the Mini Spray Dryer Büchi-191, nozzle diameter of 0.7 mm (Flawil, Switzerland) also heated to 65°C and air pressure of 0.25 MPa, with atomization inside a chilled chamber (Ultratorac, LKB - Bromma, Germany) at 10°C. [3]. The capsules were placed in Petri dishes and subjected to storage for 180 days at three different temperatures (BOD at 22°C and -18°C freezer in the absence of light and temperature of 25±3°C with light).

2.3. Encapsulation efficiency

The encapsulation efficiency was through the quantification of tocopherol by high performance liquid chromatography (HPLC). According to AOCS method Ce 8-89 [4]. The calculation was determined as follows:

$$EE\ (\%) = Quantified\ Tocopherol / Initial\ Tocopherol \quad (1)$$

2.4. Thermal Analysis

The crystallization and melting curves of the capsules at different concentrations were obtained by scanning calorimetric analysis, using a DSC Perkin Elmer TA7 (Germany), module with automatic cooling module. Analysis were conducted with approximately 10 mg capsules in aluminum pans inside an inert atmosphere (N2), according to method of AOCS Cj 1-94 (2004) [5].

2.5. X-ray diffraction

Polymorphic forms of lipid microcapsules were analyzed according to AOCS Method Cj 2-95 (2004) [5].The XRD patterns were obtained using Shimadzu model XRD 7000 (the Toky, Japan) using Bragg Brentano geometry (θ: 2θ) with Cu Kα radiation (λ = 1.54056 Å, 40 kV and 30 mA) with. measuring range in 2θ from 5 to 35 degrees, with step size of 0.02 degrees using counting time of 0.03°/sec at room temperature (23±1ºC).The polymorphic forms were identified from the crystal spacing. The α form shows a single diffraction line at 4.15 Å. The β form material is characterized by two strong diffraction lines, with a prominent line at 3.8 and 4.2 Å, while the β form is associated with a series of diffraction lines, with a prominent line at 4.6 Å and the lines of lower intensity of 3.7 and 3.8 Á. The Index of Relative Crystallinity (Ic) was quantitatively estimated according to the method proposed by Rabek [6].

3. Results & Discussion

3.1. Encapsulation efficiency

Table 1 shows the efficiencies and concentrations of tocopherol present in the microcapsules determined in trials on day after collection. The results showed a high efficiency from 90,0 to 95,8%, and concentration from 4.497,9 to 18.517,59 mg/100 g. Regarding the α-tocopherol retention after 180 days of storage (Table 2) the results were considerably higher for the studied systems ranged from 94,1 to 99,7%.

Table 1. Microencapsulation efficiency of α-tocopherol (%±SD) of the four trials

Trials	Efficiency (%)	Average Concentration (mg/100g)	SD
A	95,8 ± 2,99	9.575,18	3,11
B	92,6 ± 3,45	18.517,59	6,10
C	90,0 ± 3,81	4.497,90	4,20
D	95,4 ± 1,34	14.323,22	1,39

Table 2. Retention values of tocopherol in the microcapsules after 180 days.

Temperature of storage	Trials	% Retention	SD
22°C (BOD)	A	99,0 ± 0,42 [a]	0,45
	B	99,0 ± 3,89 [a]	4,20
	C	98,5 ± 7,35 [a]	7,16
	D	99,1 ± 3,25 [a]	5,04
25 ± 3°C (Environment)	A	94,8 ± 2,05 [a]	2,21
	B	94,1 ± 0283 [a]	0,34
	C	98,8 ± 2,55 [a]	2,90
	D	94,8 ± 1,28 [a]	1,34

Temperature of storage	Trials	% Retention	SD
-18°C (Freezer)	A	99,4 ± 1,41 [a]	0,46
	B	99,7 ± 4,17 [a]	1,27
	C	99,1 ± 6,50 [a]	7,32
	D	99,7 ± 0,78 [a]	0,80

The same lowercase letters, in the column shows no statistical difference at $p < 0.05$

The retention of tocopherol for capsules were kept at -18°C were higher, especially in relation to tests A, B and D stored at room temperature, although this difference was not significant.

The small variation observed over time in each test individually assessed indicates that the lipid matrix using interesterified and lipid FHSO, might have functioned as agent for preventing the expulsion of the core material over time and creating microstructures for a good housing of the drug consequently obtaining a higher encapsulation efficiencies with higher tocopherol retentions.

3.2. Thermal Behavior

The Differential Scanning Calorimetry (DSC) is a technique in which the difference in energy supplied to a substance and the reference is determined as a function of temperature while both are subjected to a controlled temperature program. Evaluation by DSC provides direct measurements of the energy involved in the processes of melting and crystallization of oils and fats [7].

The results of thermal properties of melting and crystallization of lipid microcapsules obtained by Differential Scanning Calorimetry (DSC) at time zero was evaluated with the objective of verify possible differences in relation to different proportions of lipid /core material, because it could have different crystallization and melting behaviors for obtained peaks. The temperature "onset", peak temperature, temperature “end” and enthalpy variation are summarized in Tables 3 and 4 and the thermograms with curves of melting and criytallization are presented in Figures 1 and 2.

Table 3. Calorimetric parameters of melting of the trials analyzed at time zero. Melting onset temperature (T_{of}), melting peak temperature (T_{pf}), melting enthalpies (ΔH_f), and melting end temperature ($T_{end\ fus}$).

Trial	T_{of} (°C)	$T_{pf}1$ (°C)	$T_{pf}2$ (°C)	ΔH_f1 (Jg^{-1})	ΔH_f2 (Jg^{-1})	$T_{end\ fus}$ (°C)
A	39,6	50,7	59,7	54,68	28,96	63,5
B	32,7	48,5	58,2	37,98	24,63	62,2
C	40,1	51.7	60,2	67,76	33,42	64,0
D	37,7	49,4	58,8	50,15	32,50	61,8

Table 4. Calorimetric parameters of crystallization of the trials analyzed at time zero. Crystallization onset temperature (T_{oc}), crystallization peak temperature (T_{pc}), crystallization enthalpy (ΔH_c), crystallization end temperature ($T_{end\ cryst}$)

Trial	T_{oc} (°C)	T_{pc} (°C)	ΔH (Jg^{-1})	$T_{end\ cryst}$ (°C)
A	37,5	35,9	60,7	-2,7
B	34,6	33,5	58,7	-7,5
C	38,4	37,0	68,4	-2,5
D	36,3	35,0	66,0	-5,1

The values obtained for the peaks recorded in both the curve of melting and crystallization indicated as being directly related to the proportion of lipid/core material used for the production of microcapsules. Table 3 shows for the evaluated trials that the melting temperature "endset" occurred over a range of 62,2°C to 64,0°C corresponding to the tests B and C respectively, which showed lower and higher amount of lipid matrix . Similarly the values of crystallization temperature curve recorded in table 4 indicate also be related to the amount of lipid in the matrix, because these values varied over a range of -2,5°C to -7,5°C corresponding to the tests C and B respectively with higher and lower amounts of lipid matrix present in the microcapsules. So even if these effects also showed an association with the respective reduction and increase in the values of melting and crystallization enthalpy (ΔH). The melting curve (Figure 1) was characterized by the formation of two distinct endothermic peaks separated by an exothermic peak could be attributed to the polymorphism of FHSO. However it is assumed that the first peak corresponds to the melting of alpha form and the second to β form. The exothermic peak between the two endothermic peaks represents the crystallization of the unstable form to a more stable form.

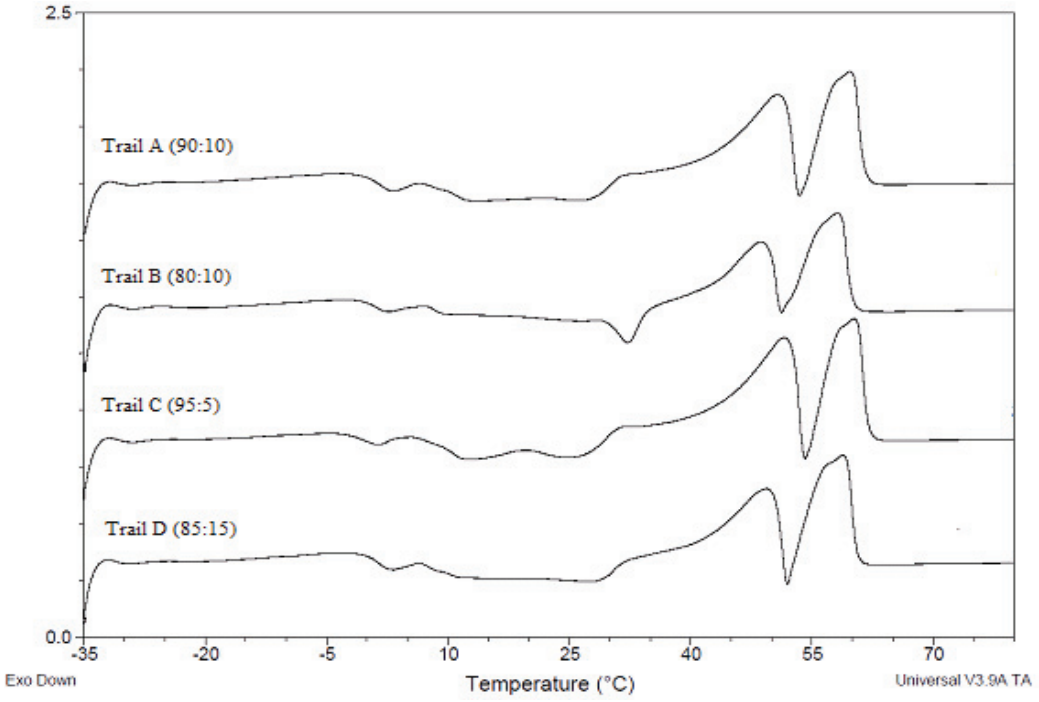

Fig. 1. Melting thermograms of the trials.

For crystallization curves (Fig. 2) the thermograms obtained were characterized by the formation of two peaks of a smaller magnitude that appears close to 0°C probably due to triglycerides from interesterified fat and another large peak near 40°C represents the crystallization of FHSO. Regarding the presence of α-tocopherol on the microcapsules, the results indicate that it did not affect the melting and crystallization of microcapsules in the tests, due to solubility that exists in the lipid matrix.

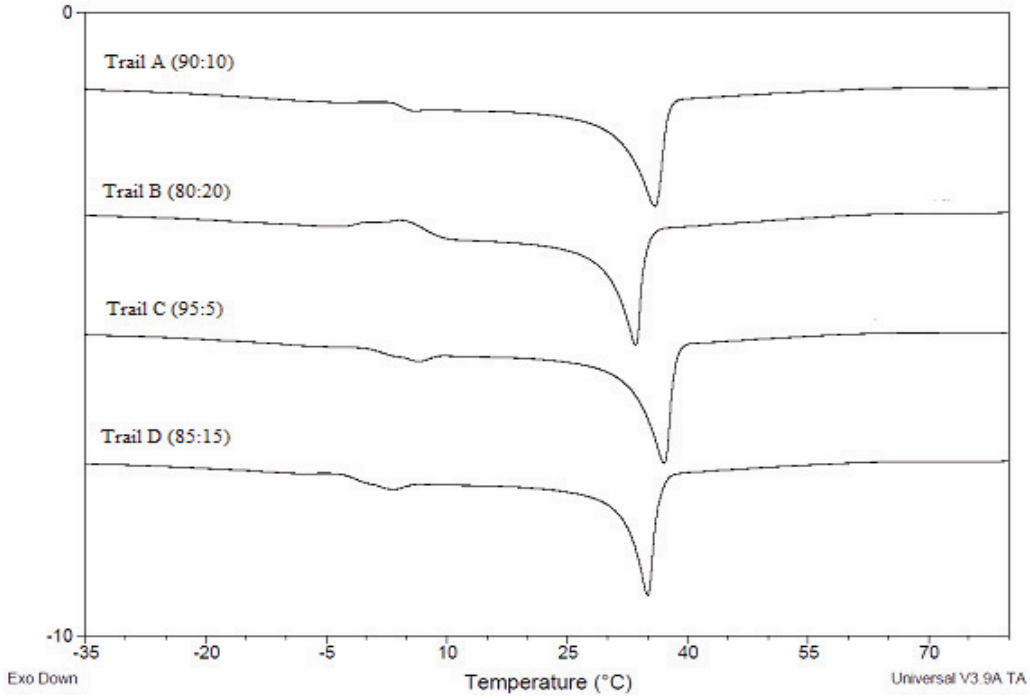

Fig. 2. Crystallization Thermograms of the trials.

3.3. X-ray diffraction

A very important point to be considered in the characterization of lipid microcapsules is the possible occurrence of different polymorphic forms. Polymorphic forms are solid phases of the same chemical composition that differ in crystal structure and due to its high level of molecular complexity, can pack in several crystalline structures relatively stable and different. The polymorphic forms of triacylglycerols can occur in three crystalline forms, α, β ' and β increasing thermodynamic stability respectively. The polymorphic reorganization for more organized and more energetically favourable is identified as a problem in the encapsulation of active material whose wall are lipids, since this reorganization could result in expulsion from the core material by producing a more crystalline material during polymorphic lipid phase transition after the production of lipid microcapsules using the spray chilling technique [8]. Diffractograms of the test capsules stored for 180 days at different temperatures showed similar specter and shape of the obtained curves at time zero. The results demonstrated the presence of three major peaks detected in the following angles 2θ = 19.3° d = 4.6Å, 2θ = 22.8° d = 3.8Å and 2θ = 23.1° d = 3.7Å. The standards were similar and literature usually associates it with the polymorphic form β, characteristic of most triacylglycerols and fatty acids. While the percentages of crystallinity of the trials of lipid microcapsules shown in Table 5 were relatively inferior to 30%, without significant difference with respect to storage time. These results were expected, whereas the lipid mixture (70% interesterified lipids, and fully hydrogenated soybean oil 30%) was the same for all tests, which only differed in the proportion of matrix / core material. The low crystallinity observed for the formulations have been driven by the presence of amorphous solids which are lipid but not crystalline that contribute to increase the retention

of core material inside the matrix. Although there are no quantitative data in the literature, the benefits of amorphous structures on microencapsulation efficiency of products are well known [9].

Table 5. Degrees of crystallinity (%) of trials evaluated according to time and temperature.

Temperature of storage	Time (days)	Trials			
		A	B	C	D
22 °C (BOD)	0	22,2	23,1	28,3	23,7
	60	24,5	24.1	28,8	24,6
	120	28,6	24,0	26,8	26.2
	180	24,7	21,4	27,4	26,5
25 ± 3 °C (Environment)	0	22,2	23,1	28,3	23,7
	60	29,4	28,3	26,1	23,6
	120	29,2	24,3	27,0	24,5
	180	26,7	21,6	24,8	20,4
-18 °C (Frezzer)	0	22,2	23,1	28,3	23,7
	180	20,5	21,0	23,7	21,8

4. Conclusion

The production of lipid microcapsules containing α-tocopherol was possible in lipid matrices using spray chilling, showing high efficiency of microencapsulation and high levels of retention of the active product. Were observed good stability over time and storage temperature indicating that the lipid matrix using interesterified fat and FHSO, may have functioned as agent for preventing the expulsion of the core material over time and creating microstructures for a good accommodation and consequently obtain higher drug encapsulation efficiencies with high concentrations of tocopherol retention.

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References

[1] Kaneko, T., Nakano, S., and Matsumoto, M. 1991. Protective e•ects of vitamin E on linoleic acid hydroperoxide induced injury to human endothelial cells. Lipids, 26, 345-348.

[2] Müller, R.H., Mader, K., & Gohla S. 2000. Solid lipid nanoparticles (SLM) for controlled drug delivery – A review of the state of the art. European Journal of Pharmaceutics and Biopharmaceutics, 50, 161-178.

[3] Leonel, J. A. 2008. Tese de mestrado. Faculdade de Engenharia de Alimentos, da Universidade Estadual de Campinas.

[4] AOCS 1989. American oil Chemists' Society. Official methods and recommended practices of the AOCS. 4a. ed. Champaign.

[5] AOCS 2004. American oil Chemists' Society. Official methods and recommended practices of the AOCS. 4a. ed. Champaign.

[6] Rabek, J.F. 1980. Experimental Methods in Polymer Chemistry: Applications of wide-Angle X-Ray Difraction (WAXD) to the Study of the Structure of Polymers, Wiley-Interscience Chichester, U.K. p. 505.

[7] MA, C. Y.; Harwalkar, V. R.; Maurice, T. J. 1990. Instrumentation and techniques of thermal analysis in food research. In: HARWALKAR, V. R. & MA, C. –Y., Thermal Analysis of Foods. London, Elsevier, cap 1, p. 1-15.

[8] Müller, R. H.; Radtke, M.; Wissing, S. A. 2002a. Nanostructured lipid matrices for improved microencapsulation of drugs. International Journal of Pharmaceutics, n.242, p. 121-128.

[9] Alves, G. P.; Santana, M. H. A. 2004. Phospholipid dry powders produced by spray drying processing: structural, thermodynamic and physical properties. Powder Technology, v. 145, p. 139-148.

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