Materials Research, Vol. 10, No. 1, 57-62, 2007

Encapsulation of the Alpha-tocopherol in a Glassy Food Model Matrix

Melina Campagnaro Farias[a], *Miriam Leite Moura*[b], *Leonardo Andrade*[c], *Maria Helena Miguez Rocha Leão*[a,d]*

[a]*Programa de Ciência de Alimentos Instituto de Química, Universidade Federal do Rio de Janeiro – UFRJ,*
[b]*Faculdade de Farmácia, Universidade Federal do Rio de Janeiro – UFRJ,*
[c]*Departamento de Histologia e Embriologia, Universidade Federal do Rio de Janeiro – UFRJ,*
[d]*Escola de Química, Universidade Federal do Rio de Janeiro – UFRJ,*
CT, Bloco E, sala 203, 21910-900 Rio de Janeiro - RJ, Brazil

Received: July 5, 2006; Revised: January 25, 2007

α-tocopherol was encapsulated in a glassy food model based on solution of maltodextrin (DE 20) and gelatin, through the use of quick freeze and freeze-drying procedures. The ratio of the maltodextrin, α-tocopherol and gelatin was 3:2:1 respectively. The morphology of the glassy food model was observed by scanning electron microscopy, whose analyses showed a slightly smooth surface and a rather fragile and porous structure due to cavities formed by ice crystals during freezing, and the absence of crystalline structure. It was observed by x ray diffraction that the material is an amorphous state. The samples stored in a specific plastic vessel isolated from gas and light held its amorphous state with no variations that concern to morphology and keeping 100% of the encapsulated α-tocopherol up to 90 days at 25 and 35 °C.

Keywords: *alpha-tocopherol, encapsulation, freeze-drying, glassy food model*

1. Introduction

Vitamin E is the generic term for tocols and tocotrienols that exhibit vitamin activity similar to α-tocopherol. Tocols are 2-methyl-2 (4', 8', 12'-trimethyltridecyl) chroman-6-ols, while tocotrienols are identical except for the presence of double bonds at positions 3',7', and 11' positions in the side chains. Tocopherol, the main compounds has vitamin E activity in foods, is derivative of tocol family, and has one or more methyl groups at 5, 7 or 8 positions of the ring structure (chromanol ring). The α, β, γ forms of tocopherol and tocotrienol differ according to the number and position of the methyl groups and thus differ significantly in vitamin E activity[1].

All tocopherols and tocotrienols are nonpolar and present mainly in the lipid phase of foods. All tocopherols and tocotrienols, when not esterified, have the ability to act as antioxidants; quench free radicals by donating the phenolic H and an electron. Tocopherols are natural constituents of all biological membranes and are thought to contribute to membrane stability through their antioxidant activity. Naturally occurring tocopherols and tocotrienols also contribute to the stability of highly unsaturated vegetable oils through this antioxidant action[1].

Vitamin E requires special transport mechanism in plasma, body fluids and cells due to its hidrofobicity. In humans, vitamin E is taken up in the proximal part of the intestine depending on the amount of food lipids, bile and pancreatic esterase. It is emulsified together with the fat-soluble components of the food. Lipolysis and emulsification of the formed lipid droplets then lead to the spontaneous formation of mixed micelles, which are absorbed at the brush border membrane of the mucosa by passive diffusion. Including triglycerides, phospholipids, cholesterol and apolipoproteins, the tocopherols are re-assembled to chylomicrons by the *Golgi* apparatus of the mucosa cells. The chylomicrons are stored as secretory granula and eventually excreted by exocytosis to the lymphatic compartment, reaching the blood stream[2].

Vitamin E compounds exhibit reasonably good stability in the absence of oxygen and oxidizing lipids. Anaerobic treatments in food processing, such as retorting of canned foods, have little effect on vitamin E activity. In contrast, the rate of vitamin E degradation increases in the presence of molecular oxygen and can be especially rapid when free radicals are also present. Oxidative degradation of vitamin E is strongly influenced by the same factors that influence oxidation of unsaturated lipids[1].

Dehydrated foods have thermal and physical properties characteristic of amorphous materials and, during dehydration, the amorphous state may encapsulate compounds such as lipids and thereby protect them from oxidation[3]. The structural stability of the glassy matrix[4-6], the diffusivity of oxygen[7,8], the moisture content[9,10], and the presence of pro-and antioxidants are among the factors to be considered when the oxidative stability of products with encapsulated lipids is evaluated.

An amorphous matrix at a temperature below the glass transition temperature has an extremely high viscosity and changes in macromolecular conformation are extremely slow. This[11] further causes low molecular mobility in the glassy state, which is why collision of reactants in the glassy matrix is limited. The glassy state is therefore expected to inhibit most chemical reactions involving reactants trapped in the glassy matrix[11]. A number of studies have investigated the diffusion of small molecules in high-viscosity carbohydrate systems[12-14], whereas others have dealt with the consequences of the physical state on the rate of chemical reactions, such as non-enzymatic browning[15-17]. General conclusions seem to be that the diffusivity of molecules in a carbohydrate system increases dramatically at temperatures close to the glass transition temperature[14,18,19].

As the stability of foods is mainly dependent on the water content and because the glass transition temperature (*T*g) is also highly

*e-mail: mhrl@eq.ufrj.br

sensitive to this parameter, the glass transition concept appeared to be a powerful tool for understanding the mechanisms of processes in food products and for controlling their shelf-life[20,21].

Indeed, the glass transition temperature was considered as a reference temperature: below *T*g, the food was expected to be stable; above this temperature, the difference ($T - Tg$) between *T*g and the storage temperature *T* was assumed to control the rate of physical, chemical and biological changes. The glass transition was also shown to allow the identification of the water content (and temperature) domains where a product could exhibit either a hard, crispy texture or a soft, rubbery or viscous one. Moreover, the variations of mechanical and transport properties in the glass transition range could contribute to a better control of some food processing operations such as drying and freeze-drying, extrusion and flaking[20].

Among various food procedures, drying is the most commonly used method to preserve and reduce the volume of the food materials. The collapse of the dehydrating food during freeze drying, stickiness of the product during spray drying, caking and agglomeration of powders during processing and storage are some of the properties, which are related to the glass transition temperature[22]. These defects also lead to structural changes on matrices like stability loses of the coated component due to an increase of the molecular mobility, the diffusivity of oxygen in the matrices, decrease of the matrices viscosity and increase of the chemical reactions involving reactants trapped in the glassy matrix[22].

The objective of the present study is to encapsulate α-tocopherol protecting against loss by oxidation during storage at 35 °C for a period less of 3 month using a hydrophilic matrix in order to obtain hydrosoluble particles. It is important to note that due to its lipophilic nature the α-tocopherolis generally is microencapsulated using hydrophobic matrices as waxes and oils[23]. However, improved experimental techniques may now be combined, and we have embarked on studies of α-tocopherol oxidation in food with glassy state. The encapsulation technique was through fast freezing followed by freeze-drying. Besides that, we developed a new way to encapsulate α-tocopherol, water-soluble one, which allowed more industrialized use of this vitamin in order to enrichment foods such as, powder milk, flours, wholemeal.

2. Material and Methods

α-tocopherol _ Sigma; Maltodextrin (De = 20) MOR REX 1920. Corn Products Brazil; Gelatin - Vetec; absolute Etanol- Vetec; Ferric Chloride – Reagen; α-α – dipyridyl – Merck (Germany); EDTA – Carlo Erba; FEMTO 482 Espectrophotometer; 002 CB – FANEM incubator;

3. Experimental

A glassy food model composed of carbohydrates and proteins was previously established[3]. Maltodextrin (5.01 g) was dissolved in distilled water at 30 °C and gelatin (1.67 g) in boiling water. After a fast cooling of the gelatin solution on tap water, the solutions were mixed and α-tocopherol (3.34 g) added. The proportion of the blend was 3:2:1, maltodextrin, α-tocopherol and gelatin, respectively. After that, the blend was emulsified for 6 minutes, divided in two parts and transferred to Falcon™ polystyrene conic bottom tubes, isolated from the air and light. The samples were frozen in liquid nitrogen and immediately freeze-dried ($p \sim 91 \times 10^{-3}$ mbar; 48 hours; -48 °C). The dried matrices were stored in an incubator at 25 and 35 °C.

4. Morphological Characterization

Scanning Electron Microscopy (SEM) was used to investigate the structure of the glassy food model and surface morphology. The dried samples were prepared through direct deposition on a conductive carbon tape covering aluminum stubs, gold-sputtered (Bal-Tec SC 050, Germany), and examined in the a Jeol Scanning Microscope (JSM-5310), operated at 15 kV with a working-distance of 10 mm.

5. X Ray Diffraction

The x ray diffraction patterns were recorded for the samples using a powder diffractometer (HGZ-4, Germany) and generator (ID 3000, Germany). The powder samples were carefully pressed on double-face tape covering aluminium trays, and exposed to CuK_x radiation ($\lambda = 15418$ Å) at diffraction angles of 2θ, from 10 °C to 100 °C (step size 0.05, time per step 1.0 s).

6. Analyses of Encapsulated α-Tocopherol

The samples were transferred to Falcon™ tubes, isolated from air and light, and stored up to 90 days in an incubator at 25 and 35 °C. The analyses were made in triplicate, periodically, up to 90 days. Colorimetric assay was used for quantitative determination of the encapsulated α-tocopherol[24]. The α-tocopherol was released from the matrices by water dissolution, which 100 mg of the encapsulate sample were dissolved in 100 mL of water at 35 °C. Aliquots of 1 mL (1 mg/mL) from this solution were transferred to 25 mL volumetric balloon and fulfilled with absolute Ethanol. From this alcoholic solution, aliquots of 1 mL were transferred to assay tubes where 1 mL of α'-α'-dipyridyl (0.6% w/v in absolute ETOH) and 1 mL of Ferric Chloride (0.25% w/v dissolved in absolute ETOH) and EDTA (0.35% w/v dissolved in distilled water) were added and mixed for 30 s. The absorbance of the mixture was read at 520 nm in a 1 cm diameter cell. A blank solution containing the reagents cited above but without the sample was read in the same wavelength and used to calculate the results. The α-tocopherol correspondent was calculated from the regression equation of the standard curve.

A quantitative determination and the oxidation rate of 2 g of the α-tocopherol sample, uncoated have been used. About 100 mg of these samples were accurately weighed periodically and submitted to a quantitative analysis, up to 45 storage days and 25 °C temperature, using the same methodology of the coated one. The results obtained from the two assays have been compared.

7. Statistical Analysis

The results were statistical analyzed by the linear model analysis of variance (ANOVA) and Tukey test ($p < 0.05$) for main effects of storage time and temperature between the matrices and interaction as fixed factors using software (ORIGIN).

8. Results and Discussion

8.1. Avaliation of the encapsulated α-tocopherol

The calibration curve used to calculate the α-tocopherol content in the matrix, was expressed by the following linear equation: $Y = 4.7629x + 0.0019$; $R^2 = 0.9973$. Where **Y** is the absorbance and **X** is the α-tocopherol concentration. Maltodextrin and gelatin were used in this work, because they are appropriate to encapsulate oil particles efficiently.

The results of the quantification analysis of the α-tocopherol stored at two different temperatures (25 and 35 °C) are similar, confirming the stability of the treatmenst (Figure 1).

The amorphous matrices may maintain its structure during storage time and protect the α-tocopherol from oxidation and losses of biological activity. This protection was observed during the storage time when the thermal treatments, 25 and 35 °C, were compared.

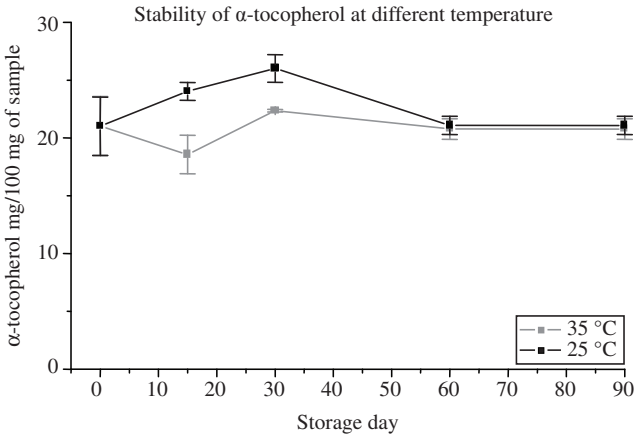


Figure 1. α-tocopherol analysis determination up to 90 days.

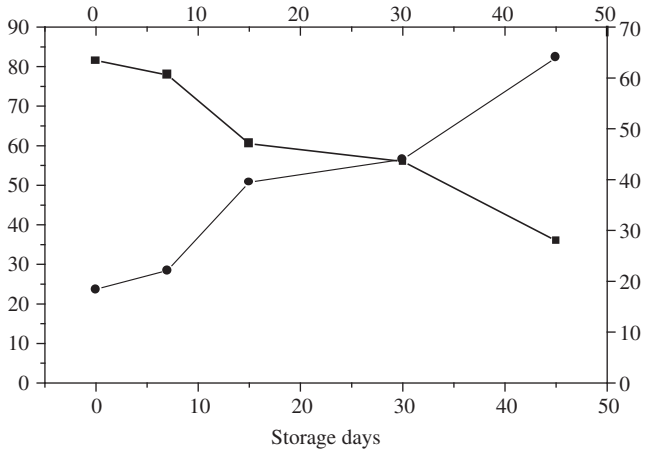


Figure 2. Degradation kinetic uncoated α-tocopherol up to 45 days. α-tocopherol mg/100 mg of sample (■); Degradation of α-tocopherol mg/100 mg of sample (•).

The Figure 2 shows the quantitative determination and the degradation kinetic of the uncoated α-tocopherol at 25 °C up to 45 storage days. This procedure was used in order to compare stability of the uncoated and coated α-tocopherol under same experimental conditions.

It was weighted 81.57 mg of α-tocopherol of initial 100 mg and degradation rate about 18.43% at the beginning of these analyses, which is explained by its exposition to light and air, that lead isomerization of its structure and loses on biological activity. After 45 days of storage uncoated α-tocopherol had quantitative determination of 36 mg of 100 mg degradation rate about 64%, indicating a possible chemical instability. On the other hand, the coated α-tocopherol shows chemical stability up to 90 storage days, and recovery rate of 85% after the coating procedure.

The results showed in the Figure 1 started from 10 g of coated α-tocopherol content on the encapsulation procedure, where 100 mg of this sample were submitted to quantitative determination up to 90 storage days, and the results showed on the Figure 2 initiate from 2 g of uncoated α-tocopherol, where 100 mg samples were submitted to quantitative determination up to 45 storage days. The content of α-tocopherol for each analysis was different comparing the same storage days, but the chemical instability of the uncoated α-tocopherol was evident.

Accordingly to[3], lipids in dried foods such as milk powder, dried soups and biscuits are often encapsulated in an amorphous food matrix of saccharides and proteins. Lipid oxidation is known to be more significant at interfaces than in bulk and, due to the large surface area of encapsulated oil, it is often found to be rather vulnerable to lipid oxidation. On the other hand, encapsulation in a glassy matrix could be expected to give some protection from oxidation by: preventing or at least limiting oxygen diffusion through the glassy matrix to the lipid phase; and by protecting the oil from oxidation through immobilizing radicals formed by pro-oxidants in the hydrophilic phase.

The collapse of freeze-dried products induces the loss of structure, the reduction of pore size and volume of the food material, which results in the loss of desirable appearance, texture and volatile substances. During the freeze-drying operation, if the temperature of the porous layer or its water content is increased (the product being then above its *T*g), the viscosity is not high enough to support the structure of the sold material and collapse occurs[25-28].

The possible changes in frozen or low-water food products are also the result of chemical or biochemical reactions such as non-enzymatic browning, oxidation or enzymatic reactions[29,30].

Karmas et al.[16] studied the temperature effect on the reaction rate constant of *non-enzymatic browning* in model food systems. They showed that the rate of the reaction is low at temperatures below *T*g and increases as the temperature difference ($T - Tg$) increases above *T*g. Those authors[16], however, underlined that the reaction is also controlled by several other factors such as structural changes or water content independently of its plasticizing effect. Roos and Himberg[31] have also studied this reaction and showed that it is not stopped by the glass-liquid transition of the maltodextrin, lysine and xylose matrix, and is possible in the glassy state.

It was observed that the glassy food model maintained the amorphous structure up to 90 days in different temperatures. Defects related to the matrix such as collapse, crystallization, agglomeration and caking that could affect the stability of the α-tocopherol were not observed.

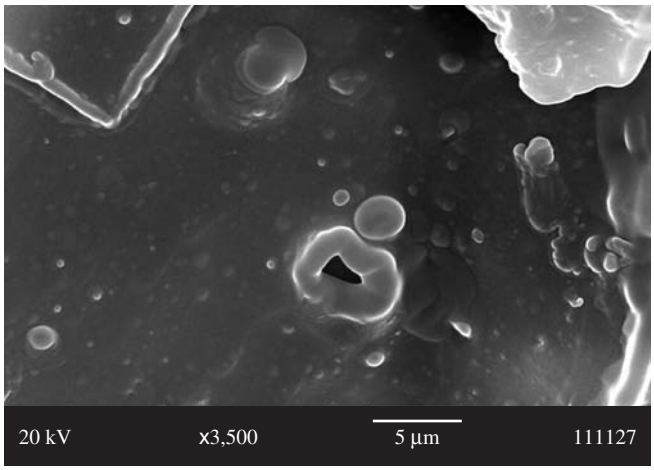
9. Morphological Characterization

The scanning electron micrographics of the flakes can be observed in the Figures 3 and 4. Images from the glassy food model had a relatively smooth surface and a rather fragile and porous structure. The porous observed were possibly formed by cavities left from ice crystals or air bubbles retained during the freezing.

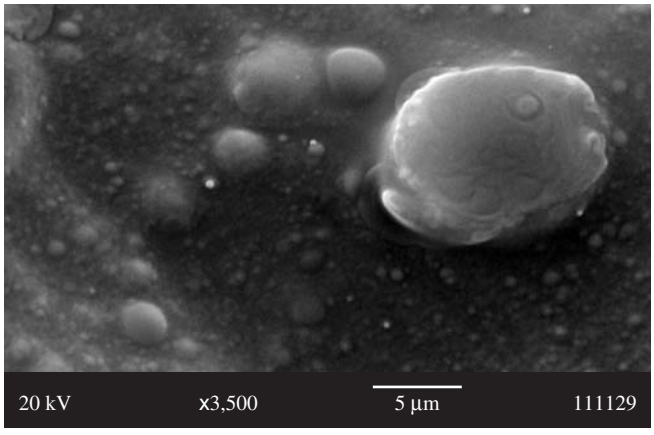
In the freeze drying process, the liquid product is first frozen and then the water is removed by sublimation. This is a low process, and has been considered superior than other drying methods in terms of product quality (taste and aroma) though being relatively expensive. While evaporating the moisture, the product becomes porous in nature and the solid network should be able to hold this porous structure. If the temperature of the dehydrating porous product is above *T*g, the viscosity of the solid material may not be enough to support the structure and "collapse" or shrinkage occurs[32,33].

Accordingly to[13,] the freezing speed will affect the crystal size and the properties of the lyophilized. Rapid cooling leads to small and heterogeneous crystals, improves freezing out effects. The crystal size is a key factor for the speed of sublimation during freeze-drying and also for the reconstitution process. A great number of small crystals and the presence of large amorphous regions may cause a fast sublimation due to formation of a very dense lyophilized network. Slow freezing leads to the formation of large crystals and results in unrestricted sublimation.

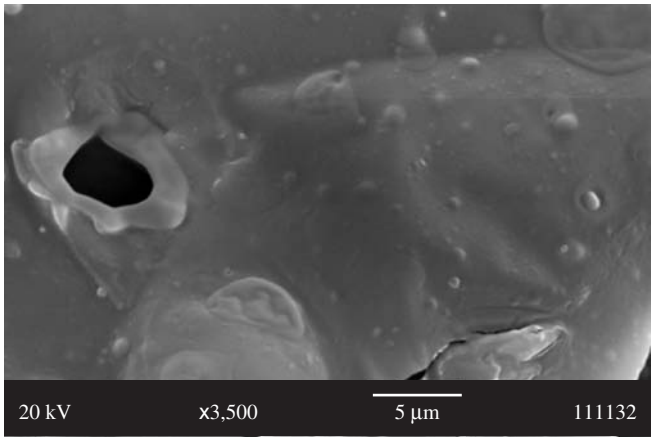
Freeze drying has been shown to be an attractive method for extending the shelf life of foods[34]. The drying of food products in freeze-



(a)

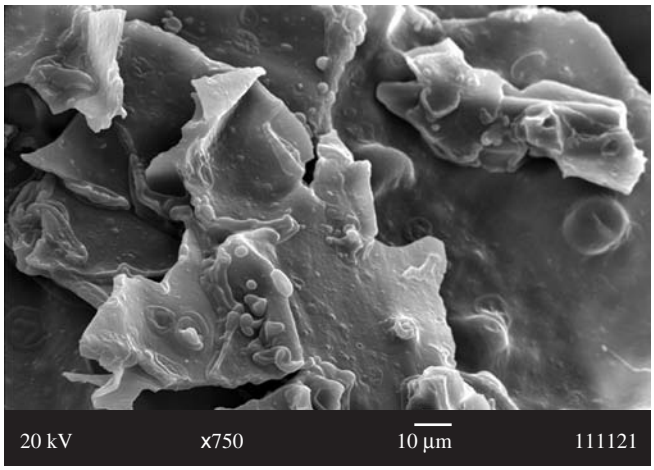


(b)

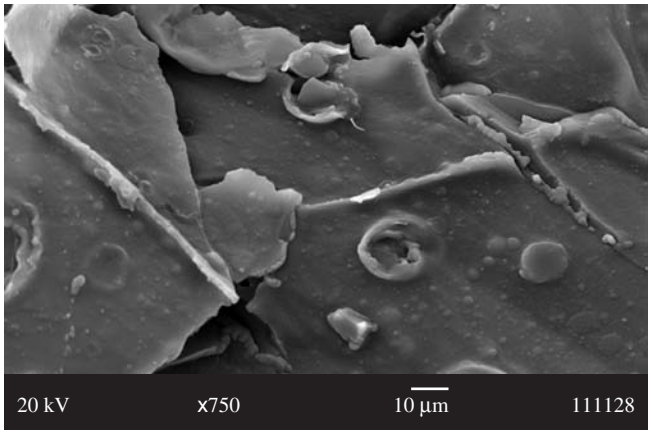


(c)

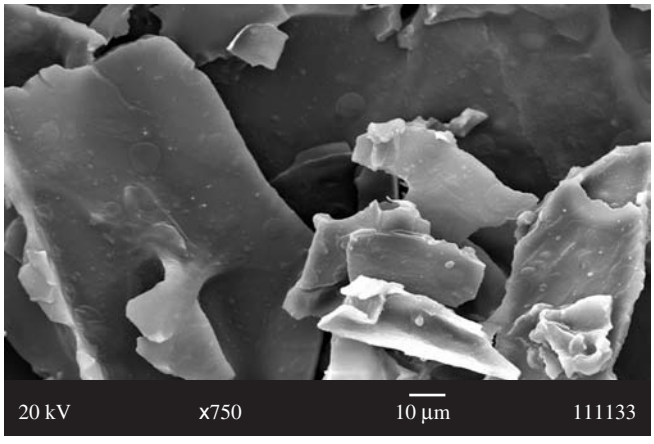
Figure 3. Scanning electron micrograph of the glassy food model at 25 °C. a) after the freeze-drying procedure; b) 60 storage days; and c) 90 storage days.



(a)



(b)



(c)

Figure 4. Scanning electron micrograph of the glassy food model at 35 °C. a) after the freeze-drying procedure; b) 60 storage days; and c) 90 storage days.

drying has two main characteristics: 1) Virtual absence of air during processing: the low processing temperature and the absence of air prevent deterioration due to oxidation or chemical modification of the product, 2) Drying at temperatures lower than ambient temperature: products that decompose or undergo changes in structure, texture, appearance, and/or flavor as a consequence of high temperature can be dried under vacuum with minimum damage[35].

The Figure 3 and 4 shows the glassy food model stored more than 90 days at 25 and 35 °C respectively. The glassy food model maintained the amorphous structure and it retained all α-tocopherol

present at the initial mixture. More over, changes in macromolecular conformation such as collapse, crystallization, agglomeration and caking were not occurred. Thus, in both temperatures the glass transition temperature of the matrix was not exceeded, allowing its chemical integrity up to storage days tested.

10. X Ray Diffraction Measurements (XRD)

The Figure 5 and 6 shows a spectrum of the glassy food model matrices storage at 25 and 35 °C respectively, with the characteristics of an amorphous structure: line broad, x ray signals without definition, x ray intensities without uniform behavior, which are peculiar of the amorphous structure. Showing up that the storage temperature does not exceed the glass transition temperature so, changes in macromolecular conformation such as collapse, crystallization that leads to structural changes on matrices and stability loses of the encapsulated α-tocopherol was not observed up to the storage time and temperature.

The Figure 6 shows a spectrum of the glassy food model matrices storage at 35 °C, which had similar profile of the Figure 5.

11. Conclusions

The morphological characterization by MEV and the x ray diffraction spectra showed the amorphicity of the matrix without crystallization. The results show that the glassy matrix is able to protect the encapsulated α-tocopherol from oxidation. The glassy food model is believed to be of value also for future investigations, since storage conditions often encountered for matrix like that during tropical condition can be imitated without collapse of structure for temperatures up to at least 35 °C.

Acknowledgments

The authors would like to thank CNPq for providing financial support necessary to carry on the present research work.

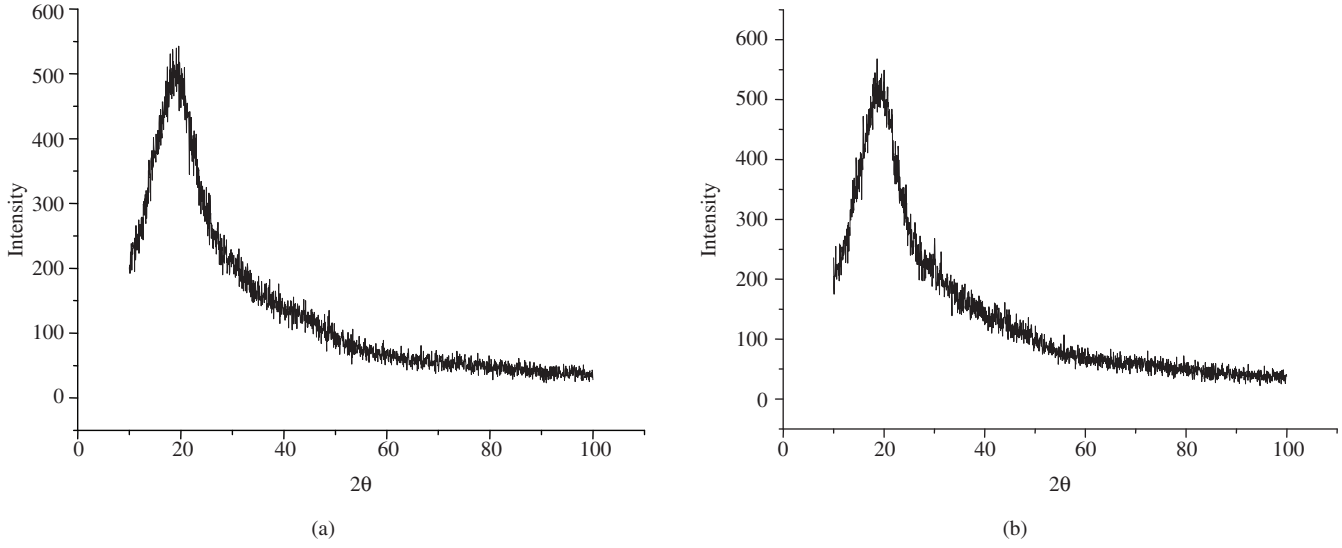


Figure 5. (XRD) spectra of the glassy food model at 25 °C storage temperature. a) after freeze-drying procedure; and b) after 90 storage days.

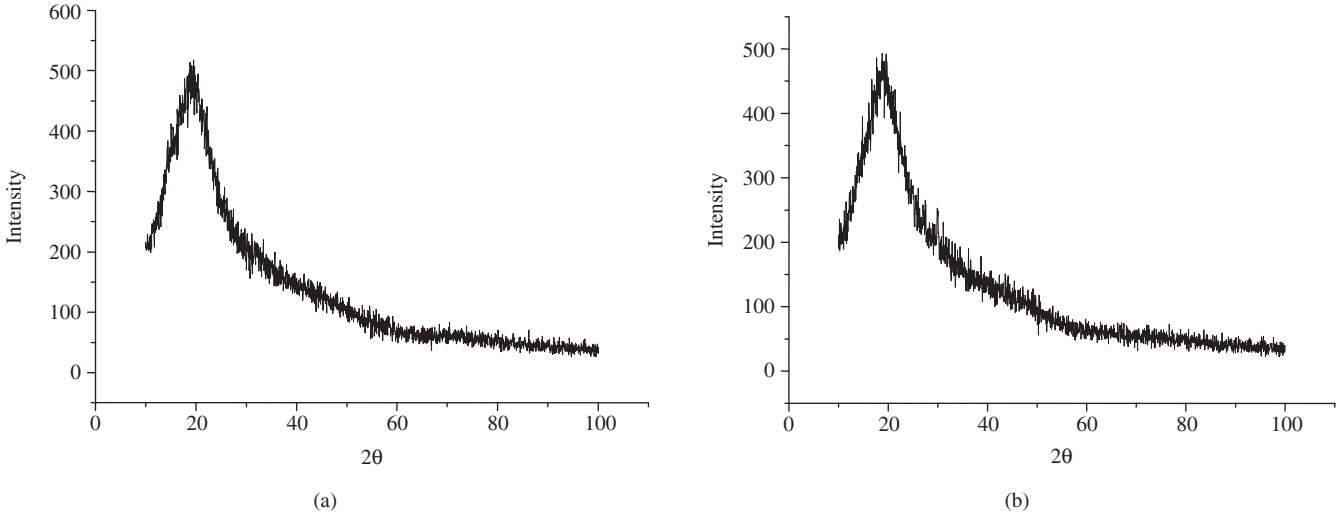


Figure 6. (XRD) spectra of the glassy food model at 35 °C storage temperature. a) after freeze-drying procedure; and b) after 90 storage days.

References

1. Fennema OR. *Food Chemistry*. 3rd ed. New York: Marcel Dekker; 1996.
2. Azzi A, Stocker A. Vitamin E: non-antioxidant roles. *Progress in Lipid Research.* 2000; 39:231-255.
3. Orlien V, Andersen AB, Sinkko T, Skibsted LH. Hydroperoxide formation in rapeseed oil encapsulated in a glassy food model as influenced by hydrophilic and lipophilic radicals. *Food Chemistry*. 2000; 68:191-199.
4. Gejl-Hansen F, Flink J. Freeze – dried carbohydrate containing oil-in-water emulsions: microstructure and fat distribution. *Journal of Food Science*.1977; 42:1049-1055.
5. Labrouse S, Roos Y, Karel M. Collapse and crystallization in amorphous matrices with encapsulated compounds. *Sciences des aliments*. 1992; 12(4):757-769.
6. Shimada Y, Roos Y, Karel M. Oxidation of methyl linoleate encapsulated in amorphous lactose-cased food model. *Journal of Agricultural and Food Chemistry*. 1991; 39:637-641.
7. Anandaram S, Reineccius GA. Stability of encapsulated orange peel oil. *Food Technology*. 1986; 11:88-92.
8. Imagi J, Muraya K, Yamashita D, Adachi S, Matsuno R. Retarded oxidation of liquids entrapped in matrixes of saccharides or proteins. *Bioscience, Biotechnology and Biochemistry*. 1992; 56:1236-1240.
9. Labuza TP, Maloney JF, Karel M. Autoxidation of methyl linoleate in freeze-dried model systems. II. Effect of water on cobalt-catalyzed oxidation *Journal of Food Science*. 1966; 31:885-891.
10. Maloney B, Labuza JF, Wallace DH, Karel MJ. Autoxidation of methyl linoleate in freeze-dried model systems. I. Effect of water on the autocatalyzed oxidation. *Journal of Food Science*. 1966; 31:878-884.
11. Slade L, Levine H. Glass transitions and water-food structure interactions. *Advances in Food and Nutrition Research*. 1995; 38:103-269.
12. Champion D, Hervet H, Blond G, Le Meste M, Simatos D. Translational diffusion in sucrose solutions in the vicinity of their glass transition temperature. *Journal of Physical Chemistry* B. 1997; 101:10674-10679.
13. Goubet I, Le Quere J, Voilley AJ. Retention of aroma compounds by carbohydrates: influence of their physicochemical characteristics and of their physical state. A review. *Journal of Agricultural and Food Chemistry*. 1998; 46:1981-1990.
14. Roozen MJGW, Hemminga MA. Molecular motion in sucrose-water mixtures in the liquid and glassy state as studied by spin probe ESR. *Journal of Food Technology*. 1990; 94:7326-7329.
15. Bell LN. Kinetics of non-enzymatic browning in amorphous solid systems: distinguishing the effects of water activity and the glass transition. *Food Research International.* 1996; 28:591-597.
16. Karmas R, Buera MDP, Karel M. Effect of glass transition on rates of nonenzymatic browning in food systems. *Journal of Agricultural and Food Chemistry*. 1992; 40:873-879.
17. Claude J, Ubbink J. Thermal degradation of carbohydrate polymers in amorphous states: A physical study including colorimetry. *Food Chemistry*. 2006; 96:402-410.
18. Roozen JP, Hemminga MA, Walstra P. Molecular motion in glassy water-malto-oligosaccharide (maltodextrin) mixtures as studied by conventional and saturation-transfer spin-probe e.s.r spectroscopy. *Carbohydrate Research*. 1991; 215:229-237.
19. Tromp RH, Parker R, Ring SG. Water diffusion in glasses of carbohydrates. *Carbohydrates Research*. 1997; 30:199-205.
20. Champion D, Le Meste M, Simatos D. Towards an improved understanding of glass transition and relaxations in foods: molecular mobility in the glass transition range. *Trends in Food Science & Technology*. 2000; 11:41-55.
21. Ubbink J, Krüger J. Physical approaches for the delivery of active ingredients in foods. *Trends in Food Science & Technology.* 2006; 17:244-254.
22. Bhandari BR, Howes T. Implication of glass transition for the drying and stability of dried foods. *Journal of Food Engineering*. 1999; 40:71-79.
23. Chang PS, Lee JH, Lee JH. Development of a new colorimetric method determining the yield of microencapsulation of α-tocopherol. *Journal of Agriculture Food Chemistry*. 2005; 53:7385-7389.
24. Pregnolato W. Normas Analíticas do Instituto Adolfo Lutz. *Métodos químicos e físicos para análise de alimentos*. 3ed. São Paulo: Instituto Adolfo Lutz; 1985.
25. Lev G, Karel M. Volumetric Shrinkage (Collapse) in Freeze-dried Carbohydrates above their Glass Transition Temperature in Food. *Res. Int.* 1995; 28:145-151.
26. Belows RJ, King CJ. Product Collapse during Freeze Drying of Liquid Foods. *Alche Sym. Ser*. 1973; 69:33-41.
27. To EC, Flink JM. Collapse a Structural Transition in Freeze Dried Carbohydrates. *Journal of Food Technology*. 1978; 13:551-594.
28. Pikal M.J, Shah S. The Collapse Temperature in Freeze Drying: Dependence on Measurement Methodology and Rate of Water Removal from The Glassy Phase. *Journal of Pharmacology*. 1990; 62:165-186.
29. Karel M, Saguy I. Effects of Water on Diffusion in Food Systems. *Water Relationships in Foods (Levine, H., Slade, L., eds)*. New York: Plenum; 1990.
30. Slade L, Levine H. Beyond Water Activity: Recent Advances Based on an Alternative Approach to the Assessment of Food Quality and Safety. *Food Science Nutrition*.1991; 30:1-360.
31. Roos YH, Himberg M J. Nonenzymatic Browing Behavior, as Related to Glass Transition, of Food Model at Chilling Temperature. *Journal of Agricultural and Food Chemistry*. 1994; 42:893-898.
32. To EC, Flink JM. Collapse, a structural transition in freeze dried carbohydrates II.Effect of solute composition. *Journal of Food Technology*. 1978; 13:567-581.
33. Tsourouflis S, Flink JM, Karel M. Loss of structure in freeze-dried carbohydrates solutions: effect of temperature, moisture content and composition. *Journal of the Science of Food and Agriculture*. 1976; 27:509-519.
34. Ma YH, Arsem H. Low pressure sublimation in combined radiant and microwave freeze drying. In A. S. Mujumdar (Ed.) *Drying`82*. New York: McGraw-Hill; 1982.
35. Longmore AP. Advances in vacuum and freeze drying. *Food Process Industry*. 1971; 40:46-49.