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Novel functional foods from vegetable matrices impregnated with biologically active compounds

Stella M. Alzamora [a,*], Daniela Salvatori [a], María S. Tapia [b], Aurelio López-Malo [c], Jorge Welti-Chanes [c], Pedro Fito [d]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina
[b] Instituto de Ciencia y Tecnología de Alimentos, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47097, Caracas 1041, Venezuela
[c] Departamento de Ingeniería Química y Alimentos, Universidad de las Américas—Puebla, Ex-Hacienda Santa Catarina Mártir, Puebla 72820, Mexico
[d] Departamento de Tecnología de Alimentos, Universidad Politécnica de Valencia, Apartado 22012, 46071 Valencia, Spain

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Abstract

Functional foods affect beneficially one or more target functions in the body, beyond adequate nutritional effects, to either improve stage of health and well-being and/or reduce the risk of disease. Lastly, the range of functional foods has grown tremendously. One of the main objectives of the multinational collaborative project entitled "Emerging preservation techniques for foods of concern in Ibero-America" (CYTED Program), carried out from 1999 to 2004, was to analyze the feasibility of atmospheric and/or in vacuum impregnation treatments to incorporate physiologically active compounds into plant tissues without destroying the initial food matrix. This contribution brings together report of progress in the development of functional fruit and vegetable matrices enriched with probiotics and minerals (calcium and zinc). Main aspects discussed are the kinetics of matrix fortification, the viability of some active compounds and the interactions between calcium, the cell structure and the mechanical properties of fruit and vegetable tissues. Vacuum and/or atmospheric impregnation techniques seem to be feasible technologies for exploitations of fruit and vegetable tissues as new matrices into which functional ingredients can be successfully incorporated, providing novel functional product categories and new commercial opportunities.
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1. Introduction

In the last few years, nutrition science, traditionally concentrated on identifying a balanced diet, has emphasized "optimised" nutrition, this is maximising life expectancy and quality by identifying food ingredients that, when added to a balanced diet, improve the capacity to resist disease and enhance health (Gibson & Williams, 2000). The development of functional foods reflects this shift in attitudes between diet and health. Because of the complexity of the term "functionality", no universally accepted definition of functional foods exists. Moreover, functional foods have to be understood as a concept rather than as a well-defined group of food products. A consensus document of the European Commission's Concerted Action on Functional Food Science in Europe (FUFOSE) have proposed that "A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more

* Corresponding author. Fax: +54 11 4576 3495.
E-mail address: alzamora@di.fcen.uba.ar (S.M. Alzamora).

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target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of the risk of disease. A functional food must remain food and it must demonstrate its effect in amounts that can normally be expected to be consumed in the diet: It is not a pill or a capsule, but part of the normal food pattern...'' (Diplock et al., 1999).

The global functional food market is estimated to be US$ 47.6 billion, being the United States the largest market segment, followed by Europe and Japan (Sloan, 2002). Lastly, the range of functional foods that have potential benefits for health has grown tremendously. Examples include baby foods, bakery and cereals, confectionery, dairy food, ready meals, snacks, soft drinks such as energy and sport drinks, meat products and spreads. These functional foods are associated with various types of benefit, looking particularly at vitamin and mineral fortification, cholesterol reduction, antioxidants, phytochemicals, dietary fibre, herbs and botanicals, and probiotics, prebiotics and symbiotics.

As part of the Science and Technology for Development in Ibero-America (CYTED) Food Preservation Subprogram, a multinational collaborative project entitled ''Emerging preservation techniques for foods of concern in Ibero-America'' was carried out from 1999 to 2003. One of the main objectives of this project was to analyse the feasibility of atmospheric and/or in vacuum impregnation treatments to incorporate physiologically active compounds (PAC) into plant tissues without destroying the initial food matrix.

Plant tissues are multiphase systems with an intricate internal microstructure formed by cells, intercellular spaces, capillaries and pores. Four major types of mature plant tissues are storage or parenchyma; conducting or vascular (composed of phloem and xylem); supporting; and protecting tissues. Edible portions of most fruit and vegetables are composed of fleshy parenchyma cells, which form the bulk of the softer parts of plants. Nutrients of importance to man are frequently stored in these thin-walled living cells. The parenchyma cells are ≅50 to 500 μm across and polyhedral or spherical in shape. They show, from out to inner, the middle lamella that glues adjacent cells; the primary cell wall; the plasma membrane; a thin layer of parietal cytoplasm containing different organelles (mitochondria, spherosomes, plastids, chloroplasts, endoplasmic reticulum, nucleus, and so on); and bound by the tonoplast membrane, one or more vacuoles that contain a watery solution of organic acids, salts, pigments, and flavours, that are responsible for the osmotic potential of the cell (Aguilera & Stanley, 1991). Cells walls are penetrated by strands of cytoplasm, the plasmodesmata, which connect the cytoplasm of adjacent cells. Cells and intercellular spaces are arranged into tissues and these last into the final organ.

Transport between cells occurs across the walls or apoplasm, via cytoplasmic strands or plasmodesmata (symplastic transport) or through tonoplast and plasmalemma membranes boundaries (transmembrane transport). Apoplastic transport involves movement of molecules through the aqueous part of the cell wall matrix, provided they are not immobilised by electrostatic or other forms of binding to the wall polymers (Brett & Waldron, 1996). Carpita, Sabularse, Montezinos, and Delmer (1979) showed that pores in the walls of parenchyma cells of vegetable tissues may be 35–55 Å in diameter and impose a restriction on the size of molecules that can penetrate. For instance, the passage of salts, sugars and amino acids is easily allowed, as well as some movement of small proteins and polysaccharides. No evidence exists for the passage of macromolecules, and large proteins are generally considered immobile. Despite the differences in composition and structure of the cell walls of various plant tissues, the limiting pore diameters appear to be similar (Carpita et al., 1979). Also, an intact plant cell wall constitute an extremely effective physical barrier against attack by microorganisms since its pores are far too small to permit bacteria (size range ≅0.1 to 5 μm), yeasts (size range ≅5 to 30 μm), mould spores (size range ≅3 to 9 μm) and even viruses (size range ≅0.02 to 0.3 μm) to penetrate through the protoplast (Alzamora et al., 2000). Penetration of microorganisms through the cell walls therefore would require physicochemical or enzymic degradation and/or alteration of wall structures.

In porous fruits and vegetables not the wall pores but the intercellular spaces may play a major role regarding the microorganisms penetration. These intercellular spaces are commonly referred to as ''pores'', and as such will be regarded in this work. Intercellular air spaces are common in parenchymatous tissue and have been estimated to be 20–25% of the total volume in apple, 15% in peach, 37–45% in mushroom, and 1% in potato. For instance, mature cells of apple parenchyma tissues may be 50–500 μm in diameter with interconnecting air spaces ranging from 210–350 μm across (Lapsley, Escher, & Hoehn, 1992). Thus, these spaces are large enough so microorganisms are able to pass through. In addition, senescing apples exhibit important changes in cell wall composition with disintegration of the middle lamella and enlargement of intercellular spaces, permitting the passage of large molecules and microorganisms (Ben-Arie, Kislev, & Frenkel, 1979).

Impregnation processes performed at atmospheric pressure (AI), under vacuum conditions (VI) or by a combination of vacuum impregnation followed by large periods at atmospheric pressure may be employed to incorporate PACs. During AI, plant cellular structure acts as a semi-permeable membrane, and the PAC is transferred from the concentrated solution to the cell by a process usually considered as diffusion driven.

When a porous tissue is immersed in a PAC concentrated solution under vacuum conditions, air is extracted from the pores and then, when atmospheric pressure is restored, the impregnation solution penetrates the intercellular spaces by capillary action and by the pressure gradients (i.e., the hydrodynamic mechanism, HDM) that are imposed to the system, helping incorporation of PACs (Fito, 1994). The substitution of internal gases by a liquid phase of adjustable composition allows direct formulation of a food by expeditious compositional modifications of the solid matrix, without exposing the food structure to the eventual stress due to long exposure to gradient solute concentration as in atmospheric process (Fito, Andrés, Chiralt, & Pardo, 1996; Mujica-Paz et al., 2002; Mujica-Paz, Valdéz-Fragoso, López-Malo, Palou, & Welti-Chanes, 2003). This in turn may result in quality enhancement of the final produce.

This contribution brings together reports of progress by some of the participants in the CYTED project in the development of functional fruit and vegetable matrices, enriched with probiotics and minerals (calcium and zinc). Main aspects concerned are the feasibility of vegetable matrices to support AI or VI with PACs; the kinetics of matrix fortification; the viability of some active compounds to be used in these foods and the interactions between calcium, the cellular structure and the mechanical properties of fruit and vegetable tissues.

2. Fortification of fruit and vegetable matrices

2.1. Probiotics

Functional foods targeted towards improving the balance and activity of the intestinal milieu currently provide the largest segment of functional market in Europe, Japan and Australia. Specific ingredients for gut health may include live microorganisms (probiotics), non-digestible carbohydrates (dietary fibre and probiotics) and bioactive compounds (e.g. phenolics). Probiotics are currently available in a variety of food products and supplements, mainly dairy products—fluid milk and fermented milk products like yoghurts. The most frequent bacteria commercially used belong to the *Lactobacillus* and *Bifidobacterium* species, although *Streptococcus thermophilus* and *Saccharomyces boulardii* are also available in certain milk products (Rastall, Fuller, Gaskins, & Gibson, 2000).

Disorders and diseases where probiotic nutritional management may have potential include mucosal vaccines and immunomodulation, infection control and eradication of multidrug-resistant microbes, treatment of candidal vaginitis, prevention of transmission of AIDS and other sexually transmitted diseases, cholesterol and blood pressure lowering, improved lactose tolerance and antimutagenic/anticarcinogenic activity (Rastall et al., 2000; Saarela, Lähteenmäki, Crittenden, Salminen, & Mattila-Sandholm, 2002).

Rodríguez (1998) conducted basic impregnation studies with different microorganisms (*Saccharomyces cerevisiae*, *Lactobacillus acidophilus* and *Phoma glomerata*) to evaluate their penetration by VI into a porous fruit tissue. Granny Smith apple was selected as a model of porous fruit, cut into cylinders and impregnated with sucrose isotonic solution containing the microorganisms. One vacuum pulse of 2 min at five different absolute pressures (75, 125, 225, 325, 425 mm Hg) at 25 °C was applied. For each microorganism, a control was prepared by maintaining the apple samples immersed into the solution for 2 min at atmospheric pressure (675 mm Hg). When comparing microbial counts of fresh apple and apple treated under atmospheric conditions, it could be observed that the simple soaking renders a significant increase in microbial counts (Table 1). This highlights the fact that capillary force and superficial adherence are very important factors that cannot be neglected in any modeling approach of immersion and impregnation operations. The lower the absolute pressure of the vacuum pulse applied, the higher the incorporation of microorganisms by HDM. Comparing with controls, counts obtained at 75 mm Hg presented increments of 0.36, 0.73 and 1.07 log for *S. cerevisiae*, *L. acidophilus* and *P. glomerata*, respectively. The model proposed by Roa, Tapia, and Millán (2001) was used for predicting microorganism incorporation into vegetable tissues. These authors simplified the model previously developed by Fito (1994) by accomplishing direct experimental determination of the volumetric fraction of sample (X) occupied by the impregnating solution as a result of HDM. Table 2 compares experimental and predicted values of *L. acidophilus* concentration after VI (C_i). The magnitude of the errors lied within acceptable limits for models that predict microbial populations as well for the results expected by the common plating methodology typical of microbial analysis of foods (Rodríguez, 1998). Similar model performance was obtained for the other microorganisms assayed.

The fortification of apple cylinders with *Bifidobacterium* spp. "Bb12" (Christian Hansen Corp.) by applying VI was investigated by Maguiña et al. (2002). Apple was impregnated with an isotonic sucrose solution containing $\cong 7.95 \log_{10}$ CFU/g of the microorganism at five different vacuum pressure levels (101, 125, 225, 325 and 425 mm Hg). Applied vacuum had a significant effect on X values as well as on incorporation of *Bifidobacterium* spp. The greater incorporation of bifidobacteria was attained at absolute pressures 101 and 125 mm Hg, which corresponded to the larger values of the volumetric fraction determined experimentally. In all cases the microorganism was incorporated at levels higher than 10^7 cells/g. The *Bifidobacterium* spp. concentration in the final product was also satisfactorily predicted by

Table 1
Microbial impregnation of apple cylinders[a] at different vacuum pressures and at atmospheric pressure

Microorganism	Pulse pressure[b] (mm Hg)	Inoculum ($\log_{10}$CFU/g)[c]	Fresh fruit ($\log_{10}$CFU/g)[c]	Impregnated fruit ($\log_{10}$CFU/g)[d]
Saccharomyces cerevisiae	75			5.05*a*
	125			5.01*a*
	225	5.74	3.08	4.84*b*
	325			4.70*c*
	425			4.59*d*
	675[e]			4.61*d*
Lactobacillus acidophilus	75			5.40*a*
	125			5.05*b*
	225	5.84	3.59	4.90*c*
	325			4.84*d*
	425			4.80*e*
	675[e]			4.67*f*
Phoma glomerata	75			5.28*a*
	125			4.88*b*
	225	5.76	<1.00	4.82*c*
	325			4.40*d*
	425			4.27*e*
	675[e]			4.21*f*

a–f: Similar letters indicate non-statistical difference ($p < 0.05$) was conducted on non-log values of microbial counts.
[a] Length: 2.58 ± 0.01 cm; diameter: 2.27 ± 0.01 cm.
[b] Absolute pressure.
[c] Average standard deviation. Results of 20 replicates.
[d] Average standard deviation. Results of 7 replicates.
[e] Atmospheric pressure (controls).

Table 2
Prediction of concentration of *L. acidophilus* of vacuum impregnated apple cylinders C_i (expressed as $\log_{10}$CFU/g) and comparison with experimental values

Pulse pressure (mbar)	X	C_i		Error
		Experimental	Predicted[a]	
75	0.1195	5.40	5.07	0.33
125	0.0983	5.05	5.02	0.03
225	0.0598	4.90	4.90	0.00
325	0.0292	4.84	4.76	0.08
425	0.0090	4.80	4.63	0.17

[a] Predicted from equation: $C_i = (C_{iF} - C_{iS})(F/M) + C_{iS}$, where C_{iF} = concentration of microorganisms in the fresh fruit; C_{iS} = concentration of microorganisms in the impregnation solution; F = mass of fresh sample; M = mass of vacuum impregnated sample.

the Roa, Tapia and Millán's model. Maintaining the viability, stability and functionality of probiotics not only during processing but during storage is essential to delivering the health benefits of these microorganisms to consumers (Mattila-Sandholm et al., 2002; Saarela et al., 2002). Viability evaluation of *Bifidobacterium* spp. in apple pieces stored in anaerobiosis at 4 °C for 12 days revealed that viable populations decreased only by a log cycle after the sixth day, and remained in that level until the end of storage. Apple samples impregnated at 325 and 425 mm Hg and stored during six days showed the highest sensory scores regarding colour, odour and flavour, while control fruit pieces had less acceptability. Visualisation of the microorganism into the fruit pores made by scanning electron microscopy (SEM) verified the introduction of impregnation liquid into cellular spaces of apple tissue (Fig. 1a).

Similar experiments were performed by Ortiz et al. (2002), who used the HDM to fortify with *Bifidobacterium* spp. guava (*Psidium guajava* L., Dominica red var.) (X = 13.5%; 400 mm Hg; 5 min). Impregnated guava pieces contained around 10^7 CFU/g. Counts of *Bifidobacterium* spp. decreased in 3 log cycles after 12 days of storage at 5 °C because no special anaerobic packaging was used. Viability was expected to be sustained with consideration of proper packaging.

Probiotic-enriched dried apple by VI was developed by Betoret et al. (2003). Apple cylinders were impregnated either with commercial apple juice containing *S. cerevisiae* or with whole milk or apple juice containing 10^7–10^8 CFU/ml of *Lactobacillus casei* (spp. *rhamnosus*). Impregnated apple samples contained around 10^7 CFU/g of each microorganism. Fig. 1b corresponds to cryo-SEM microscopic observations of parenchymatous

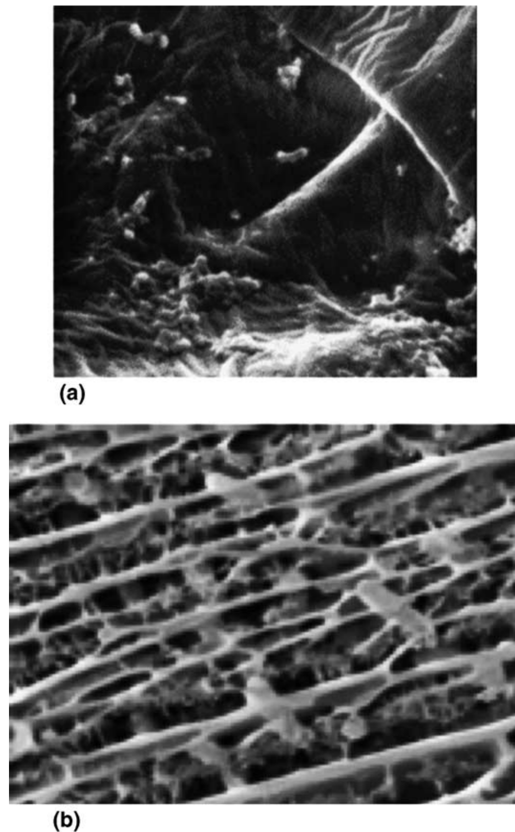

Fig. 1. (a) SEM micrograph showing cells of *Bifidobacterium* spp. inside intercellular spaces of parenchymatous tissue of apple after a vacuum pulse of 400 mm Hg for 5 min. (b) Cryo-SEM micrograph showing cells of *L. casei* inside an intercellular space of parenchymatous tissue of apple full of impregnation liquid after a vacuum pulse of 50 mbar for 10 min (from Bertoret et al., 2003; Maguiña et al., 2002).

apple tissue after impregnation treatment with milk inoculated with *L. casei*. Dendritic structures observed in the intercellular space confirm that gas has been replaced by impregnation liquid. Enriched apples were air dried at 40 °C to a water content of 0.037 kg water/kg dry matter and stored at room temperature for two months. At the end of the storage, *L. casei* concentration in dried product was greater than 10^6 CFU/g, being very similar to the levels usually found in commercial probiotic dairy products.

2.2. Minerals

Mineral supplementation has become a very popular method of fortifying inadequate diets. In particular, calcium and zinc are essential elements for survival. Adequate calcium intake has been associated with reduced risk of osteoporosis, hypertension, colon cancer, kidney stones and lead absorption. Modern man in industrialised nations obtains most of his calcium intake from dairy products. However, because preferences toward foods other than milk, many individuals ingest inadequate calcium (Weaver, 1998). Zinc is essential for our immune system, for reproduction, growth, wound repair, taste and smell, ensuring the proper functioning of more than 300 enzymes involved in maintenance of the structural integrity of proteins and in the regulation of gene expression. Recent analysis of diet and nutritional needs have led researchers to estimate that a staggering 48% of the population of the world is at risk from zinc deficiency, in spite of the low value of its recommended dietary allowance (RDA_{Zn} = 2–14 mg/day, according to life storage group) (Institute of Medicine, 1998).

Calcium impregnation capacity of parenchymatous apple tissue by different impregnation techniques (AI or VI) and the effect of these treatments on mechanical properties were studied by Alzamora, Anino, and Salvatori (2001), Anino, Salvatori, and Alzamora (2003, chap. 4) and Anino, Salvatori, Castro, and Alzamora (2002). Apple cylinders (1.5 cm in diameter and 2 cm in length) were immersed with forced convection at room temperature into isotonic glucose aqueous solution containing 5.24% (w/w) Ca^{2+} salts (5266 ppm Ca^{2+}). A mixture of Ca^{2+} lactate and Ca^{2+} gluconate was selected because its relatively high solubility and the neutral taste imparted to the food. For VI, a pressure of 30 mm Hg was applied to the system for 10 min and after that atmospheric pressure was restored and maintained for 10 min. For AI, fruit samples were taken out of the solution at different immersion times (0, 2, 6, 10 and 22 h). Compression behaviour (force-deformation curve pattern, force in rupture point and modulus of deformability) of calcium-impregnated apples exhibited some differences compared to fresh fruit: a decrease in the rupture force (F_{rup}) values (Fig. 2) and fracture mostly occurred over a period of deformation, as in ductile materials. Ca^{2+} content significantly increased along the AI treatment, reaching 1300 ppm in samples treated for 6 h and 3100 ppm after 22 h. Calcium incorporation in 200 g of fruit would satisfy about 41–62% of the Adequate Intake, AI_{Ca} (1000 mg/day) (Institute of Medicine, 1998). Structural changes of calcium treated apples were recorded by light microscopy (LM) and transmission electron microscopy (TEM). Calcium incorporation resulted in darkly stained cell walls, with a middle lamella clearly reinforced (Fig. 3C–H) but extensive folding of cell walls occurred. At 6 h of immersion the cytoplasm appeared separated from the wall and in some cells membranes looked broken with vesicle formation. After 22 h immersion, cell membranes were completely disrupted. After 2 h immersion, calcium crystals appeared between the cell wall and the plasmalemma, detaching the cytoplasm and pushing it further into the cell. Afterwards, crystallization seemed to proceed also in the other side of the plasmalemma and into the cytoplasm. After 22 h immersion, crystals of calcium salt appeared deposited in the intercellular spaces, along the walls and in the lumen of the cells.

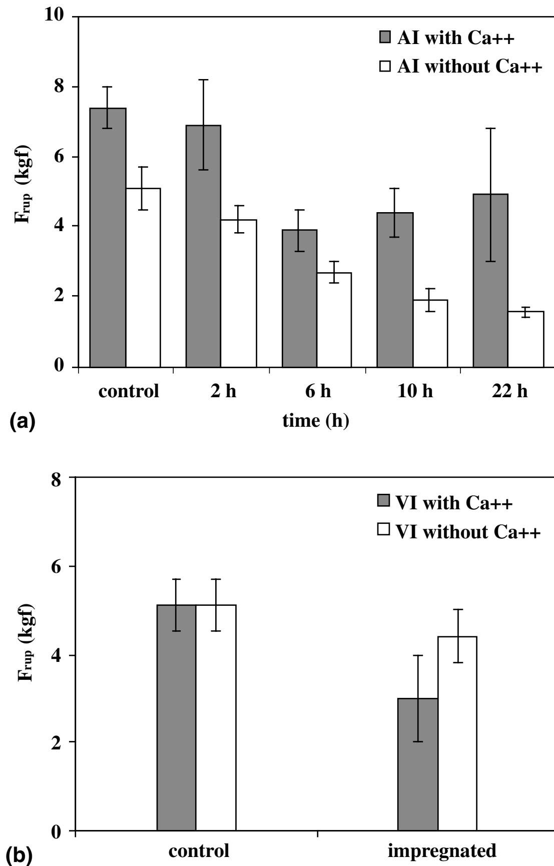

Fig. 2. Effect of calcium incorporation on the rupture force of apples subjected to (a) atmospheric impregnation (AI), (b) vacuum impregnation (VI).

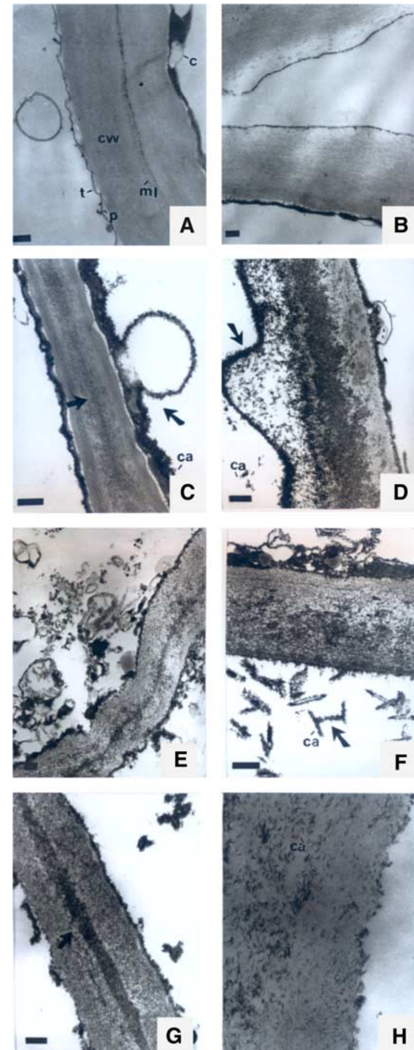

Fig. 3. MET micrographs of Granny Smith apple tissue along immersion time in a isotonic solution with calcium (5.24% calcium lactate–gluconate). A, B: control; C, D: after 2 h immersion; E, F: after 6 h immersion; G, H: after 22 h immersion. Scale: A, C, D, F, G: 500 μm; B, E, H: 200 μm (from Anino et al., 2002).

Calcium fortification of different histological tissues (eggplant, oyster mushroom and carrot) by applying VI was performed by Gras, Vidal-Brotons, Betoret, Chiralt, and Fito (2002). These authors analysed in detail the Ca-plant tissue interaction and the modification of mechanical and vacuum impregnation responses. VI (37.5 mm Hg for 10 min and immersion at atmospheric pressure for 10 min) was made in sucrose isotonic aqueous solutions containing calcium lactate. A slight influence of Ca^{2+} presence on the impregnation behaviour could be observed from sample impregnation and deformation data. Compression response of eggplant and carrot was notably affected by calcium but not significant effects were observed in oyster mushroom. Egg plant ($X = 51$–62%) and oyster mushroom ($X = 41\%$) appeared to be highly suitable for obtaining fortified products by using small concentrations of PACs in the impregnating solution. Calcium distribution in the tissues was analysed by energy depressive X-ray microanalysis. Calcium incorporation mainly occurred in the intercellular spaces of egg plant and oyster mushroom and in xylem of carrot, and, to a much lesser extent, inside the cells in egg plant. This distribution is not in total agreement with the results found by Anino et al. (2002) previously reported in this paper. In addition to differences in tissue matrix, this discrepancy could be attributed to the different calcium concentrations assayed in each case, since Gras et al. (2002) worked with lower calcium concentration solutions.

In other study, VI was applied to melon (*Cucumis melo* L.) cylinders (2.2 cm diameter and 2 cm high length) to incorporate calcium and zinc (Tapia, Schulz, Gómez, López-Malo, & Welti-Chanes, 2003). Impregnation of minerals was evaluated by using different sucrose syrups (8%, 29% and 50%) containing 1000 ppm calcium (as $CaCl_2 \cdot 2H_2O$) and 375 ppm zinc (as $ZnSO_4 \cdot 7H_2O$), at different vacuum pressures (0, 25 and 50 cm Hg) and relaxation times (3, 24 and 45 min at atmospheric pressure), as well as different fruit: syrup (w/w) ratios (1:3 and 1:10) according to a Box-Behnken

experimental design, fixing the vacuum pulse in 10 min. Maximum average concentration of calcium and zinc in melon were 228 and 51 ppm, respectively at atmospheric pressure, while levels rose to 322 and 62 ppm with previous VI. A favourable effect on calcium incorporation was obtained with an increase in vacuum pressure and relaxation time, while in the case of zinc, total time of the process seemed to be the main variable affecting its final concentration in melon. Maximum levels of both minerals would represent 2.2–2.9% of the AI_{Ca} and more than 100% of the RDA_{Zn} with servings of 100 g of impregnated melon. Fig. 4 illustrates the corresponding surface responses for calcium impregnation when fruit: syrup ratio was 1:3. Sensory evaluation (9 points hedonic scale, with colour, aroma, taste, texture and general acceptation) was performed on impregnated melons with the highest concentrations of calcium and zinc attained for each of the three sucrose concentrations assayed and with fresh melon. All enriched melons were accepted better than the fresh sample. VI in sucrose syrups seemed to enhance sensory attributes of melon.

Kinetics of incorporation of Ca^{2+} to the apple matrix was dependent on the pressure(s) of the system along impregnation and on the structure of the tissue. Evolution of calcium concentration was studied for different treatments: VI (50 mm Hg pressure for selected periods of time t_1: 0, 5, 10, 15, 180 min); AI under conditions of internal control, in which samples were out at different times t_2 (0, 0.75, 1.5, 3, 4.5, 6, 7.5 h); or AI with previous VI during 15 min (González Fesler, Salvatori, Weisttaub, Portela, & Alzamora, 2002). Ca^{2+} content increased considerably along the treatment at atmospheric pressure, reaching 835 ppm in samples treated for 3 h and ≅1300 ppm after 7.5 h (Fig. 5). These results would indicate that the fruit matrix was still far away from achieving equilibrium with the impregnation medium. The values reported correspond to the mean between samples from apple 1 and apple 2. Somehow it is

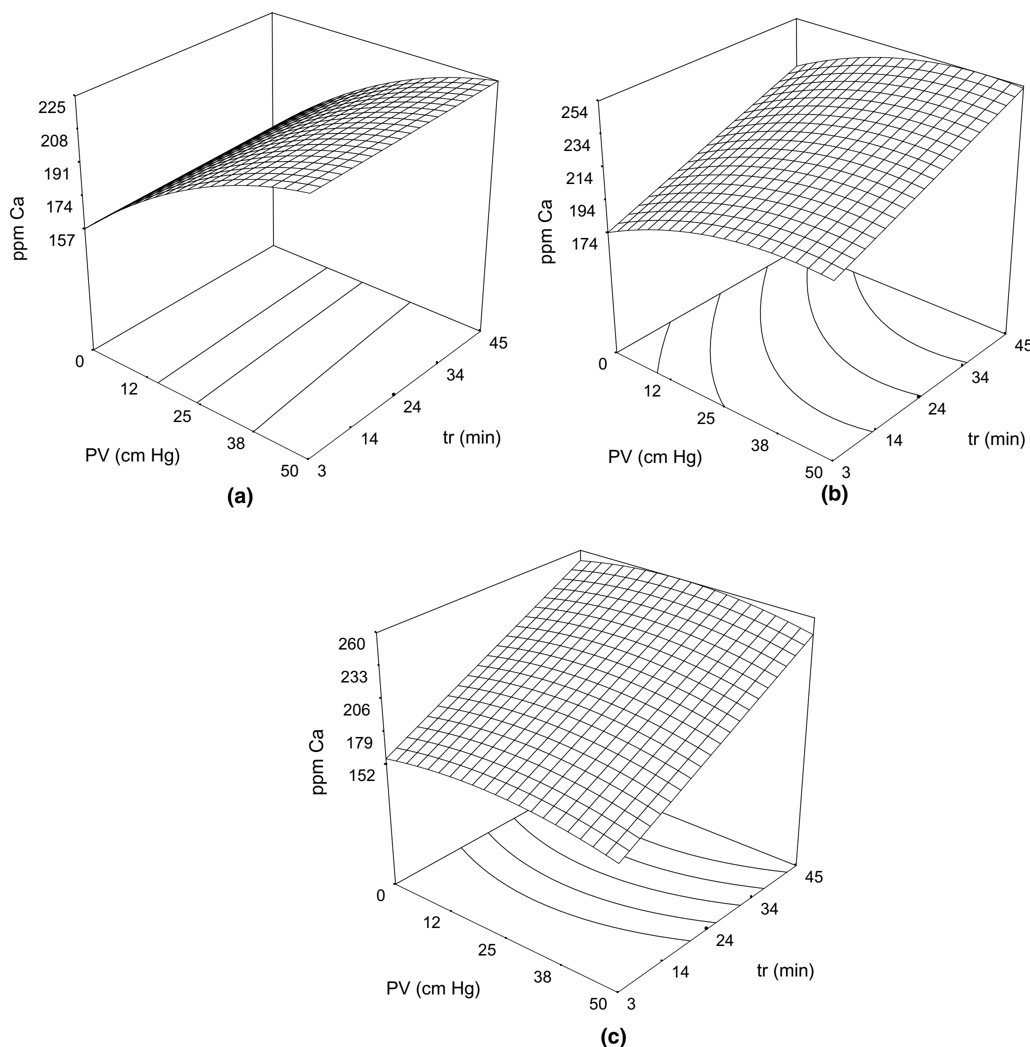

Fig. 4. Surface responses of the effect of vacuum pressure (PV) and relaxation time (t_r) on calcium concentration (Ca) in melon cylinders ($X = 16\%$) impregnated with calcium and zinc at different percentages of sucrose (w/w) in the syrup (a—8%; b—29%; c—50%) with a fruit:syrup ratio 1:3.

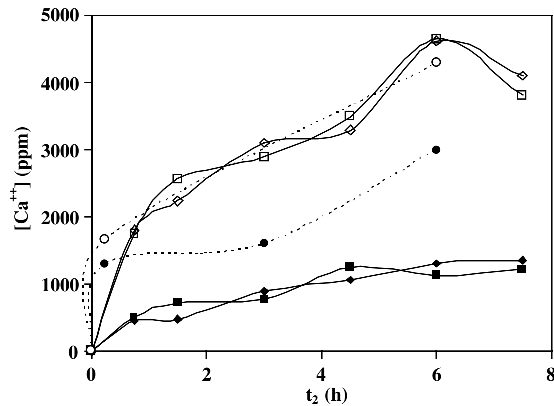

Fig. 5. Calcium concentration (ppm) of apples subjected to atmospheric impregnation (AI) throughout time t_2 for different values of time at vacuum t_1 (0,15 min). ♦: $t_1 = 0$ min (apple 1), ■: $t_1 = 0$ min (apple 2), ●: $t_1 = 15$ min, ◇: $t_1 = 0$ min (blanched apple 1), □: $t_1 = 0$ min (blanched apple 2), ○: $t_1 = 15$ min (blanched apple).

interesting to note that, in spite that the dispersions observed in concentration data, the tendency of both curves throughout t_2 is practically the same, indicating variability between apples from the same lot used in the experiments is not important when considering Ca^{2+} impregnation. According to Ca^{2+} quantity incorporated after 7.5 h of atmospheric treatment the amount consumed in 200 g of fruit would satisfy 26% of the AI_{Ca}, although after 3 h a 17% was already achieved. The length of time under vacuum was found to have little effect on Ca^{2+} content for short periods of time (0–15 min) as well as after a long period of time (180 min) (data not shown). The values increased from 7.4 ppm (Ca^{2+} concentration of fresh fruit) to ≅1000 ppm.

Blanching treatment in fruits often produce profound structural alterations (swelling of cell walls, disruption of membranes, etc.) which affect mass transport phenomena, resulting in the extensive uptake of solute inside the cytoplasm of parenchyma cells. In fact, it was possible to reach higher Ca^{2+} contents in the samples previously blanched (2 min in saturated vapour and cooling in water) after only 45 min of AI (≅1800 ppm) (Fig. 5). Then, Ca^{2+} continued increasing with time to concentrations of 4000–4600 ppm (near the equilibrium status) at the end of the process, with less variability between the apple samples. These high values for Ca^{2+} concentration would imply 80–92% of the AI_{Ca}. However, the blanching treatment slightly improved the incorporation of the mineral by VI (data not shown). As can be observed in Fig. 5, for AI the mild thermal treatment not only modified in a great extension the apple matrix resistance to calcium flux affecting Ca^{2+} uptake kinetics but significantly enhanced its impregnation capability.

The behaviour of mushrooms (*Agaricus bisporus*) previously blanched during calcium impregnation under vacuum was apparently different compared with the response of apple tissue. Due to the high impregnated liquid fraction ($X = 17$–40%), calculated from the sample weight before and after each treatment, a high final Ca^{+2} content was reached in the mushroom matrix (⩾2400 ppm Ca^{2+}) (Ortiz, Salvatori, & Alzamora, 2001; Ortiz, Salvatori, & Alzamora, 2003) (Fig. 6). When mushroom was water-blanched, significant weight losses after heating step occurred, implying native fluids flew out due to high temperature exposition. After cooling, a weight loss was again taking place coupled with the inflow of external solution by capillary mechanisms. When samples were subjected to VI, although negative net values of X were obtained, significant amount of Ca^{2+} were incorporated due to HDM. This Ca^{2+} concentration would be the result of exchange between internal fluids trapped into the matrix pores (native liquid or cooling solution) and external impregnation solution. In spite of the significant mass loss and sample deformation due to heating followed by VI, Ca^{2+} was incorporated during VI, as well as during the cooling stage. Thus, blanching in boiling water would enhance impregnation capabilities of mushroom tissue by increasing native liquid loss (Ortiz, Salvatori, & Alzamora, 2002).

Low temperature blanching, usually employed to improve final firmness of several processed fruit and vegetables by activating native pectin methyl esterase, was also applied by Pérez-López, Welti-Chanes, López-Malo, Palou, and Ibarz (2002) to incorporate calcium in papaya. They evaluated the effect of temperature (45, 55 or 65 °C), calcium chloride concentration (0.2%, 0.6% or 1.0%), and treatment times (20, 40 or 60 min) following a Box-Behnken experimental design. Papaya pieces were submerged in the pre-heated calcium solution and then the fruit was cooled with water. Calcium incorporation and texture characteristics of papaya were significantly affected ($p < 0.05$) by the independent variables, as well as by their interactions. Fig. 7 shows, as an example, the effect of temperature and time

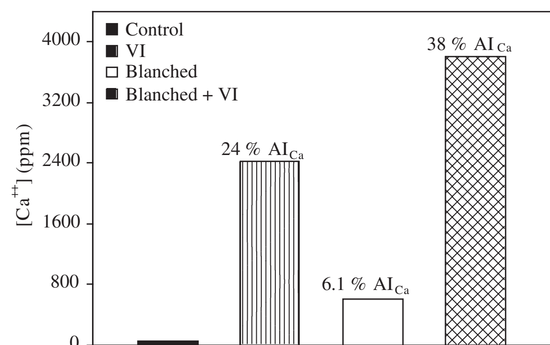

Fig. 6. Calcium concentration (ppm) of raw (control) and vacuum impregnated (VI) mushrooms with and without previous blanching in water at 100 °C during 2 min and cooling in the calcium impregnation solution.

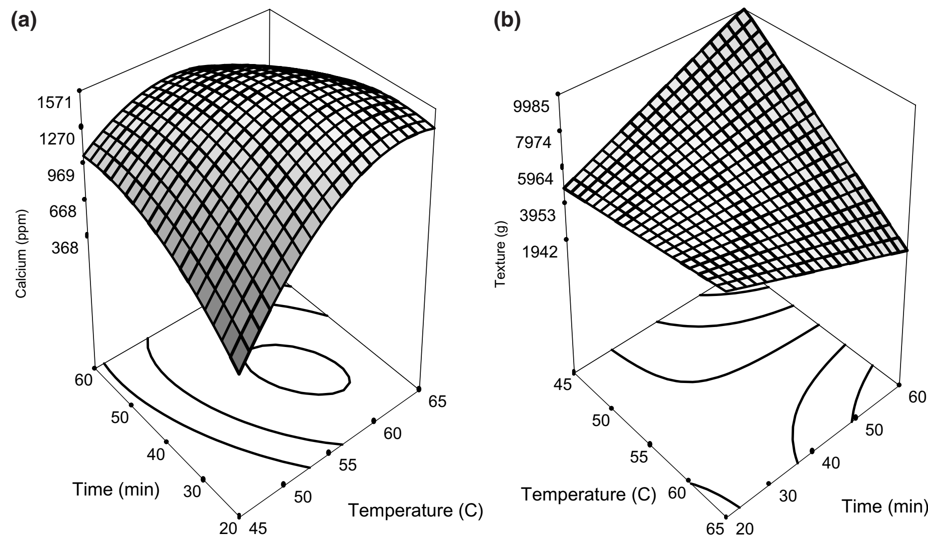

Fig. 7. Surface responses of (a) calcium concentration and (b) maximum compression force of papaya as affected by low temperature blanching (average compression force for raw papaya: 1443 g).

on Ca^{2+} incorporation and texture of papaya when the concentration of impregnation solution is 1.0% $CaCl_2$. Statistical design was performed to select a treatment which maximises calcium incorporation and texture retention.

The calcium contribution of a food depends on both the content of calcium in a serving and the bioavailability of calcium. Bioavailability comprises absorption, transport, and utilisation of a nutrient. In the case of calcium, absorption indicates bioavailability, since any absorbed ions are available for body processes and any calcium stored in bone is functionally used (Weaver, 1998). Many factors affect calcium absorption, among others, the load of the meal, the solubility of the calcium salt and the presence of inhibitors (such as oxalic and phytic acids). Various techniques have been proposed for measurement of calcium absorption in humans in the last few years. However they have many methodological difficulties. Therefore, absorption determination in growing rats is accepted as an alternative method to study the influence of dietary factors on calcium absorption (Greger, 1992). González Fesler, Salvatori, Weisttaub, Portela, and Alzamora (2003) studied in rats the relative absorption of calcium incorporated in apple matrix, taking calcium carbonate as reference substance. AI of calcium was performed by immersing apple cylinders into agitated isotonic glucose aqueous solution containing 5.24% (w/w) Ca^{2+} salts (lactate + gluconate). The following experimental conditions were selected: 1.5 h under AI; 1.5 h under AI with previous blanching (2 min in saturated vapour and cooling 5 min in water) and 6 h under AI with previous blanching. Apparent absorption values with respect to calcium carbonate were 80.2%, 74.2% and 86.8%, respectively, indicating apple matrix impregnated with Ca^{2+} lactate/gluconate was a vehicle to provide easily absorbable calcium. Also, the results would imply a tendency to a high calcium absorption when matrices were impregnated for long times with previous blanching.

3. Conclusions

The development and consumption of functional foods, or foods that promote health beyond providing basic nutrition, are on the rise. VI and/or AI seem to be feasible technologies for exploitation of fruit and vegetable tissues as new matrices into which functional ingredients can be successfully incorporated, providing novel functional product categories and new commercial opportunities. The impregnation behaviour of vegetable and fruit matrices is highly produce-specific and processing and storage of these functional foods may have profound effect on health benefits. Fruit and vegetable matrices will certainly be an important research and development area for future functional food markets. Knowledge to assess the effects of these matrices in the body is also a highly desirable goal.

References

Aguilera, J. M., & Stanley, D. W. (1991). *Microstructural principles of food engineering & processing*. New York: Elsevier Applied Science.

Alzamora, S. M., Anino, S., & Salvatori, D. M. (2001). Impregnation techniques for the incorporation of calcium to apple matrix. In *2001 IFT annual meeting*. New Orleans, USA: Institute of Food Technologists.

Alzamora, S. M., Tapia, M. S., Leúnda, A., Guerrero, S. N., Rojas, A. M., Gerschenson, L. N., & Parada-Arias, E. (2000). Relevant results on minimal preservation of fruits in the context of the

multinational project XI.3 of CYTED, an Ibero-American R&D cooperative program. In J. Lozano, M. C. Añón, & G. Barbosa Cánovas (Eds.), *Trends in food engineering*. Gathersburg, MD: Aspen Publishers.

Anino, S., Salvatori, D., & Alzamora, S. M. (2003). Apple matrix with calcium incorporated by impregnation processes. In P. Fito, A. Mulet, A. Chiralt, & A. Andrés (Eds.), *Ingeniería de alimentos: Nuevas fronteras en el siglo XXI. Proceedings del III congreso Iberoamericano de ingeniería de alimentos (CIBIA 2001)* (Vol. 4). Valencia, España: Servicio de Publicaciones de la Universidad Politécnica de Valencia.

Anino, S., Salvatori, D. M., Castro, M. A., & Alzamora, S. M. (2002). Cambios estructurales en tejido de manzana fortificado con calcio. In *IX congreso argentino de ciencia y tecnología de alimentos*. Buenos Aires, Argentina: Asociación Argentina de Tecnólogos Alimentarios.

Ben-Arie, R., Kislev, N., & Frenkel, C. (1979). Ultrastructural changes in the cell walls of ripening apple and pear fruit. *Plant Physiology, 64*, 197–202.

Betoret, N., Puente, L., Díaz, M. J., Pagan, M. J., García, M. J., Gras, M. L., Martínez-Monzó, J., & Fito, P. (2003). Development of probiotic-enriched dried fruits by vacuum impregnation. *Journal of Food Engineering, 56*, 273–277.

Brett, C. T., & Waldron, K. W. (1996). *Physiology and biochemistry of plant cell walls*. Cambridge: Chapman and Hall.

Carpita, N. C., Sabularse, D., Montezinos, D., & Delmer, D. P. (1979). Determination of pore size of cell walls of living plant cells. *Science, 205*, 1144–1147.

Diplock, A. T., Aggett, P. J., Ashwell, M., Bornet, F., Fern, F. B., & Roberfroid, M. B. (1999). Scientific concepts of functional food in Europe: Consensus document. *British Journal of Nutrition, 81*(Suppl. 1), S1–S28.

Fito, P. (1994). Modelling of vacuum osmotic dehydration of foods. *Journal of Food Engineering, 23*, 313–328.

Fito, P., Andrés, A., Chiralt, A., & Pardo, P. (1996). Coupling of hydrodynamic mechanism and deformation–relaxation phenomena during vacuum treatments in solid porous food-liquid systems. *Journal of Food Engineering, 27*, 229–240.

Gibson, G. R., & Williams, C. M. (2000). *Functional foods. Concept to product* (pp. 1–27). Cornwall: Woodhead Publishing Limited.

González Fesler, M., Salvatori, D. M., Weisttaub, A., Portela, M. L., & Alzamora, S. M. (2002). Calcium impregnation kinetics of apple matrix. In *2002 IFT annual meeting*. Anaheim, CA: Institute of Food Technologists.

González Fesler, M., Salvatori, D. M., Weisttaub, A., Portela, M. L., & Alzamora, S. M. (2003). Personal communication.

Gras, M., Vidal-Brotons, D., Betoret, N., Chiralt, A., & Fito, P. (2002). The response of some vegetables to vacuum impregnation. *Innovative Food Science and Emerging Technologies, 3*, 263–269.

Greger, J. L. (1992). Using animals to assess bioavailability of minerals: Implications for human nutrition. *Journal of Nutrition, 122*, 2047–2052.

Institute of Medicine (1998). *Dietary references intake (DRI) for calcium, phosphorus, magnesium, vitamin D and fluoride* (p. 432). Food and Nutrition Board, Standing Committee on the Scientific Evaluation of Dietary References Intakes. Washington, DC: National Academic Press.

Lapsley, K. G., Escher, F. E., & Hoehn, E. (1992). The cellular structure of selected apple varieties. *Food Structure, 11*, 339–349.

Maguiña, G., Tapia, M. S., Briceño, A. G., Rodríguez, C., Sánchez, D., Roa, V., & López-Malo, A. (2002). Incorporación of *Bifidobacterium* spp. en una matriz porosa de fruta por el mecanismo hidrodinámico (PF-08). In *Actas del 2° Congreso Español de Ingeniería de Alimentos* (pp. 1–4). Lleida, España: Universitat de Lleida.

Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., & Saarela, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal, 12*, 173–182.

Mujica-Paz, H., Hernández-Fuentes, M. A., López-Malo, A., Palou, E., Valdez-Fragoso, A., & Welti-Chanes, J. (2002). Incorporation of minerals to apple slabs through vacuum impregnation and osmotic dehydration (306-22). In *2002 IFT annual meeting book of abstracts* (p. 74), Anaheim, CA.

Mujica-Paz, H., Valdéz-Fragoso, A., López-Malo, A., Palou, E., & Welti-Chanes, J. (2003). Impregnation properties of some fruits at vacuum pressure. *Journal of Food Engineering, 56*, 307–314.

Ortiz, C., Salvatori, D. M., & Alzamora, S. M. (2001). Mushroom tissue as matrix for calcium incorporation by vacuum impregnation. In *Proceedings of EMPROMER Vol. III—3rd Mercosur congress on process systems engineering—1st Mercosur congress on chemical engineering*, Santa Fe, Argentina (pp. 1639–1644).

Ortiz, C., Salvatori, D. M., & Alzamora, S. M. (2002). Combined blanching and vacuum impregnation for enhancing calcium fortification in mushroom tissue. In *2002 IFT annual meeting*. Anaheim, CA: Institute of Food Technologists.

Ortiz, C., Salvatori, D., & Alzamora, S. M. (2003). Fortification of mushroom with calcium by vacuum impregnation. *Latin American Applied Research, 33*, 191–197.

Ortiz, E., Sánchez, D., Rodríguez, C., Tapia, M., Medina, V., & Sánchez, C. (2002). Incorporación de *Bifidobacterium* sp. en guayaba (*Psidium guajava* L.) mediante impregnación a vacío. In *Libro de resúmenes del III congreso venezolano de ciencia y tecnología de alimentos y II congreso panamericano de calidad sanitaria de los alimentos*, Caracas, Venezuela (p. 37).

Pérez-López, I., Welti-Chanes, J., López-Malo, A., Palou, E., & Ibarz, A. (2002). Calcium incorporation in papaya by low temperature blanching. In *2002 IFT Annual Meeting Book of Abstracts* (306-22), Anaheim, CA (p. 74).

Rastall, R. A., Fuller, R., Gaskins, H. R., & Gibson, G. R. (2000). Colonic functional foods. In G. R. Gibson & C. M. Williams (Eds.), *Functional foods*. Cambridge: Woodhead Publishing Limited.

Roa, V., Tapia, M. S., & Millán, F. (2001). Mass balances in porous foods impregnation. *Journal of Food Science, 66*, 1332–1336.

Rodríguez, M. I. (1998). *Estudio de la penetración de microorganismos en frutas mediante el modelo Hidrodinámico (HDM)*. Thesis. Instituto de Ciencia y Tecnología de Alimentos, Universidad Central de Venezuela.

Saarela, M., Lähteenmäki, L., Crittenden, R., Salminen, S., & Mattila-Sandholm, T. (2002). Gut bacteria and health foods—the European perspective. *International Journal of Food Microbiology, 78*, 99–117.

Sloan, E. (2002). The top 10 functional food trends. The next generation. *Food Technology, 56*(4), 32–57.

Tapia, M. S., Schulz, E., Gómez, V., López-Malo, A., & Welti-Chanes, J. (2003). A new approach to vacuum impregnation and functional foods: Melon impregnated with calcium and zinc. In *2003 IFT annual meeting technical program abstracts (60D-2)*, Chicago, USA.

Weaver, C. (1998). Calcium in food fortification strategies. *International Dairy Journal, 8*, 443–449.