

EVOLUTION OF PROBIOTIC CONTENT AND COLOR OF APPLES IMPREGNATED WITH LACTIC ACID BACTERIA

EVOLUCIÓN DEL CONTENIDO PROBIÓTICO Y EL COLOR DE MANZANAS IMPREGNADAS CON BACTERIAS ÁCIDO LÁCTICAS

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ABSTRACT

An important issue related with functional foods development is the stability of physiologically active compounds. For example vitamins, minerals, probiotic bacteria are very sensitive to the technological processes involved in their obtention. The objective of this paper is to evaluate the viability of a probiotic strain (*Lactobacillus rhamnosus*) inside a food matrix (Granny smith apple) and physical parameters such as color ($L^* a^* b^*$) of vacuum impregnated apple cylinders during a refrigerated and frozen storage. The results show that the viable probiotic bacteria inside food matrix maintain the minimal value to be considered as probiotic functional food (10^6 CFU/g), and the color parameters which are critical in refrigerated samples during their storage.

Keywords: functional foods, probiotics, *Lactobacillus*, apple.

RESUMEN

Un importante tema relacionado con el desarrollo de alimentos funcionales es la estabilidad de los componentes fisiológicamente activos como vitaminas, minerales, bacterias probióticas y otros, los cuales pueden ser muy sensibles al proceso tecnológico de obtención. El objetivo de este trabajo es evaluar la viabilidad de una cepa probiótica (*Lactobacillus rhamnosus*) en el interior de una matriz alimentaria (manzana *Granny smith*) y los parámetros de color ($L^* a^* b^*$) en cilindros de manzana impregnados al vacío durante el almacenamiento bajo refrigeración y congelación. Los resultados muestran una viabilidad de la bacteria probiótica en el interior de la matriz alimentaria, manteniendo los valores mínimos para ser considerados como alimentos funcionales probióticos (10^6 UFC/g) y los parámetros de color son críticos en las muestras refrigeradas durante el almacenamiento.

Palabras clave: alimentos funcionales, probióticos, *Lactobacillus*, manzana.

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INTRODUCTION

The concept of functional food can be defined as an ingredient or food supplement with the essential function of improving the health and wellbeing of the consumer. Fuller (1989) gave a widely accepted definition of probiotic (1) who defined probiotic as “live microbial feed supplements which beneficially affect the host by improving its intestinal balance”. The beneficial effects and applications of probiotics are the focus of active research and have been the subject of several reviews (2-6).

Probiotic microorganisms belong to different microbial genera, *Lactococcus*, *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Bacillus*, *Enterococcus*, *Pediococcus*, *Leuconostoc* among others (2, 7-11). Lactic acid bacteria also belong to this classification. Within this group are natural microbiota of human intestine with about 100 billions of bacteria from 400 different strains. More than 95% of this microbial population lives in the surface of a healthy colon (12-15). Colonic microorganism can produce vitamins and short chain fatty acids and can also metabolize some non digestible nutrients, to stimulate immune response and growth of other beneficial microorganisms, decrease the cholesterol level in the blood, prevent the entrance of pathogens and decrease the risk of intestinal diseases by colonization among other beneficial effects (16-22).

The development of novel probiotic foods have been focused mainly on dairy products. The consumption of these foods shows a progressive increase in the last decade due to changes in habits and trends of consumers attracted by the benefits from the consumption of this “new functional foods”. Nowadays the development of fruits and vegetables with probiotic content is a topic of high interest for the probiotic-food consumers. However, the available information is very limited.

The use of vacuum impregnation is a relatively recent technique in the food industry within the new concept of “Matrix Engineering” (23). This technique is based on the highly porous structure of some foods. Therefore they have spaces inside (intercellular spaces) with gases inside, that could be removed by means of vacuum pressures and then replaced by a dissolution with some interest compounds, such as physiologically active compounds, microorganisms, minerals and other

compounds. This technique has demonstrated to be very versatile making possible to develop many functional foods containing diverse kind of substances (24-32).

The aim of this paper is to obtain stable apple based probiotic-functional food and to evaluate its time stability. Besides changes in color parameters as quality measurement are studied.

MATERIALS AND METHODS

Microorganisms and culture media

Lactobacillus rhamnosus (CECT 275) from Spanish Collection of Culture Type (Burjassot-Valencia) were grown and maintained on De Man, Rogosa and Sharpe media (MRS, Scharlau Chemie S.A).

Raw material

Apples variety *Granny smith* from Valencia (Spain) bought in local markets, with commercial ripening grade, was used to obtain cylinders 18 mm wide and 40 mm long. Grape must (GM) at pH 5.0 was used as impregnation vehicle to inoculate *Lactobacillus rhamnosus* on this material.

Physicochemical properties

The moisture content was determined according to the official method AOAC 20.013; 1980 (33). The pH with a potentiometer Crison model 507 (potentiometric method) (34) and the water activity (a_w) with a hygrometer dew point 25°C (AquaLAB decagon model CX-3) (AOAC 978.18M, 1996) (35).

Vacuum impregnation

Vacuum impregnation procedure was carried out according to Betoret *et al* (14) using grape must inoculated with *L. rhamnosus*. Vacuum time was 10 minutes at 50 mbar and then 10 minutes at atmospheric pressure (1013-1018 mbar). The procedure and equations to calculate impregnation parameters, volumetric fraction of incorporated liquid, volumetric deformation and effective porosity (X , γ and ϵ_c respectively) has been described by other authors (30, 36). Figure 1 show a flow chart of the vacuum impregnation procedure.

Impregnated fresh apple samples were packed into plastic bags and stored under refrigeration and freezing conditions. Storage conditions and sample analysis frequency are shown on table 1.

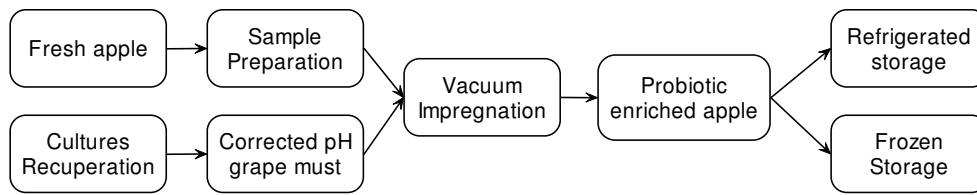


Figure 1. Operations to obtain probiotic-enriched apple.

Stabilization of impregnated samples

Table 1. Storage conditions and frequency sampling.

Treatment	Frequency sampling	Storage conditions
Frozen	Every 15 days, until reach 90 days	-26 °C ± 1
Refrigerated	Every 3 days, until reach 60 days	4 °C ± 1

Viable plate counts

After the stabilization treatment, plate counts of viable microorganisms were done in every process stages (impregnation dissolution, impregnated apples, refrigerated and frozen samples) in double layer MRS agar incubated at 37°C during 72 hours.

Color measurement

Color of apple samples was measured with a Minolta CM-2002 spectrophotometer (Minolta camera Co., Osaka, Japan) using a D65 illuminant, and a vision angle of 10°. For lectures low reflectance Minolta crystals were used (CR-A5/1829-752M). Color values were expressed according to CIE L*a*b* scale.

Electron Microscopy

To verify the presence of the probiotic strain inside the apple tissue, ultrastructural assays were performed with impregnated apple samples. For this purpose Cryo-SEM observations were done. Sample preparation included small sections from the central zone of apple cylinders obtained with a stainless steel sample holder, and frozen using liquid nitrogen. Frozen samples were placed inside a chamber at -180°C under vacuum conditions and prepared for observation. Ice from vegetable tissue was eliminated by freeze-drying holding samples 15 minutes at -85°C under high vacuum. Then surface of samples was covered with gold. Samples prepared were analyzed in a JEOL JSM-5410 microscope.

RESULTS

Table 2 shows the average values and their respective standard deviations of impregnation parameters X , γ and ϵ_c , in impregnated apples with grape must inoculated with *L. rhamnosus*.

Table 2. Response to vacuum impregnation of apple with *L. rhamnosus*.

X $\text{m}^3 \text{GM}_{\text{inoculated}} / \text{m}^3$ fresh apple	γ m^3 / m^3 fresh apple	ϵ_c $\text{m}^3 \text{ aire} / \text{m}^3$ fresh apple
10.3 ± 1.18	1.0 ± 0.6	21.2 ± 1.3

Results obtained of the X parameter are slightly inferior that other works with the same food matrix using isotonic dissolutions (37), vitamin E emulsions (30), apple juice inoculated with *S. cerevisiae* and *L. rhamnosus* (38), and similar to other experiments using *Red chieff* apple inoculated with *L. rhamnosus* in grape must (38). The positives values of γ show a deformation of the food structure, as a consequence of the hydrodynamic mechanism (HDM) (39). On the other hand, effective porosity of apple samples show that apple structure is very suitable for impregnation procedures. The response to vacuum impregnation depends on HDM, deformation-relaxation phenomena of the food structure (DRP), as well as viscosity of vacuum impregnation dissolution, and chemical composition (39-41).

Moisture content and water activity showed high values in all samples as expected, since this behavior is typical in most of fruit and vegetables tissue. The pH of fresh apple decreased when vacuum impregnation was applied. The lowest value was observed in frozen samples probably due to cryoconcentrations phenomena. The color was a more sensitive parameter, mainly the L* values were more affected by the vacuum impregnation

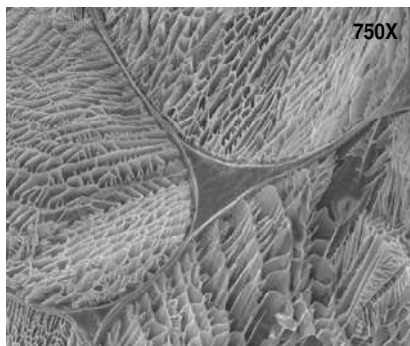
treatment since the samples become more translucent and dark. Frozen samples showed L^* and b^* values closer to the fresh samples (table 3).

Figure 2A shows the oval cells of parenchymatic fresh apple tissue, which are usually arranged into a 5 or 6 cell clusters leaving intercellular spaces in the center of the agrupation cellular wall limits cellular structures (42). Intercellular spaces (dark regions in the picture) have a triangular shape, typical for most of vegetal tissues. These spaces content gases inside.

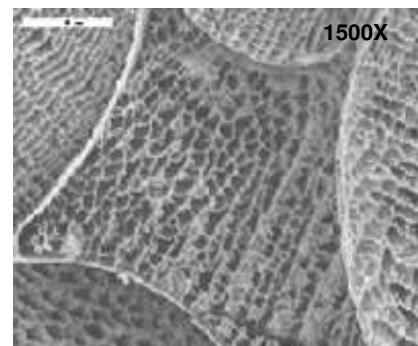
In the micrograph they appear totally empty, which is indicative of no liquid content. Figure 2B shows a detail of an intercellular space full of liquid as consequence of the vacuum impregnation operation. The eutectic artifact inside the space is the evidence of the penetration of liquid inside the fruit structure, after the freeze-drying process. Figure 2C shows microbial cells of *Lactobacillus rhamnosus* inside vegetable structure. It could be observed that microorganisms are uniformly distributed along the sample.

Table 3. Characterization of apple tissue subjected to different conditions.

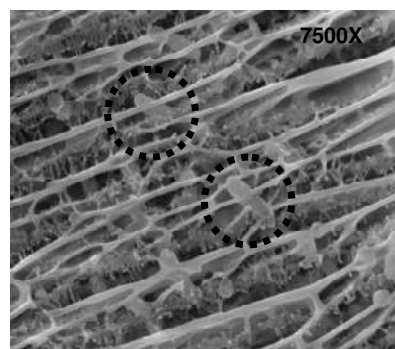
Parameter	APPLE SAMPLE			
	Fresh	Impregnated	Refrigerated	Frozen
a_w	0.985 ± 0.001	0.980 ± 0.003	0.980 ± 0.001	0.982 ± 0.004
Moisture (%)	87.72 ± 0.18	88.02 ± 0.50	88.02 ± 0.50	85.34 ± 0.30
pH	3.94 ± 0.07	3.5 ± 0.04	3.5 ± 0.07	2.46 ± 0.05
Color	L^*	66.21 ± 3.37	30.97 ± 2.80	48.22 ± 6.41
	a^*	-3.83 ± 0.31	-2.41 ± 0.34	-2.20 ± 0.29
	b^*	13.51 ± 1.71	8.38 ± 0.85	8.38 ± 0.48



(A)



(B)



(C)

Figure 2. Cryo-SEM images of apple tissue. A) Fresh non-impregnated apple X 750. B) Vacuum impregnated apple (intercellular space detail) X 1500. C) *Lactobacillus rhamnosus* cells inside apple tissue X 7500.

Figure 3A shows that the probiotic counts practically did not change during the 60 days of storage time, maintaining around a 10^9 CFU/g. This value is far above the minimum required for a functional food to produce probiotic effect (43). In the case of frozen samples (Figure 3B), microbial counts were closer to 10^9 CFU/g until day 45, when experienced a decrease of around 1

logarithmic cycle. However, the bacterial counts after 90 days of storage were high enough for label the product as with probiotic content. The observed decrease maybe due to the long storage period under freezing conditions, although the known tolerance of *Lactobacillus* to low temperatures may account for the stable number of *L. rhamnosus* in the first 45 days of the storage.

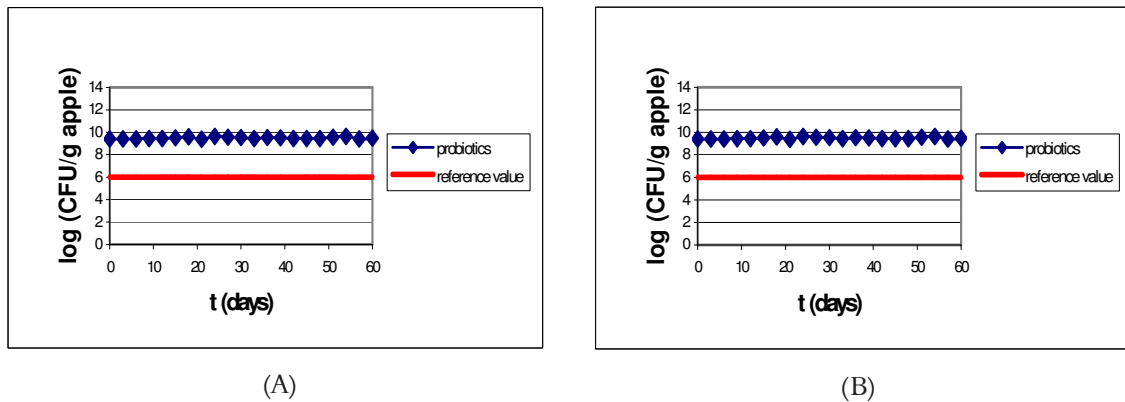


Figure 3. Evolution of probiotic microbial count in apple samples during storage period A) refrigerated storage, B) frozen storage.

Nowadays there are few research papers on the development of fruit probiotic. Some authors have experimented with the incorporation of native strain *Lactobacillus plantarum* and strain *Lactobacillus casei* in capegooseberries, using the same methodology reached at storage conditions 4°C and 15 days; $1.52 \pm 0.6 \times 10^9$ y $2.20 \pm 0.59 \times 10^9$ CFU/100 g fresh

cape-gooseberries, respectively (44). Other authors have impregnated mango with *Lactobacillus casei*, reaching between 5 and 7 Log CFU/g after dried a 35°C (45). In the case of cape-gooseberry, impregnations made with *Lactobacillus acidophilus* reached levels between 4.1 and 4.7 Log CFU/g fresh cape-gooseberry (46).

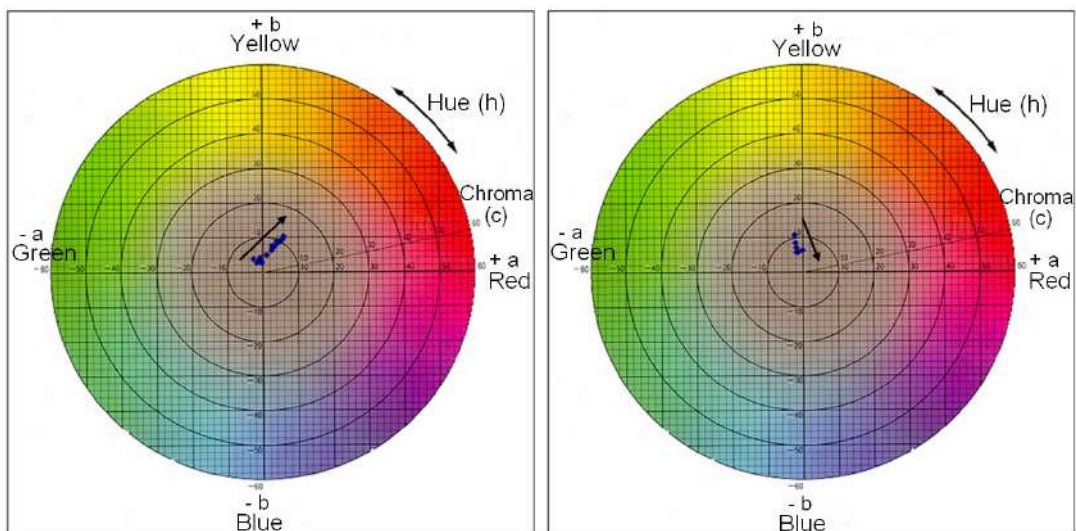


Figure 4. Representation of the evolution of a^*b^* coordinates in a Cartesian space from apple samples. A) refrigerated storage, B) frozen storage.

Figure 4 shows the evolution of a^* and b^* values in color plane formed by Cartesian coordinates in CIE $L^*a^*b^*$ space during the storage time. In the progress of these parameters in refrigerated samples (Figure 4A) a trend to yellow-red colors was observed. Small variations on the first days of the storage obey to a typical variability of raw material. In frozen storage (Figure 4B) the values of both parameters (a^* and b^*) tends to the chromatic center, which shows that color properties of frozen apples are more stable than those observed in refrigeration treatment. L^* value did not present important changes for both storage conditions.

CONCLUSIONS

The probiotic strain in this study (*Lactobacillus rhamnosus*) shows to be very stable under storage for both conditions. This fact means that the obtained product keeps a number of viable probiotic microorganisms to be considered as functional food with probiotic content during all the storage.

The color is a critical parameter in the quality of the obtained product. The early changes in color showed that this parameter is the main shelf-life limitant.

REFERENCES

- Fuller R. Probiotics in man and animals. *J Appl Bacteriol.* 1989; 66 (1): 365-378.
- Reuter G, Klein G, Goldberg M. Identification of probiotic cultures in food samples. *Food Res Int.* 2002; 35 (2-3): 117-124.
- Koop-Hoolihan L. Prophylactic and therapeutic uses of probiotics: A review. *J Am Diet Assoc.* 2001; 2 (101): 229-238.
- Sanders ME, Klaenhammer TR. Invited review: The scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *J Dairy Sci.* 2001; 84 (1): 319-331.
- Goldin B. Health benefits of probiotics. *Brit J Nutr.* 1998; 80 (2): S203-S207.
- Reddy B. Prevention of colon cancer by pre- and probiotics: Evidence from laboratory studies. *Brit J Nutr.* 1998; 80 (2): S219-S223.
- Prado F, Parada J, Pandey A, Saccol C. Trends in non-dairy probiotic beverages. *Food Res Int.* 2008; 41(2): 111-123.
- Shah N. Review functional cultures and health benefits. *Int Dairy J.* 2007; 17 (11): 1262-1277.
- Isolauri E, Salminen S, Ouwehand A. Probiotics. *Best Pract Res Cl Ga.* 2004; 18 (2): 299- 313.
- Fooks L, Gibson G. In vitro investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. *FEMS Microbiol Ecol.* 2002; 39 (1): 67-75.
- Gomes A, Malcata F. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends Food Sci Tech.* 1999; 10 (4-5): 139-157.
- Hammes W, Hertel C. Research approaches for pre- and probiotics: challenges and outlook. *Food Res Int.* 2002; 35 (2-3): 165-170.
- Silvi S, Verdenelli MC, Orpianesi C, Cresci A. EU project Crownalife: functional foods, gut microflora and healthy ageing isolation and identification of *Lactobacillus* and *Bifidobacterium* strains from faecal samples of elderly subjects for a possible probiotic use in functional foods. *J Food Eng.* 2003; 56 (2-3): 195-200.
- Betoret N, Puente L, Díaz MJ, Pagán MJ, García MJ, Grass ML, et al. Development of probiotic-enriched dried fruits by vacuum impregnation. *J Food Eng.* 2003; 56 (2-3): 273-277.
- Ortega R, Marcos A, Aranceta, J, Mateos J, Requejo A, Serra L. *Alimentos funcionales. Probióticos.* Madrid: Editorial Médica Panamericana; 2002.
- Collado M, Meriluoto J, Salminen S. Measurement of aggregation properties between probiotics and pathogens: *in vitro* evaluation of different methods. *J Microbiol Meth.* 2007; 71 (1): 71- 74.
- Jenkins B, Holsten S, Bengmark S, Martindale R. Probiotics: A Practical Review of Their Role in Specific Clinical Scenarios. *Nutr Clin Pract.* 2005; 20 (2): 262-270.
- Pennacchia C, Ercolini D, Blaiotta G, Pepe O, Mauriello G, Villani F. Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. *Meat Sci.* 2004, 67 (2): 309-317.
- Fasoli S, Marzotto M, Rizzotti L, Rossi F, Dellaglio F, Torriani S. Bacterial composition of commercial probiotic products as evaluated by PCR-DGGE analysis. *Int J Food Microbiol.* 2003; 82 (1): 59-70.
- Reid G, Jass J, Sebulsky M, McCormick J. Potential uses of probiotics in clinical practice. *Clin Microbiol Rev.* 2003; 16 (4): 658-672.
- Tuhoy MK, Probert HM, Smejkal CW, Gibson GR. Using probiotics and prebiotic to improve gut health. *Drug Discov Today.* 2003; 8 (15): 692-700.
- Charalampopoulos D, Wang R, Pandiella S, Webb C. Application of cereals and cereal components in functional foods: a review. *Int J Food Microbiol.* 2002; 79 (1-2): 131-141.
- Fito P, Chiralt A, Betoret N, Gras M, Cháfer M, Martínez-Monzó J. Vacuum impregnation and osmotic dehydration in matrix engineering. Application in functional fresh food development. *J Food Eng.* 2001; 49 (2-3): 175-183.
- Restrepo AM, Cortés M, Márquez CJ. Uchuvas (*Physalis peruviana* L.) mínimamente procesadas fortificadas con vitamina E. *Vitae.* 2009; 16 (1): 19-30.
- Cortés M, Chiralt A. Cinética de los cambios de color en manzana deshidratada por aire fortificada con vitamina E. *Vitae.* 2008; 15 (1): 8-16.
- Restrepo A. Nuevas perspectivas de consumo de frutas: uchuva (*Physalis peruviana* L.) y fresa (*Fragaria vesca* L.) mínimamente procesadas fortificadas con vitamina E [Tesis de maestría]. Medellín: Universidad Nacional de Colombia; 2008.
- Cortés M, García A, Suárez H. Fortificación de hongos comestibles (*Pleurotus ostreatus*) con calcio, selenio y vitamina C. *Vitae.* 2007, 14 (1): 16-24.
- Cortés M, Guardiola L, Pacheco R. Aplicación de la ingeniería de matrices en la obtención de mango mínimamente procesado enriquecido con calcio. *DYNA.* 2007; 74 (153): 19-26.
- Cortés M, Osorio A, García E. Manzana deshidratada fortificada con vitamina E utilizando la Ingeniería de Matrices. *Vitae.* 2007; 14 (2): 17-26.
- Cortés M. Desarrollo de productos de manzana deshidratados enriquecidos con vitamina E. [Tesis doctoral]. Valencia: Universidad Politécnica de Valencia 2004.
- Grass M, Vidal-Brotons D, Betoret N, Chiralt A, Fito P. Calcium fortification of vegetables by vacuum impregnation interactions with cellular matrix. *J Food Eng.* 2003; 56 (2-3): 279-284.
- Grass M, Vidal-Brotons D, Betoret N, Chiralt A, Fito P. The response of some vegetables to vacuum impregnation. *Innovat Food Sci Emerg Tech.* 2002; 3 (3): 263-269.

33. Association of Official Analytical Chemists. AOAC. Official Methods of Analysis. Washington: W. Horwitz (Ed.); 1980.
34. Association of Official Analytical Chemists. AOAC. Official Methods of Analysis. 15th ed Arlington, VA AOAC; 1990.
35. Association of Official Analytical Chemists. AOAC. Official Methods of Analysis. Basic calculations for chemical and biological analyses Bassey. Arlington, VA: J.S. Efiok; 1996.
36. Salvatori D, Andrés A, Chiralt A, Fito P. The response of some properties of fruits to vacuum impregnation. *J Food Eng.* 1998; 21 (1): 59-73.
37. Fito P, Chiralt A, Betoret N, Grass M, Cháfer M, Martínez-Monzó J, *et al.* Vacuum impregnation and osmotic dehydration in matrix engineering application in functional fresh food development. *J Food Eng.* 2001; 49 (2-3): 175-183.
38. Puente L. Aplicación de la técnica de impregnación a vacío en la obtención de alimentos funcionales con contenido probiótico a partir de manzana. [Tesis doctoral]. Valencia, España: Universidad Politécnica de Valencia 2003.
39. Fito P, Andrés A, Chiralt A, Pardo P. Coupling of Hydrodynamic Mechanism and Deformation-Relaxion Phenomena During Vacuum Treatments in Solid Porous Food-Liquid Systems. *J Food Eng.* 1996; 27 (3): 229-240.
40. Chiralt A, Fito P, Andrés A, Barat JM, Martínez-Monzó J, Martínez-Navarrete N. Vacuum impregnation: a tool in minimally processing of foods. In: F.A.R. Oliveira y J.C. Oliveira (Eds). *Processing of Foods: Quality Optimization and Process Assesment.* Boca Ratón: CRC Press; 1999.
41. Martínez-Monzó J. Cambios físico-químicos en manzanas *Granny smith* asociados a la impregnación a vacío. Aplicaciones en congelación. [Tesis Doctoral]. España: Universidad Politécnica de Valencia; 1998.
42. Paniagua R, Martín de Serrano M, Sesma P, Álvarez-Uria M, Anadón R, Fraile B, *et al.* Citología e histología vegetal y animal. *Biología de las células y tejidos animales y vegetales.* Madrid: McGraw-Hill Interamericana; 1997.
43. Ouwehand A, Kirjavainen P, Shortt C, Salminen S. Probiotics: mechanisms and stabilized effect. *Int Dairy J.* 1999; 9 (1): 43-52.
44. Marín ZT. Viabilidad de desarrollo de uchuva (*Physalis peruviana L.*) mínimamente procesada enriquecida con microorganismos probióticos a partir de la ingeniería de matrices [Tesis de maestría]. Medellín: Universidad Nacional de Colombia; 2009. 152 p.
45. Giraldo G, Duque AL, Padilla L. Impregnación de mango con probióticos. VIII Congreso de estudiantes de Química. Cali: Universidad del Valle; 2007.
46. Cueto MC, Pardo JM, García DA, Quintero JP, Posada IA. Supervivencia de bacterias ácido lácticas en uchuvas impregnadas y deshidratadas con aire caliente. III Jornadas de Socialización de Resultados de Investigación, septiembre 10 – 11, 2008, Universidad de la Sabana. [Website] Available at: <http://enif.unisabana.edu.co/InvestigacionWeb/WFPonenciaPublica.aspx?PON=39>. Consulted: 1 december 2008.

**GRUPO DE EXTENSIÓN SOLIDARIA E INVESTIGACIÓN
EN SEGURIDAD ALIMENTARIA PARA LA REGIÓN
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