# INFLUENCE OF STORAGE CONDITIONS ON FREEZE-DRIED APPLE FORTIFIED WITH VITAMIN E

# INFLUENCIA DE LAS CONDICIONES DE ALMACENAMIENTO SOBRE MANZANA LIOFILIZADA FORTIFICADA CON VITAMINA E

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Recibido: Octubre 8 de 2008 Aceptado: Marzo 30 de 2009

# ABSTRACT

The effect of different storage conditions (temperature: 4, 20 and 30 °C; time: 30, 60, 90 and 180 days; package: with and without vacuum), is evaluated in terms of the stability of vitamin E, color and texture of freeze-dried apple fortified with vitamin E, using matrix engineering as a methodology for obtaining functional foods. The vitamin's quantification was carried out using gas chromatography. The color's by coordinates CIE- L\*a\*b\*, hue and chroma and texture by penetration test. Degradation of vitamin E was modeled using a first order kinetics. However the kinetic constants weren't fitted by Arrhenius equation, due to an abrupt change in them between 4 and 20°C, the effect of the transition from glassy to rubbery state in the product. At 4°C, the color was acceptable at 180 days, whereas browning is observed at 20°C and 30°C, and is higher according to temperature and time. Vacuum packaging showed a negative effect in the samples color, probably due to the mechanical effects. Textural changes, caused by progressive moisture gain, are related to permeability of packaging material, generating loss of crunch, which was well evaluated at the initial control time (30 day).

Keywords: Functional food, vitamin E, vacuum impregnation, storage, fortified freeze-dried apple.

# RESUMEN

El efecto de diferentes condiciones de almacenamiento (temperatura: 4, 20 y 30°C; tiempo: 30, 60, 90 y 180 días; envasado: con y sin vacío), se evalúa en función de la estabilidad de la vitamina E, el color y la textura de manzana liofilizada fortificada con vitamina E, utilizando la ingeniería de matrices como metodología de obtención de alimentos funcionales. La vitamina se cuantifica por cromatografía de gases, el color a partir de las coordenadas CIE-L\*a\*b\*, tono y croma y la textura por ensayos de punción. La degradación de la vitamina E modeliza a una cinética de primer orden; sin embargo, las constantes cinéticas no se ajustan a la ecuación de Arrhenius, debido a un cambio brusco de éstas entre 4 y 20°C, por efecto de la transición del estado vítreo a gomoso en el producto. A 4°C, el color fue aceptable a los 180 días, mientras que a 20 y 30°C se observa pardeamiento, siendo mayor cuanto mayores son la temperatura y el tiempo. El envasado al vacío tiene un efecto negativo en el color de las muestras, debido a los efectos mecánicos. Los cambios texturales debidos a la progresiva ganancia de humedad, tienen que ver con la permeabilidad del material de empaque e inducen la pérdida de crujencia, la cual fue bien valorada a los 30 días.

**Palabras clave**: alimentos funcionales, vitamina E, impregnación al vacío, almacenamiento, manzana liofilizada fortificada.

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#### **INTRODUCTION**

As time passes, nourishing habits have shown changes. Every day customers demand are a healthier diet or products with physiological activity compounds to allow a better physical and mental health, reducing the illness risks and prolonging quality life. These are the functional foods (1, 2, 3). This situation is driving to a faster growth in the market and marketing of functional food in the world and the food industry must evolve everyday to satisfy customers' needs (4, 5, 6).

The tocopherols are the principal compound exhibiting vitamin E activity and they are the most important natural antioxidants allowed for use in food to prevent or interrupt the chain reactions produced by the free radicals, neutralizing them by donation of their phenolics hydrogen. These are very unstable species which have a despaired electron that can react with any other molecules like fat acids of the cell's membrane, fats which circulate in blood, proteins, vitamins, gene's nucleic acids, etc. (7, 8, 9, 10). The effect of different factors, such as alkaline medium, light and cations (Fe<sup>+3</sup>, Cu<sup>+2</sup>), in tocopherol degradation rate has been studied in model systems of dehydrated foods (11, 12, 13, 14). Several authors (15) studied the tocopherol stability in different food matrixes (wheat flour, rye meal, biscuits, margarine, jams). Stability in rice (16) and infant milks (17) was also analyzed. Nevertheless, studies about the stability of vitamin E impregnated in dehydrated food matrices have not been found. The degradation rate of the compounds with vitamin E activity depends on oxygen availability, temperature, water activity and storage time, as well as on the fat content and food composition (18).

The preservation of this kind of products can be carried out by drying methods. Several dehydration methods have been used in fruit preservation, each one affecting in different ways the final product properties such as color, texture, density and porosity (19, 20, 21). Likewise, degradation of physiologically active compounds component (native or incorporated to the product) can be affected by drying conditions. Product fortification with dl- $\alpha$ -tocopherol acetate or other forms of vitamin E or other antioxidants can be a required practice in manufacturing of functional foods. The darkening of the fruits and vegetables during the drying and the storage represents a very important consideration in the product's shelf life determination, as enzymatic browning reactions

as non enzymatic are present (21, 22, 23). On the other hand, reactions complex's formation with metallic ions like copper and iron, also operate in the browning with a diminution of the lightness ( $L^*$ ) and increase of a<sup>\*</sup>(> chromaticity redness) (24).

There are many reasons why this work studied the freeze-drying in the development of functional food: to increase the shelf-life of products and reduce chemical degradation during storage, to reduce the moisture of products until a level near to the bond water to improve the characteristic crunchiness of products.

Vacuum impregnation, through the action of hydrodynamic mechanism (25, 26) has been described as an effective technique for enriching porous matrixes (27) since it is an alternative application in the food industry for the production of new functional foods, because of its advantages: kinetics of transference of fast masses, higher gain of solutes in short times, better color conservation and color improvement in some products, taste and scentconservation of the fresh product (28). The aim of this work is evaluating the effect of different storage conditions (temperature, time and packing) on freeze-dried apple fortified with vitamin E, in terms of the stability of vitamin E, color and texture.

## MATERIALS AND METHODS

The criteria for fortification was to add enough amounts of components to assure that 100 % of recommended daily intake (RDI) was present in 200 g of fresh apple which is equivalent to 100 % of the recommended dietary allowance (RDA) in USA law (33 mg of dl- $\alpha$ -tocopherol acetate) (The National Academy of Sciences, <u>www.nap.edu</u>) (29).

Quarter slices of *Granny Smith* apples variety with a weight of 3 g approximately were used (inside and outside diameter 22.4 and 66.7 mm respectively and 5 mm in thickness). The apples were obtained from the local market (Valencia, Spain) taking into account homogeneity in size, shape and apparent ripeness, and they were stored at 4°C before being used. Two lots of apples were used during the study of vitamin activity compounds degradation and 75 samples for each lot. The initial conditions were for lots 1 and 2: moisture content ( $0.862 \pm 0.009$ ,  $0.856 \pm 0.011$ ), water activity ( $0.991 \pm 0.003$ ,  $0.990 \pm 0.004$ ) and soluble solids ( $12.5 \pm 1.3$ ,  $11.3 \pm 1.7$ ) respectively. The dl- $\alpha$ -tocopherol acetate (0.065%) was emulsified in an isotonic glucose solution (9°Brix) containing: 0.051% tween 80<sup>®</sup>, 0.049% Span 60<sup>®</sup>, 0.1% Arabic gum and 1.7% of CaCl<sub>2</sub> to reinforce fruit texture (30). Batchs of the 250 mL emulsions were prepared in a vacuum homogenizer (Ultraturrax T25 - Janke & Kunkel IKA - Labortechnik) for 20 minutes to 24000 rpm. A glass container with recirculation and a jacket refrigeration was adapted. The analysis for Cryo-SEM Techniques allowed to get the diameter of the drop in the emulsion (1 - 1.5  $\mu$ m) and the values of the density of emulsions were 1045 ± 1 kg/m<sup>3</sup> (31).

The concentration of dl- $\alpha$ -tocopherol acetate in the emulsion was determined in order to reach the defined concentration in the product, taking into account the fruit response to vacuum impregnation. VI experiments were carried out in specially designed equipment (32, 33) and the parameter evaluated was the volume fraction of emulsion, X (m<sup>3</sup> emulsion / m<sup>3</sup> fruit), the mass ratio, X<sub>HDM</sub> (Kg emulsion / Kg impregnated fruit) and the effective porosity of the sample ( $\epsilon$ ) available to the hydrodynamic mechanism (HDM). This was quantified by applying a previously described methodology (26), which is through the control of mass and density sample before and after impregnation process. Therefore in the impregnation process, the vacuum (50 mbars) step deformation was considered insignificant and it doesn't have any atmospheric pressure (32, 33).

The Freeze-Dried Equipment was a laboratory lyophilizer LIOALFA 6–80, TELSTAR for 48 h, condenser temperature:  $-45^{\circ}$ C, hot plate temperature:  $25^{\circ}$ C and vacuum:  $1.2 \times 10^{-2}$  mbar. Moisture level in dried samples ranged between 5-7%.

The extraction process used (31) is a modification of Kmostak's method (34), by incorporation of ultrasound treatment instead of agitation. The analytical determination was carried out in a gas chromatography (CG-FID) – Fisons Instrument model NPD 800. The dl- $\alpha$ -tocopherol acetate was quantified from calibrations curves. To obtain it, we prepared dissolutions with 0.250, 0.5 y 1 mg/ml internal standard intern (squalane) and standard reference (dl- $\alpha$ -tocopheryl acetate, 99.1% Supelco or dl- $\alpha$ -tocopherol, 99.8% Supelco). Operating conditions: detector and injector temperature: 320°C and 300°C respectively, initial column temperature, 120°C, initial ramp programmed at 20 °C/min to 240°C; end ramp programmed at 5°C/min to 300°C and keeping 2 min at 300°C. Carrier gas: Helium 5.0, ratio split: 1/11.4, Flow velocity: 1.2 mL/min.; make-up pressure (N<sub>2</sub>): 120 kPa. Chromatographic column: DB-5, J&W Scientific, 30m x 0.32 mm I.D.: 0.25  $\mu$ m. The injection in the CG-FID was 2  $\mu$ L per sample. Under these conditions, squalane, dl- $\alpha$ -tocopherol and dl- $\alpha$ -tocopherol acetate had retention times of 12.5, 17.9 y 18.5 min respectively. For the study, each condition was made by triplets.

The color was tested with a Minolta Spectra Magic CM–3600D spectrocolorimeter, illuminant D65 for the 10° observer. The color parameters values of lightness (L<sup>\*</sup>), chromaticity greenness / redness (a<sup>\*</sup>), chromaticity blueness/yellowness (b<sup>\*</sup>), hue ( $h_{ab}^{*}$ ) and chroma ( $C_{ab}^{*}$ ) were recorded. The samples were evaluated thirty six times for each storage condition.

The mechanical tests were carried out at 25 °C with a Universal Texture Analyzer TA.XT2 (Stable Micro Systems). A penetration test was carried out with plastic probe 10 mm diameter, strain 95% and test rate 2 mm/sec. From the force – % relative deformation curves obtained, force deformation at 95% ( $F_{95\%}$ ), force deformation constant ( $F_f$ ) and the % initial relative deformation ( $\gamma^\circ$ ) and end ( $\gamma^f$ ), keeping  $F_f$ . Six samples were evaluated for each storage condition.

The temperature's determination of vitreous transition ( $T_g$ ) of the freeze-dried emulsion was made in a differential scanning calorimeter (DSC) made by Seiko Instruments, model DSC-5200CO, Chiba, Japan. The samples were equilibrated to 20°C according to the procedure described for the obtention of the sorption's isotherms (35). The study samples' size was approximately 25 mg and they were set in crucibles of sealed aluminum. The interval of temperature's scanning was between –110 y 60°C, depending on the samples and the velocity of the scanning in the warning's stage was 5°C/min.

The samples were packed in plastic bags: Polyamide / Polyetilen (reference RCA 20-70 Eurobag) with oxygen permeability ( $O_2$ ), 40-50 cm<sup>3</sup>/m<sup>2</sup>; water permeability, 2.6 g/m<sup>2</sup>; nitrogen permeability ( $N_2$ ), 10 cm<sup>3</sup>/m<sup>2</sup> and oxygen permeability carbon dioxide (CO<sub>2</sub>), 150 cm<sup>3</sup>/m<sup>2</sup>. The samples were stored under vacuum (CV) and at atmospheric conditions (SV). The products were stored at 4, 20 y 30°C and controls at 0, 30, 60, 90 and 180 days were made. The results were analyzed from ANOVA, using the method LSD (least significant difference) as a method of multiple comparisons, with a confidence level of 95%. The analysis of variance was performed with the statistical package Statgraphics Plus version 5.1. Beside, samples were evaluated in triplicate for each condition of storage.

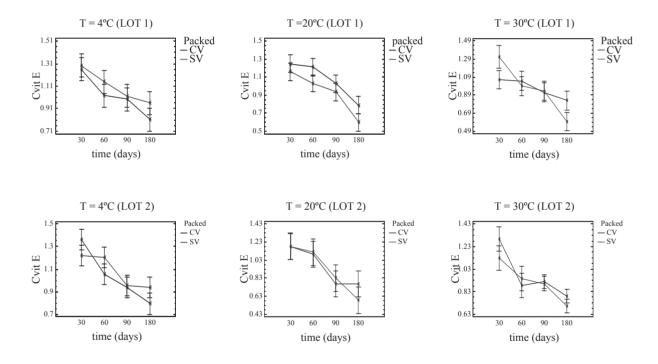
## **RESULTS AND DISCUSSION**

#### Vacuum Impregnation

The characterization of response to impregnation showed an excellent reproducibility for two lots, X =  $0.09 \pm 0.03$ , X<sub>HDM</sub> =  $0.11 \pm 0.03$  and  $\varepsilon = 0.10 \pm$ 0.03. The impregnation levels are less than to those obtained by using isotonic sucrose solutions (36), this is attributable mainly for to the interactions of pectins in the apple with  $Ca^{+2}$  ions present in the emulsion (37), that block the inside flow because of jellification. Also, the structure and the mechanicals properties of solid matrix (32) help at the contraction of porous during process for the coupling of the impregnation–deformation phenomena during the pressure change.

#### **Product's Stability**

Figure 1 shows the average values (and LSD intervals, 95%) of concentration of dl- $\alpha$ -tocopherol acetate reached at each storage time and temperature for samples stored under vacuum and at atmospheric conditions.



**Figure 1**. Medium values (and LSD intervals, 95%) of concentration of dl-α-tocopherol acetate (CVit.E) reached at each storage time and temperature for samples stored under vacuum (CV) and at atmospheric conditions (SV).

In two lots, the multifactor ANOVA did not reflect significant differences between the concentrations of dl- $\alpha$ -tocopherol acetate (C<sub>Vit.E</sub>) associated with the vacuum packing. This is attributable to the protection that could be having the molecules of the component with vitamin activity when they remain encapsulated

by the peptic components' jellification of the cellular wall with the  $Ca^{+2}$ . Therefore, mean values (SV and CV) of the corresponding values at each temperature-time condition were considered for kinetic analysis. The table 1 show the medium values of  $C_{Vit,E}$  and %RDI and its standard deviations for of both lots.

Time (days)	Lot	4°C		20°C		30°C	
		C <sub>vit.E</sub>	%RDI	C <sub>vit.E</sub>	%RDI	C <sub>vit.E</sub>	%RDI
0	1	1.34 ± 0.14	131.2 ± 15.9	1.34 ± 0.14	131.2 ± 15.9	1.34 ± 0.14	131.2 ± 15.9
	2	1.38 ± 0.14	134.6 ± 16.3	1.38 ± 0.14	134.6 ± 16.3	1.38 ± 0.14	134.6 ± 16.3
30	1	1.29 ± 0.16	145.3 ± 16.9	1.18 ± 0.22	136.6 ± 30.3	1.21 ± 0.14	137.6 ± 17.2
	2	1.28 ± 0.09	119.5 ± 9.3	1.20 ± 0.09	110.6 ± 7.0	1.16 ± 0.14	109.2 ± 12.8
60	1	1.13 ± 0.18	110.9 ± 11.8	1.11 ± 0.12	107.0 ± 12.5	0.92 ± 0.06	90.3 ± 5.1
	2	1.09 ± 0.12	110.0 ± 12.2	1.12 ± 0.12	114.5 ± 19.0	1.02 ± 0.18	101.3 ± 23.5
90	1	0.95 ± 0.06	95.6 ± 7.3	0.80 ± 0.12	79.6 ± 11.9	0.98 ± 0.09	97.3 ± 12.5
	2	1.01 ± 0.15	102.0 ± 14.5	0.98 ± 0.13	100.3 ± 14.6	0.93 ± 0.04	91.6 ± 5.3
180	1	0.86 ± 0.09	89.9 ± 8.4	0.68 ± 0.16	72.2 ± 15.2	0.70 ± 0.15	73.6 ± 14.8
	2	0.89 ± 0.10	92.2 ± 8.6	0.69 ± 0.16	74.0 ± 15.5	0.71 ± 0.20	75.4 ± 15.2

Table 1. Medium Values of C<sub>Vit F</sub> and % RDI and its standard deviation for lots 1 and 2.

 $C_{Vit.E}$  data variability is high and it is due to the differences in the impregnation levels of the different samples which take to initial concentrations different in every one of them. Moreover, the oxidation process can also present fluctuations from some samples to others because of the catalytic effect of some factors as metal presence, free radicals, etc. which can present variability from some samples to others. Otherwise, the tocopherol extraction process for its analysis, presents a source of variability, as the right to the proper analysis.

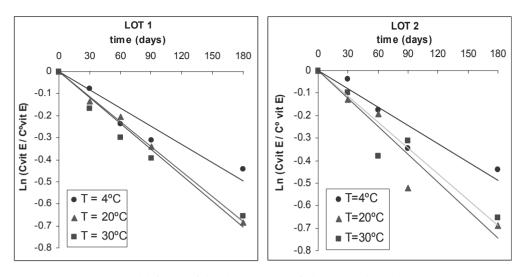
It is observed that some medium values of the reached %DRI are superior to the estimated in the theoretic calculus (100%). The reason is that principally during vacuum stage, in the impregnation process; part of the native apple liquid (in the intercellular spaces or in the open cellules) comes out from the structures and is replaced by a mayor quantity of emulsion. The evaluated impregnation quantity, by difference of weight in the samples, before and after the in vacuum operation, does not take into account this liquid interchange. Due to this, the quantity of gained emulsion is evaluated by change. This effect becomes very important, when the increase in the relation surface / volume allows space to a bigger number of cut cells and to a shorter run of the native liquid in the intercellular spaces during their expulsion by vacuum.

The temperature influence over the  $C_{Vit.}$ <sub>E</sub>, showed that exists significant differences, presenting two homogenous groups in both lots, for the lot one: 4-20°C and 20-30°C and for the lot two, 4°C and 20-30°C. In terms of time, for both lots there are very significant differences, which are explained by the fast diffusion of the air to the interior of the product's porous structure, as well as the synergy with the temperature becomes mayor with the increase of this, also favoring the oxidation reactions and the decomposition.

#### **Kinetic results**

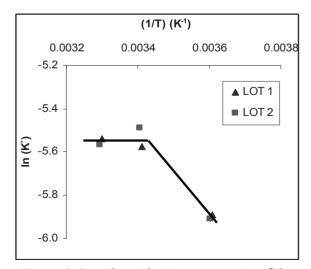
The first order velocity equation (equation 1) (38) was the one which presented the best description of the dl- $\alpha$ -tocopherol acetate losses regarding time. Figure 2 shows the kinetics adjustment of the data and their straight to 4, 20 and 30°C for each one of the lots. The K' pendant in the graphic represents the constant of degradation velocity dl- $\alpha$ -tocopherol acetate, being C<sup>t</sup><sub>Vit.E</sub> y C<sup>o</sup><sub>Vit.E</sub> the concentrations of dl- $\alpha$ -tocopherol acetate to time t and to time zero, respectively.

$$\frac{d C_{Vit.E}}{dt} = K' C_{Vit.E}$$
 Ecuación 1.



**Figure 2.** Kinetic model fitted of the degradation of dl-α-tocopherol acetate at 4, 20 y30°C as a function of temperature and storage time.

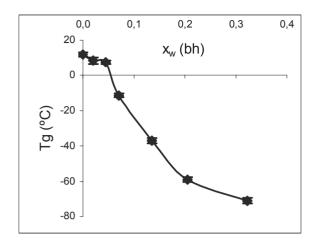
The kinetics constants (K') to both lots increased with temperature, that can be attributed to high samples' porosity which makes easier the biggest oxygen's diffusion to the intercellular spaces of the tissue where it is found the dl- $\alpha$ -tocopherol acetate distributed by the impregnation process. Figure 3, presents the Arrhenius' graphic, where it is observed an abrupt change in the behavior when passing from 4 to 20°C, which does not allow a good adjustment in its equation. The fraction of the soluble apples' (in which it is encapsulated the dl- $\alpha$ -tocopherol acetate in the intercellular spaces) with the presented humidity (5-7%), it can present a vitreous transition between 4 y 20°C, which would imply a drastic in the diffusion properties of the matrix and as a consequence, in the product stability (39).



**Figure 3**. Semi-logarithmic representation of the kinetic constant (*K*') vs. 1/T (K<sup>-1</sup>) on freeze-dried apple fortified with vitamin E.

Figure 4, presents the evolution of vitreous transition temperature values  $(T_{\alpha})$  taken in the transition interval middle point of the thermograms obtained from the impregnated freeze-dried emulsion, as will be found in the freeze-dried apple's porous and according to humidity content. The  $T_{\alpha}$  diminished with the increase of the sample's a due to plasticizer effect water has (39). The behaviour of the  $T_{\alpha}$  in low humidity (a<sub>w</sub> between 0.112 y 0.225) presents a very low influence of the moisture, while the plasticizer effect intensifies with  $a_w$  values over 0.225. In equilibrium state to the humidity conditions of the final product, the product would have a  $a_w$  between 0.11 y 0.22, with values of  $T_{a}$  of 8.3 and 7.5°C respectively. It supposes that in inferior temperature conditions the emulsion solid, which encapsulates the component with vitamin E, will be vitreous, while in higher temperatures it will be rubbery. The kinetics for the oxygen diffusion in this last case will be much faster, with the consequent increase of the degradation velocity.

These results are coherent with the storage's temperature influence in the degradation kinetics of the vitamin E, which accelerates a lot to 20 y 30 °C in relation to 4°C, in which solids would be in vitreous state conferring much higher stability to the encapsulated vitamin. The possibility of mayor stability in higher storage temperatures would imply the emulsion's reformulation to obtain anhydrous solids with higher value of  $T_g$ , which assures their vitreous state in the required storage temperature.



**Figure 4.** Curve of the vitreous transition against the humidity of the freeze-dried emulsion.

#### Color

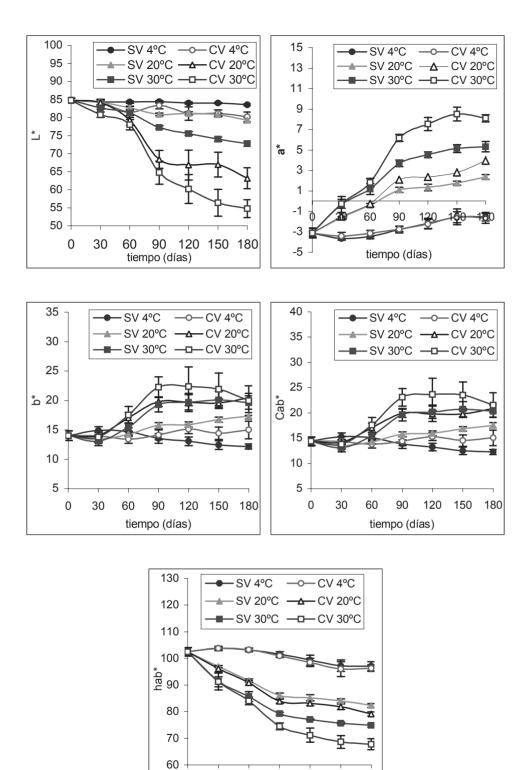
Figure 5 illustrates the L<sup>\*</sup>, a<sup>\*</sup>, b<sup>\*</sup>, C<sub>ab</sub><sup>\*</sup> y h<sub>ab</sub><sup>\*</sup> evolution during the 180 days of storage to 4, 20 y 30°C packing, with and without vacuum. The samples of apples fortified with vitamin E present, in the beginning, high luminosity values, a greenish color (>  $h_{ab}^*$ ) and a little intense (<  $C_{ab}^*$ ). During the storage, the porosity seems to play a decisive role in the color deterioration, probably when favoring the oxidations.

To 4°C exist significant differences in terms of time in all the parameters, except in L\* (samples SV). The decrease of L\* (samples CV) can be attributed to volumetric contractions of the samples and their compactation because of the vacuum's mechanic effect. The samples presented higher matrix's contraction, as much as the storage time was higher. This is due to the high samples' porosity and to the solid matrix's rubbery induced by the progressive adsorption of the water through the pack, with certain permeability to the vapor of water. The color purity increased progressively with the storage (associate to an increase of  $b^*$ ) much more notable in the CV samples. The a<sup>\*</sup> and h<sub>ab</sub> \* parameters did not present significant differences associated to the packing type (CV y SV).

To 20°C exist significant differences in terms of time and packing's conditions, being the luminosity  $(L^*)$  so similar during the first 30 days and after an abrupt decrease in the samples CV according to the SV ones, here the samples' contraction also play an important role in this evolution. The CV and SV samples start to foster more the reddish yellow colors, reflecting in the increasing of a<sup>\*</sup> y b<sup>\*</sup>.

To 30°C it exist differences highly significant in all the color's parameters because all the factors. In the SV samples the velocity of luminosity loss was smaller. The b<sup>\*</sup> parameter in the SV and CV samples presented and increase to 90 days and from here the curves tend to be asymptotic, while a<sup>\*</sup> always was crescent in the SV and CV samples being promoted the reddish yellow colors in the CV samples against the SV ones.

Figure 6 presents the color's evolution with the time in the chromatics planes  $a^*b^*$  and  $L^*C_{ab}^*$ . It is observed that to 4°C the variation in both graphics is not very critic to total color change, while to 20 y 30°C in the  $a^*b^*$ 's plane it is observed a tendency to pass from a greenish yellow to an orange. This color's trajectory is also present to the grey zone because of the low purity and the low reached levels of L\*. In the  $L^*C_{ab}^*$ 's plane it is clearly observed the pass from pale colors to dark ones while the time and the temperature of storage increase.



**Figure 5**. Color parameters L<sup>\*</sup>, a<sup>\*</sup>, b<sup>\*</sup>,  $C_{ab^*}$  y  $h_{ab}^*$  in storage's time function.

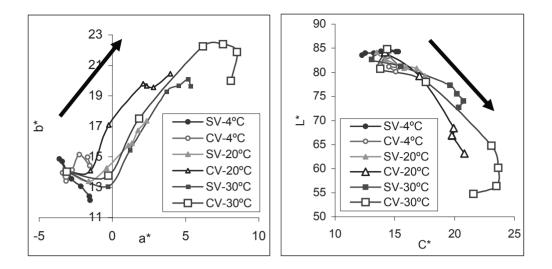
0

30

60 90 120 tiempo (días)

150

180



**Figure 6**. Evolution of the experimental points in the  $a^*b^* y L^*C_{ab}^*$  color's planes, during the storage.

Food color is affected by multiple factors (process, process conditions, storage, etc.) (40, 41, 42, 20). The application of the IV process modifies the food optical properties, increasing the absorbed light over the surface which makes the samples look lighter ( $<L^*$ ) (43, 44).

Previous researches in freeze-dried apple fortified with vitamin E using conventional dried, don't show significative changes during storage to 4°C and 180 days, whereas to 20 and 30°C, the browning phenomena become stronger, rising the L<sup>\*</sup> and a<sup>\*</sup> values and decreasing b<sup>\*</sup>(45, 46). The color parameters in freeze-dried fortified with vitamin E samples were lighter (>L<sup>\*</sup>), with more greenness (<a<sup>\*</sup>), and less yellow (>b<sup>\*</sup>).

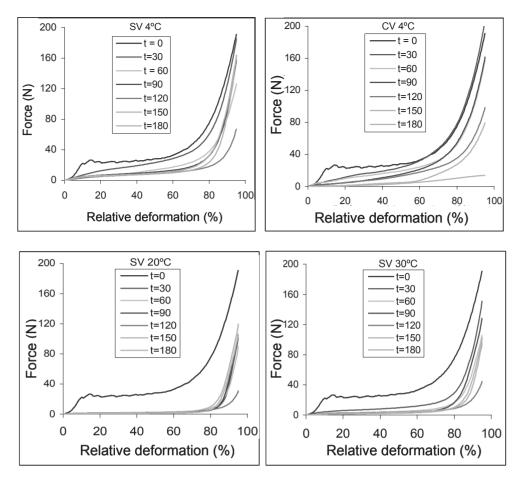
## Texture

During the storage some samples presented structural changes due to the vacuum effect applied in packing and for the progressive humectation through the package. The CV samples stored at 20°C and 30°C presented, after 30 days, an approximated volumetric contraction from the 70 to the 80%, associated to the mechanic action of the vacuum due to the limited mechanic resistance of the matrix solid to those temperatures. To 4°C the effects were smaller which is explained by the higher matrix's rigidity in those temperatures. In figure 7 it is given the representatives curves to 4°C (SV y CV), SV 20°C and SV 30°C compared with a control sample at the beginning of the storage where the product presents crunchy characteristics ( $F_{95}$  = 202.0 ± 10.7 N,  $F_f$  = 29.1 ± 3.1 N,  $\gamma^{\circ}$  = 14.5 ± 2.8 % and  $\gamma^{f}$  = 47.7 ± 8.5%). It is observed that the samples to 4°C, in the first control (30 days) already present a sigmoidal form which reflexes the loss of the crunchy characteristics of the product due to the hygroscopicity of the samples and the permeability to vapor water of the package commented before. This phenomenon becomes more critical in the samples storage to 20 and 30°C.

Rubbery texture characteristics have been found in air-dried apple fortified with vitamin E (46) making different the freeze-dried samples, by presenting a crunchy texture at the beginning of the storage.

## Shelf life's prediction

The shelf life time ( $t_s$ ) in constant temperature conditions and water activity, can be predicted with regard to a final wished concentration of dl- $\alpha$ -tocopherol acetate ( $C^{f}_{Vit E}$ ). For this study,  $t_s$ has been evaluated in base to the reduction of the concentration of dl- $\alpha$ -tocopherol acetate in 50%, that means,  $t_{1/2}$  ( $C^{f}_{Vit E} = 0.5 C^{o}_{Vit E}$ ). The average of  $t_{1/2}$  in the two lots of the products freeze-dried at 4, 20 y 30°C were 253, 175 y 179 days respectively. In terms of color, to 4°C the freeze-dried samples were acceptable until the 180 days, but the samples storage to 20 and 30°C in any condition of SV and CV packing just would be acceptable until 30 days.



**Figure 7**. Comprehension test (force against % relative deformation) in the samples to 4°C (SV and CV), SV 20°C and SV 30°C.

# CONCLUSIONS

- Vacuum impregnation of apple samples allows us their fortification with vitamin E previously emulsified in an adequate media. Freeze-drying of samples did not imply loss of vitamin but its degradation occurs progressively, depending on temperature, when packed in polyamide/ polyethylene bags with or without vacuum.
- Storage temperature higher than a critical value (between 4 and 20 °C) implied faster degradation of vitamin E. The level of vitamin E at 180 days was of 92, 74 y 75 % RDI at temperatures 4, 20 y 30°C, respectively.
- The samples fortified with vitamin E and freezedried are very opaque and very reflective due to gas occluded in the porous, what makes them look so light at the beginning, moreover the velocity of luminosity loss and browning is too high, so much in packing conditions (CV). To 4°C the samples present an acceptable color until the 180 days, but the samples stored at 20 and

30°C in any condition of SV and CV packing would be acceptable until the 30 days.

The texture is affected negatively by the vacuum packing due to the package used, with some water permeability. Low temperatures (4°C) help to preserve the textural properties of the freeze-dried products, but it is needed an impermeable package to water to avoid the problems of humectation.

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