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Ascorbic acid and calcium uptake in pineapple tissue through different sucrose concentrations of impregnation solution

P.R. Fernández[a], R.H. Mascheroni[b], L.A. Ramallo[a,*]

[a] IMAM (CONICET-Universidad Nacional de Misiones) and FCEQYN-UNaM, Misiones, Argentina
[b] CIDCA (CONICET-Universidad Nacional de La Plata and CICPBA) and FI-UNLP, La Plata, Argentina

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ABSTRACT

Incorporation of ascorbic acid and calcium in pineapple slices through impregnation processes in isotonic (~14 °Bx) and hypertonic (50 °Bx) aqueous solution of sucrose was studied. Changes in water content, soluble solids, pH, mechanical and structural properties, ascorbic acid and calcium content in fruit in function of dipping time and impregnation syrup concentration were evaluated. Isotonic treatment was more effective than hypertonic treatment for the calcium and ascorbic acid incorporation in the plant tissue. Under same conditions, the ascorbic acid uptake was greater than calcium uptake. The fruit firmness was reduced during isotonic treatment and increased by osmotic dehydration, without significant effect of the nutrients addition to the impregnation solution. Additionally, changes in some physicochemical properties of impregnation solution in function of the time have been studied. Nutrient content in impregnation solutions did not register significant changes, which would allow its reuse with some conditioning of the soluble solids concentration.

1. Introduction

There is currently a trend towards the incorporation of healthy products into the daily diet (Villaño et al., 2016), including enriched foods, that is, those that have been significantly supplemented in their natural content of essential nutrients. Pineapple fruit contains approximately 47.8 mg/100 g of ascorbic acid and 13 mg/100 g of calcium (USDA, 2017), apart from carbohydrates, fibers and minerals. Although pineapple fruit is high in vitamin C, it has been shown that up to 60% is lost during osmotic dehydration and drying processes (Ramallo and Mascheroni, 2010). The incorporation of ascorbic acid previously to these processes would compensate the loss. Likewise, in order to prevent several diseases, nowadays numerous foods are fortified with calcium to increase consumer calcium intake.

Different results on fruit fortification by impregnation processes at atmospheric pressure or under vacuum conditions have been published (Gras et al., 2003; Anino et al., 2006, Silva et al., 2014a). So, vacuum impregnation has been defined as an operation that allows the incorporation of any ingredient in a food porous structure to confer it functional properties (Fito et al., 2001). However, Anino et al. (2006) found that the process of apples impregnation with calcium carried out without vacuum application was more efficient than the impregnation process with vacuum application. Isotonic and hypertonic aqueous solutions, previously nutrients-enriched, have been used as impregnation medium (Anino et al., 2006; Lovera et al., 2014; Silva et al., 2014b; Mauro et al., 2016). However, the possibility of increasing the fruit nutrient content through impregnation in isotonic solutions has been less studied than through impregnation treatment in hypertonic solutions. The obtained product through isotonic impregnation preserves its sensory characteristics and can be enriched with nutritionally valuable components, which make it attractive to the consumer and can positively affect health.

Moreover, development of fruits impregnation process with nutrients can be limited due to the problems with management of enriched solution and the economic feasibility of this process probably depends on the reuse of the impregnation medium.

Process of osmotic dehydration can cause physical and chemical changes in cellular tissues that vary with the plant material, process temperature and solutes content, among other variables. Also, mechanical properties of fruits and vegetables can be altered by the increase in calcium content in plant tissue, since the presence of this compound affects the integrity of the cellular structure (Anino et al., 2006; Mauro et al., 2016). The ascorbic acid uptake can affect the cell membrane permeability as a consequence of different effects, including lowering the pH (Mauro et al., 2016). Mass transfer during osmotic dehydration is linked to the selective permeability of cell membrane, the intercellular spaces and the liquids composition of these spaces, among others. Thus, kinetics of pineapple osmotic dehydration can be

* Corresponding author.
E-mail addresses: laura.ana.ram@gmail.com, laura.ana.ram@gmail.com (L.A. Ramallo).

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altered by changes in the content of calcium and ascorbic acid of plant tissue.

The objective of this work was to compare the effectiveness of two means of impregnation, isotonic and hypertonic sucrose solution, for the development of pineapple fruit fortified with calcium and ascorbic acid, based on the incorporation of nutrients in plant tissue, and changes in mechanical, chemical and structural properties. Furthermore, in order to evaluate the feasibility of syrup reuse, composition changes of impregnation solutions was analyzed.

2. Materials and methods

2.1. Sample preparation

Ananá comosus fruits (Smooth Cayenne variety), with commercial ripeness degree (14.0 ± 1.4 °Bx) were washed and manually peeled, cored with a cork borer (25 mm diameter), cut into half rings of 6.0 ± 0.5 mm thickness and of 115.0 ± 5.0 mm diameter. Due to variation in the content of native compounds between the base and the top of the fruit (Ramallo and Mascheroni, 2012) only the central portion of each pineapple fruit was used.

2.2. Impregnation process

Hipertonic and isotonic aqueous solutions of sucrose (commercial sugar and distilled water), with addition of ascorbic acid (food grade, Parafarm, Argentine) and pentahydrated calcium lactate (food grade, Parafarm, Argentine) were used as impregnation medium. In order to evaluate the effect of the immersion process at 40 °C on the mass transfer and mechanical properties of the pineapple fruit, two other treatments with the same sucrose aqueous solutions without calcium and ascorbic acid were performed. Denominations and specific conditions of treatments are shown in Table 1. In treatments *IT* and IT_{CaAA} the solution was isotonic respect to the soluble solids content of fresh fruit.

In each essay, pineapple samples were placed in beakers containing impregnation solution in a ratio 4/1 of solution mass to fruit mass. The system remained into a shaken thermostatic bath (Dubnoff, Vicking, Argentine) at constant temperature (40 °C), with constant stirring (60 rpm) during 24 h. After 1, 2, 3, 6 and 24 h the samples were randomly taken away, rinsed with demineralized water and the excess of surface water was removed with tissue paper. Weight of samples was individually measured before and after treatment.

Dipping solutions were monitored during the assays by evaluating the content of soluble solids, ascorbic acid and calcium, pH and titratable acidity (Table 4).

2.3. Mass transfer parameters

Variation of mass (ΔM), water (ΔW), calcium (ΔCa), ascorbic acid (ΔAA) and sugar (ΔS) in the fruit, with the simplification that fruit natives solutes do not migrate into sucrose solution, were calculated according to Equations (1)–(5) respectively (Mauro et al., 2016).

Table 1
Labeling of treatments and composition of solutions used for pineapple impregnation.

Treatment	Sucrose (% W/W)	Calcium (% W/W)	Ascorbic acid (% W/W)
IT	~14[a]	0	0
$\boldsymbol{IT_{CaAA}}$	~14[a]	2	1
HT	50	0	0
$\boldsymbol{HT_{CaAA}}$	50	2	1

[a] Defined concentration for each fruit, differing according its native content of soluble solids (°Bx).

$$\Delta M(\%) = \frac{M_t - M_0}{M_0} * 100 \quad (1)$$

$$\Delta W(\%) = \frac{M_t * W_t - M_0 * W_0}{M_0} * 100 \quad (2)$$

$$\Delta Ca(\%) = \frac{Ca_t * M_t - Ca_0 * M_0}{M_0} * 100 \quad (3)$$

$$\Delta AA(\%) = \frac{AA_t * M_t - AA_0 * M_0}{M_0} * 100 \quad (4)$$

$$\Delta S(\%) = \Delta M - \Delta W - \Delta Ca - \Delta AA \quad (5)$$

where: *M* is the mass of the fruit (g); *W, Ca* and *AA* are the content of water, calcium and ascorbic acid respectively (g/g of fruit). The subscript 0 corresponds to initial time (t = 0), and t corresponds to a given time *t*.

2.4. Analytical methods

2.4.1. Physicochemical properties

Moisture content of the fresh and treated samples was determined gravimetrically by drying in an oven at 75 ± 2 °C to constant weight (approximately 48 h). Soluble solids content (*SS*) was measured through reading the refractive index of the juice, obtained by fruit mechanical compression, with a digital refractometer (HI96801, Hanna Instruments Inc, Romania).

Pineapple pulp (10 g) obtained from 3 different samples was crushed, with addition of 100 mL distilled water, by a mixer (HR 1364-600W, Philips, Argentine). The resulting product was analyzed to determine pH by a digital glass electrode (TPA-III, Altronix, Argentine) and citric acid content (*CA*), measured as titratable acidity through titration with 0.1 N sodium hydroxide. Results were expressed as g citric acid per 100 g of fruit. To measure the titratable acidity of syrup, 10 mL aliquot of the impregnation solution was leveled to 100 mL with distilled water; results were expressed as g citric acid per 100 mL of syrup.

Optical transmittance of the impregnation solutions were evaluated through a UV-VIS spectrophotometer (UV-2550, Shimadzu, Japan) at λ = 600 nm.

Measurements were performed in triplicate.

2.4.2. L-ascorbic acid determination

Extraction process. Each fruit sample (≈3 g) was weighed and crushed in mixer for 1 min, with addition of 50 mL of buffer solution (the same used in mobile phase). This mixture was transferred to a dark flask, subjected to ultrasound for 15 min and then filtered. To quantify the ascorbic acid content in the impregnation solution, 5 mL sample solution was diluted with 5 mL buffer, filtered and injected into the chromatograph.

Quantification. It was performed using liquid chromatography, HPLC (Shimadzu Co., Japan), with Alltima C-18 column (250 mm × 4.6 mm, 5 μm particle size) and UV detector (λ = 254 nm). A mobile phase of buffer (potassium phosphate 0.02 M, pH 2.5, adjusted with phosphoric acid): acetonitrile (98:2 v/v) at a flux rate of 0.1 mL/min was utilized.

Analyses were performed in duplicate and the results were expressed in mg AA/g fresh fruit and in mg AA/mL solution.

2.4.3. Calcium determination

Calcium concentrations of fresh and treated fruit were measured in duplicate using atomic absorption spectrometer (Perkin Elmer 3110, Perkin Elmer Inc., USA). The samples (≈2 g) were calcined at 550 °C in a muffle furnace (ORL, Argentine), until obtaining white ash (6 h). Ashes were dissolved in 10 mL of 2N HCl and the system was boiled for 5 min. The resulting solution was filtered in a 25 mL flask, 2 mL solution of 10% w/w lanthanum oxide-2.2% w/w potassium chloride and distilled water were added until the volume was completed. Operating

conditions were: wavelength 422.7 nm, slit width 0.7 nm, and fuel/oxidant ratio 2.5/4.5. Results are expressed in mg Ca/g fresh fruit and mg Ca/mL solution.

2.5. Mechanical properties

Samples of fresh and treated fruit were sectioned with cork borer in discs of 25 mm diameter. Mechanical properties were evaluated through a universal texturometer (TA.XT2 Plus Texture Analyzer, Stable Micro Systems, USA), with a load cell of 50 N. Uniaxial compression tests were carried out on the cylindrical samples using a stainless steel probe of circular cross-section of 75 mm diameter, at a constant speed of 0.5 mm/s, until 70% sample deformation of the original height.

Force F_t (N) and height H_t (mm) data were recorded. Stress (σ), strain (ε) and modulus of elasticity (E) were calculated according to Equations (6)–(8), respectively. The elasticity modulus was calculated from the slope of the initial linear portion of the stress-strain curve up to 6% compression (Ramallo and Mascheroni, 2012). Stress (σ_{rup}) and strain (ε_{rup}) at rupture were determined at the maximum point of stress-strain curve (Chiralt et al., 2001; Pereira et al., 2006). Reported values were the average of nine measurements.

$$\sigma = \frac{F * H_t}{H_0 * A_0} \tag{6}$$

$$\varepsilon = -\ln\left(\frac{H_t}{H_0}\right) \tag{7}$$

$$E = \left(\frac{d\sigma}{d\varepsilon}\right)_{\varepsilon \to 0} \tag{8}$$

where A_0 (m^2) is the initial contact area of the sample, H_0 and H_t (mm) are the sample height at start and after a time t of the compression test, respectively.

2.6. Microscopic analysis

Microstructure of fresh and treated fruits were observed through an environmental scanning electron microscope (ESEM, FEI ESEM Quanta 200, USA). Samples cut in cubes of 5 mm^3 were fixed in formaldehyde-acetic acid-alcohol solution for 24 h and then dehydrated by dip in ethanol solutions of increasing concentration (30, 50, 60, 70, 85 and 95%) for 15 min in each one and followed by dipping 20 min in pure ethanol (Cicarelli, Reagents S.A., Argentine).

Samples were dried using the critical point drying technique and then covered with a thin layer of gold. The conditions used in the microscope were high vacuum, at an acceleration voltage of 15 kV.

In addition, a microanalysis of the relative contents of calcium was determined in different tissue zones of treated samples, through the energy dispersive x-ray spectroscopy (EDS) (EDAX SDD Apollo 40, USA) coupled to the scanning electron microscope.

3. Results and discussion

3.1. Water loss and sucrose gain

Fresh fruit of pineapple had an average content of ascorbic acid and calcium of 40.0 ± 12.5 and 13.1 ± 3.7 mg/100 g fruit respectively; water content of 86.4 ± 1.9 g/100 g fruit; soluble solids content of 14.02 ± 1.37 °Bx; pH of 3.78 ± 0.04 and citric acid content of 0.69 ± 0.10 g/100 g fruit.

Experimental data of water loss (ΔW) and sucrose gain (ΔS) of pineapple slices during treatments described in Table 1 are presented in Fig. 1.

When the impregnation solution has the same solute concentration to the fruit (isotonic solution), the water flow from the fruit to the solution is not significant, unlike what happens when sucrose concentration is higher in the solution than in the plant tissue (hypertonic solution): water leaves the fruit and the osmotic dehydration take place. However, slight increase in water loss during the fruit dipping in isotonic solution was observed (Fig. 1). Since the plant tissue moisture content remained without significant changes during the isotonic process (Table 2), it can be assumed that mass change quantified as a water loss according to Equations (1) and (2), was in fact a mass loss due to material disintegration, native solutes loss, etc. These observations are in agreement with the transmittance readings (Table 4) of impregnation solutions, since the reduction of transmittance values at the end of the process was greater in the isotonic solution than in the hypertonic solution.

Addition of calcium and ascorbic acid to the syrup did not significantly affect the water loss during osmotic dehydration at 50 °Bx. This behavior was repeated in isotonic medium at short treatment times, but after 3 h of dipping the water loss was greater when the syrup contained calcium and ascorbic acid.

Sucrose gain was increased with the addition of calcium and ascorbic acid to hypertonic solution (Fig. 1), but no significant differences in sucrose gain by nutrients addition in isotonic solution were recorded. These results agree with those presented by Silva et al. (2014b), where the impregnation with sucrose and calcium was favored by the presence of ascorbic acid in osmotic solution. Likewise, Silva et al. (2014a) concluded that calcium addition into osmotic syrup tends to restrict the sucrose gain in pineapple matrix. This restrictive behavior of calcium about the sucrose gain is similar to that observed by Mavroudis et al. (2012) in osmotic dehydration of apples and by Pereira et al. (2006) during guavas osmotic dehydration. Mauro et al. (2016) pointed out that ascorbic acid addition to osmotic solution affected the porosity of the cell walls in apple tissues.

3.2. Ascorbic acid and calcium impregnation

Results of the ascorbic acid and calcium content in pineapple samples treated by two impregnation conditions (IT_{CaAA} and HT_{CaAA}), in function of dipping time, are shown in Fig. 2. The incorporation of ascorbic acid to the pineapple tissue did not differ between the isotonic and hypertonic treatments, during the first 3 h of immersion. Next, a marked increase of ascorbic acid content in samples processed in isotonic solution was recorded.

As can be seen in Fig. 2b, incorporating calcium into the plant matrix was favored by decrease of sucrose content in the impregnation solution along all process. The pineapple calcium content raised continuously during the isotonic process; on the contrary, these values remained practically unchanged after the first hour of treatment in hypertonic solution. This behavior could be due to formation of a crust with high sucrose content in the samples surface, as is corroborated by micrograph of Fig. 3. Giraldo et al. (2003) have also observed crust formation during osmotic dehydration. However, the ascorbic acid incorporation in pineapple through hypertonic solution was not limited by crusting, possibly because the molecular weight of ascorbic acid is lower than the molecular weight of calcium lactate (Phisut, 2012).

Effect of syrup concentration on the incorporation of calcium and ascorbic acid in fruits varies according to chemical and structural characteristics of the plant tissue. Silva et al. (2014b) determined that lower concentration of sucrose favors the gain of ascorbic acid and calcium during pineapple osmotic dehydration. A study of melons impregnation with calcium lactate during osmotic dehydration obtained greater incorporation of the mineral with the lowest concentration of osmotic solution (Ferrari et al., 2010). Nagai et al. (2015) found the sucrose concentration of syrup did not influence significantly the ascorbic acid gain of mangoes.

About fruit impregnation processes through isotonic solution, positive results regarding the incorporation of calcium in apples (Anino et al., 2006) and papaya (Lovera et al., 2014) were reported. Studies of

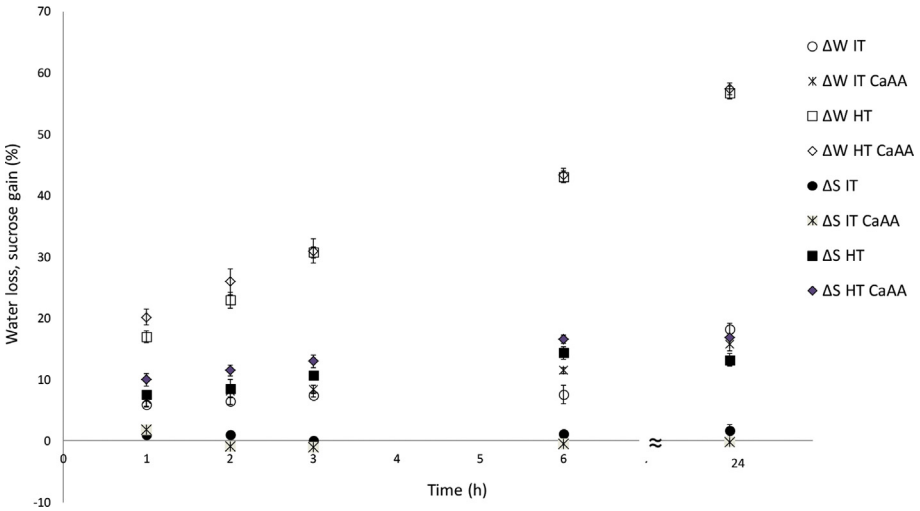

Fig. 1. Variation of water loss (ΔW) and sucrose gain (ΔS) of pineapple during dipping treatment in isotonic and hypertonic solution, with and without ascorbic acid and calcium lactate.

Table 2
Physicochemical properties of fresh and processed pineapple samples.

Fruit sample		*SS* (°Bx)	pH	*CA* (g/100 g)	*Moisture* (g/g)
Treatment	Time (h)				
FrFr	0	14.02 ± 1.37 ^{a}	3.78 ± 0.04 ^{bc}	0.63 ± 0.10 ^{cde}	0.86 ± 0.08 ^{cd}
IT	3	14.34 ± 0.73 ^{a}	3.76 ± 0.06 ^{bc}	0.48 ± 0.03 ^{b}	0.85 ± 0.01 ^{cd}
	6	14.52 ± 0.80 ^{a}	3.94 ± 0.03 ^{d}	0.38 ± 0.01 ^{a}	0.84 ± 0.05 ^{c}
IT_{CaAA}	3	15.30 ± 0.70 ^{a}	3.91 ± 0.03 ^{d}	0.52 ± 0.02 ^{bc}	0.84 ± 0.01 ^{c}
	6	15.60 ± 0.67 ^{a}	3.93 ± 0.02 ^{d}	0.51 ± 0.04 ^{bc}	0.84 ± 0.01 ^{c}
HT	3	31.68 ± 1.00 ^{b}	3.92 ± 0.03 ^{d}	0.54 ± 0.02 ^{c}	0.68 ± 0.02 ^{b}
	6	37.78 ± 1.00 ^{c}	3.66 ± 0.02 ^{a}	0.68 ± 0.05 ^{e}	0.61 ± 0.03 ^{a}
HT_{CaAA}	3	30.60 ± 1.80 ^{b}	3.82 ± 0.02 ^{c}	0.61 ± 0.01 ^{d}	0.67 ± 0.02 ^{b}
	6	39.70 ± 3.20 ^{c}	3.84 ± 0.03 ^{c}	0.77 ± 0.02 ^{f}	0.61 ± 0.04 ^{a}

*Means values in the same column with the same letter do not differ significantly ($p > 0.05$)
* SS = soluble solids content; CA = citric acid content.

pineapple impregnation with calcium and ascorbic acid applying isotonic solutions were not found.

3.3. Physicochemical properties

3.3.1. Nutritional intake

Intake recommendations for nutrients are provided in the Dietary Reference Intakes (DRI) developed by the Food and Nutrition Board. 50 g of pineapple fruits with 2 h dipping treatment in isotonic or hypertonic solution (Fig. 2a) could cover roughly 150% of the recommended daily intake (DRI) of vitamin C for adult male (90 mg/d) (IOM, 2000).

However, the same portion of fruit would cover less than 4% of calcium daily requirement, considering that recommended daily calcium intake is 1000 mg (IOM, 2011). Although the calcium content of fruit treated for 6 h in isotonic solution was six times higher than calcium content of fresh fruit (Figs. 2b), 50 g of this food would cover around 5.5% of DRI for calcium.

Difficulties to cover calcium requirements from fruits impregnated with this mineral have already been reported in other studies: calcium content of apples with 6 h impregnation in isotonic solution containing Ca^{2+} lactate and Ca^{2+} gluconate was 130 mg/100 g; therefore, 50 g of treated fruit could cover only 6.5% of calcium DRI (Anino et al., 2006); calcium content of pineapple samples was 90 mg/100 g after 6 h immersing in sucrose syrup added with calcium lactate, hence 50 g of

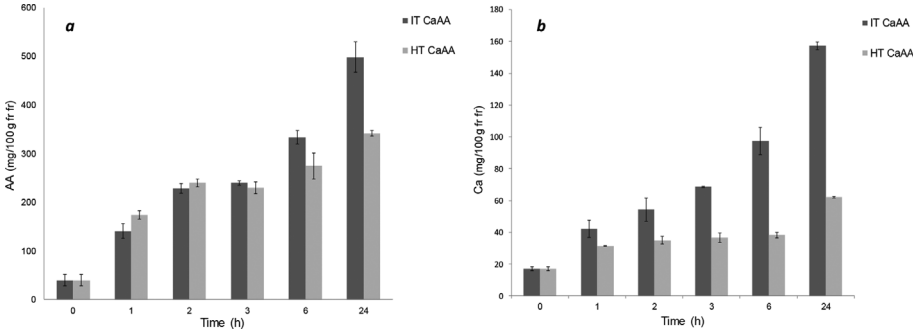

Fig. 2. Variation of (a) ascorbic acid and (b) calcium content of pineapple fruit during impregnation treatment in isotonic solution (IT_{CaAA}) and in hypertonic solution (HT_{CaAA}).

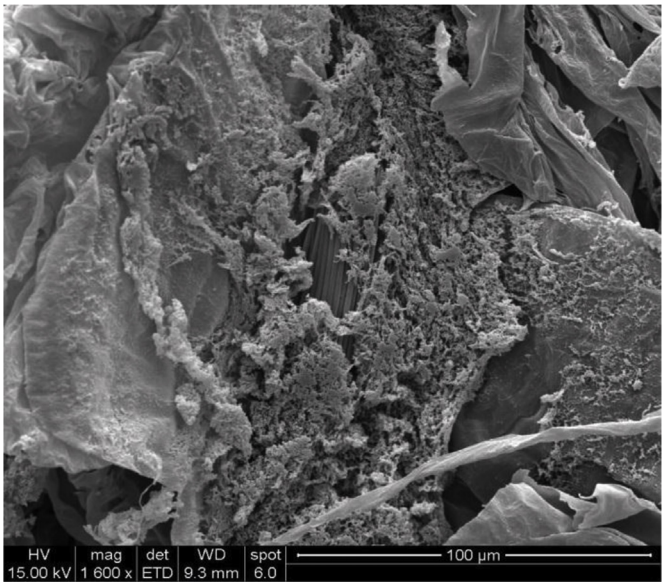

Fig. 3. SEM micrograph of pineapple sample with 3 h of dipping hypertonic solution illustrating the surface layer of high sucrose content or crusting.

product would cover approximately 5% calcium DRI (Silva et al., 2014a).

Immersion treatment by itself could affect the physicochemical characteristics of the fruit; the syrup concentration also induces modifications in chemical composition of dehydrated pineapple. Average and standard deviation values of pH, moisture content, soluble solids (*SS*) and citric acid content (*CA*) of the fresh and treated fruit in isotonic solution with and without nutrients and in hypertonic solution with and without nutrients are shown in Table 2.

As expected, values of soluble solids content was increased significantly in samples treated in hypertonic solution, due to the osmotic process. Also, the addition of calcium lactate and ascorbic acid to the sucrose solution (isotonic and hypertonic) did not affect moisture and soluble solids content of the fruit.

The citric acid content increase during hypertonic treatments may be due to the concentration of native organic acids by the osmotic dehydration of pineapple tissue. Conversely, the reduction of *CA* values in fruit samples during immersion processes in isotonic solution could be due to the leaching of these acids. The addition of calcium lactate and ascorbic acid to isotonic solution restricted the titratable acidity reduction, possibly by increasing the ascorbic acid content.

Although there was variation in pH values of fruits during the different treatments, there was no trend of these changes, in agreement with other studies of pineapple osmotic dehydration (Ramallo et al., 2013).

3.3.2. Mechanical properties

Uniaxial compression tests on fresh and treated fruit were performed to evaluate the effect of calcium and ascorbic acid incorporation on the mechanical behavior of plant tissue. Elasticity modulus (E_0), stress (ε^o_{rup}) and strain (σ^o_{rup}) at rupture of fresh pineapple fruit were 37.81 ± 12 kPa, 45.60 ± 6.30 kPa and 0.30 ± 0.06, respectively. In order to compare the results of tests from different fruits, mechanical properties were expressed as ratio of the current value to that of the respective fresh fruit, as shown in Table 3.

Although values of stress at rupture (σ_{rup}) of treated samples in isotonic solution decreased gradually with dipping time no significant effect ($p > 0.05$) of the treatment time and the addition of calcium and ascorbic acid was found. In contrast, treatments in hypertonic solution favor the increase of σ_{rup} values, an effect that was enhanced by dipping time and addition of calcium and ascorbic acid to the syrup. These

Table 3
Mechanical properties of pineapple during different dipping treatments, as the relationship between experimental data at different immersion times and the same one in fresh fruit.

Time (h)	*IT*			IT_{CaAA}			*HT*			HT_{CaAA}		
	$\sigma_{rup}/\sigma^o_{rup}$	$\varepsilon_{rup}/\varepsilon^o_{rup}$	*E/Eo*	$\sigma_{rup}/\sigma^o_{rup}$	$\varepsilon_{rup}/\varepsilon^o_{rup}$	*E/Eo*	$\sigma_{rup}/\sigma^o_{rup}$	$\varepsilon_{rup}/\varepsilon^o_{rup}$	*E/Eo*	$\sigma_{rup}/\sigma^o_{rup}$	$\varepsilon_{rup}/\varepsilon^o_{rup}$	*E/Eo*
0	1^{b}	1^{a}	1^{a}	1^{b}	1^{a}	1^{c}	1^{a}	1^{a}	1^{b}	1^{a}	1^{a}	1^{b}
3	$0.93^{a,A}$ (0.05)	$1.25^{a,A}$ (0.27)	$0.92^{a,B}$ (0.16)	$0.86^{a,A}$ (0.06)	$1.10^{ab,A}$ (0.20)	$0.80^{c,B}$ (0.25)	$0.92^{a,A}$ (0.08)	$1.63^{b,B}$ (0.21)	$0.30^{a,A}$ (0.09)	$1.07^{b,B}$ (0.05)	$1.14^{b,A}$ (0.12)	$0.34^{a,A}$ (0.15)
6	$0.85^{a,A}$ (0.07)	$1.90^{b,B}$ (0.40)	$0.90^{a,C}$ (0.28)	$0.88^{a,A}$ (0.08)	$1.48^{b,AB}$ (0.30)	$0.47^{b,B}$ (0.18)	$1.22^{b,B}$ (0.06)	$1.72^{b,B}$ (0.18)	$0.19^{a,A}$ (0.08)	$1.30^{c,B}$ (0.08)	$1.38^{c,A}$ (0.10)	$0.20^{a,A}$ (0.08)
24	–	–	–	$0.88^{a,A}$ (0.13)	$1.66^{b,A}$ (0.13)	$0.14^{a,A}$ (0.05)	–	–	–	$1.77^{d,B}$ (0.17)	$1.72^{d,A}$ (0.07)	$0.11^{a,A}$ (0.05)

* Values in parentheses correspond to standard deviation.
** Means values in the same column with the same lower case letter do not differ significantly ($p > 0.05$).
*** Means values in the same row and for the same parameter with the same capital letter do not differ significantly ($p > 0.05$).

results indicate the osmotic dehydration contributed to the rise of tissue firmness while the dipping treatment at 40 °C contributed to reduce the firmness. In the early period of treatment with hypertonic solution such opposite effects result in samples firmness values similar to those of fresh fruit. After 6 h of treatment, the osmotic dehydration exerts a preponderant effect on the fruit firmness.

The influence of impregnation with calcium salts on food mechanical properties has been studied by several authors. In many cases, calcium in plant tissue can prevent firmness loss during treatments but the relationship of calcium uptake and tissue firmness depends on composition and structure of the tissue. Calcium content significantly affected the mechanical properties of apple (Anino et al., 2006) and papaya (Lovera et al., 2014); tissue firmness was increased with the calcium gain in carrots and eggplants, whereas the calcium content did not affect the mushroom firmness values (Gras et al., 2003). Silva et al. (2014a) found that calcium impregnation did not influence the values of stress at rupture of pineapple.

To clarify the relationship between calcium uptake and firmness of pineapple, data of Fig. 2 and Table 3 were compared. After 6 and 24 h treatment in isotonic solution the fruit calcium content increased 5.7 and 9 times, respectively, while the stress at rupture was reduced around 12% and remained unchanged throughout the process. Instead, in 6 and 24 h of hypertonic treatment, the pineapple calcium content increased 2.2 and 3.7 times, respectively, meanwhile the stress at rupture increased continuously with dipping time reaching values 30 and 70% higher than the same one in fresh fruit. So, in isotonic solution the pineapple calcium content increases continuously with dipping time without affecting the product firmness. This fact may be due to a low or absent formation of calcium pectates during the treatment, as a consequence of the fruit composition. Previous studies indicate that the pineapple cell walls contain only small amounts of pectic polysaccharides (Smith and Harris, 1995).

A drastic decrease in the modulus of elasticity (E) of pineapple was caused by osmotic dehydration (Table 3). This effect was more important at beginning of processing. The addition of calcium and ascorbic acid to the hypertonic solution had no significant impact on E parameter. There was a greater loss of turgor during hypertonic treatment than during isotonic process. The state of turgidity of tissues may be due to the water content and not to the calcium content. In isotonic treatments, the addition of calcium and ascorbic acid favors the turgor loss.

Strain at failure ε_{rup} was increased with treatment time and was reduced with the addition of calcium and ascorbic acid. Also, no significant effect of the sucrose concentration was observed.

3.3.3. Microscopic analysis

In agreement with the mechanical properties results, microscopic observations showed that sucrose content of dipping solution affected the structural characteristics of the pineapple fruit tissue. Microphotographs of fruit, fresh and treated for 3 h in isotonic and hypertonic solution with addition of calcium lactate and ascorbic acid are shown in Fig. 4. The microstructure of fresh fruits showed typical plant cells of polyhedral shape, assembled in a non-homogenous network pattern (Fig. 4a). Pineapple tissues treated for 3 h in sucrose isotonic solution with calcium lactate and ascorbic acid presented cells more rounded than those of fresh fruit, without shrinkage, non-uniform size, with thin cell walls but unbroken and presence of intercellular spaces (Fig. 4b). There were no differences between samples treated in isotonic solution with and without calcium and ascorbic acid addition (microphotographs are not shown).

However, collapsed cells and reduction of intercellular spaces were observed in pineapple tissues treated in hypertonic solution with calcium lactate and ascorbic acid (Fig. 4c), possibly due to the water loss during the osmotic process. Vacuoles volume was reduced in osmotically dehydrated fruit samples and preserved in fruits with isotonic treatment, as expected since this cell organelle includes the greatest amount of water from the plant tissue. Similar results in osmotically dehydrated strawberry tissue were reported by Moreno et al. (2012). Ferrari et al. (2010) observed cells more deformed, contracted and collapsed due to the water loss throughout the osmotic process of melon impregnated with calcium lactate.

Energy dispersive X-ray microanalysis (EDX), coupled to the microscopic observation, allows detecting the presence of components on the analyzed material surface. Carbon and oxygen, typical elements of biological material, gold due to the coating used, and calcium were detected (Fig. 5). Calcium concentration on the surface of samples treated during 3 h in isotonic and hypertonic solution, quantified by EDX method, were 2.29% and 0.87% w/w respectively. As expected, these values are much higher than those found in the integral volume of the sample by the atomic absorption spectrophotometry method, but the ratio between values of calcium concentration of samples treated in isotonic and hypertonic solution was equivalent.

In addition, the surface microanalysis of an internal section of the samples showed a markedly lower calcium content than on the external surface, and this would demonstrate the existence of a calcium concentration profile (Fig. 5). So, EDX technique could be used to quantify the calcium concentration profile in the solid during impregnation processes and thus study transport mechanisms and develop specific models.

Gras et al. (2003) used this technique to determine the relative content of ions and their location in fresh and vacuum impregnated vegetables in isotonic solution with calcium.

3.4. Modifications of impregnation solution

To take into account possible reuse the impregnation solution in industrial applications, changes in impregnation solutions were evaluated in the course of dipping treatments. Experimental values of soluble solids content (SS), citric acid content (CA), pH, light transmittance (LT) and changes in content of ascorbic acid (AA/AA_0) and calcium (Ca/Ca_0) before and during impregnation process in isotonic and hypertonic solutions are shown in Table 4.

Soluble solids content of solutions was slightly modified during impregnation process. Decreasing of SS in hypertonic solution can be

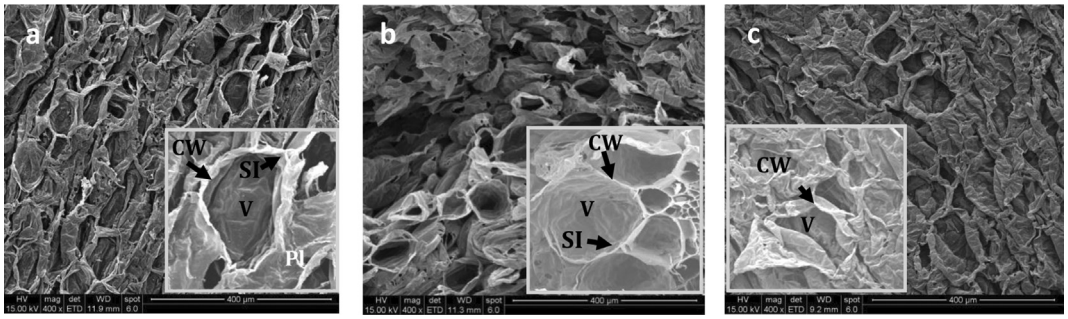

Fig. 4. SEM microphotographs of pineapple tissue (a) fresh, (b) with 3 h of IT_{CaAA}, and (c) with 3 h of HT_{CaAA}. CW: cell wall; SI: intercellular space; V: vacuole.

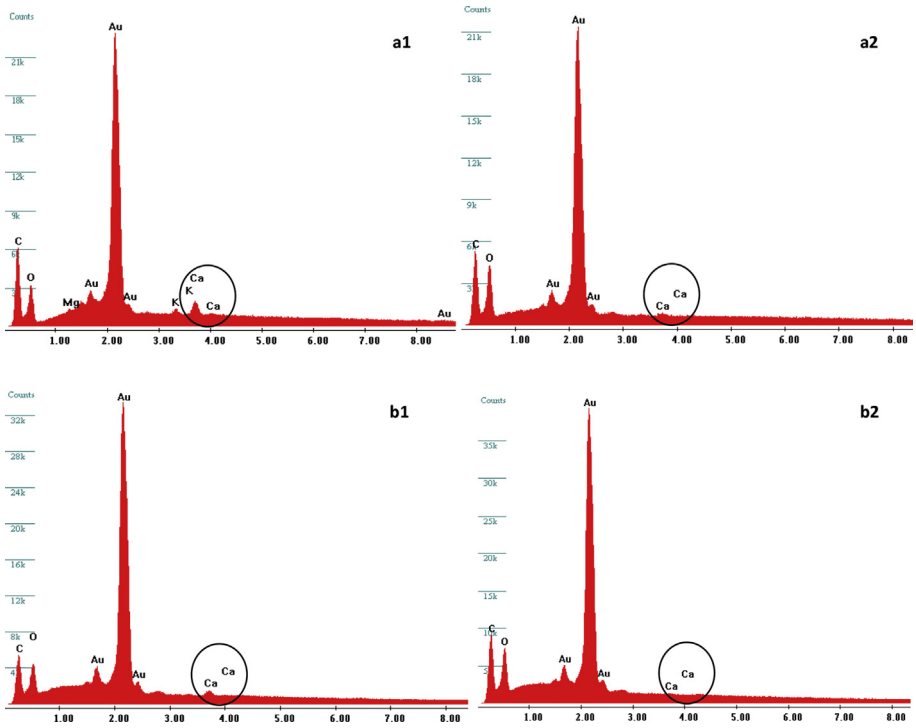

Fig. 5. EDX spectra of (a1 and b1) external surface and (a2 and b2) surface of internal section of pineapple sample treated for 3 h in (a) isotonic solution and (b) hypertonic solution.

due to the water flow from the fruit; and its increase in isotonic solution can be due to different factors such as leaching of fruit components and water evaporation. Moreover, values of light transmittance were reduced to a greater extent in isotonic solutions that hypertonic solutions, which could be due to increased migration of components from the fruit to the solution or a plant tissue disaggregation in the isotonic medium; this reduction is more severe in the absence of calcium and ascorbic acid. The calcium effect on the transmittance values was less noticeable for hypertonic solution than for isotonic solution, possibly for the reason that osmotic dehydration process itself gives mechanical resistance to the fruit, as can be seen in Table 3.

A decrease in solution pH to values close to those of the fruit was observed, possibly due to the migration of organic acids from the fruit to the solution. This effect was more marked in the solutions without ascorbic acid and calcium. The presence of ascorbic acid reduces the initial pH of the solution.

It is known that exposure time and temperature affect negatively the stability of ascorbic acid. However, a non-meaningful reduction of this component in 6 h treatment was observed. On the other hand, the calcium content remained without substantial changes because it is not a thermolabile compound and calcium mass absorbed by the fruit did not significantly modify the calcium content in solution. In view of an industrial application, foregoing results show the possibility of reusing the solution in successive impregnation cycles in order to reduce the process waste and improve the industrial profitability.

Table 4
Mean values and standard deviation of physicochemical properties of sucrose solutions during impregnation treatments.

Treatment	Time (h)	*SS* (°Bx)	pH	*CA* (g/100 mL)	AA/AA_0	Ca/Ca_0	*LT* (%)
IT	0	14.72 ± 0.28 ^{a}	4.92 ± 0.20 ^{e}	0.00 ± 0.00 ^{a}	–	–	94.08 ± 0.23^{e}
	1	15.00 ± 0.18 ^{a}	3.77 ± 0.03 ^{bc}	0.02 ± 0.01 ^{b}	–	–	–
	3	15.13 ± 0.10 ^{b}	3.62 ± 0.05 ^{a}	0.05 ± 0.01 ^{c}	–	–	–
	6	15.90 ± 0.09 ^{e}	3.67 ± 0.01 ^{a}	0.06 ± 0.01 ^{c}	–	–	40.25 ± 1.53 ^{a}
IT_{CaAA}	0	14.70 ± 0.32 ^{a}	3.87 ± 0.03 ^{d}	0.35 ± 0.01 ^{d}	1	1	95.54 ± 0.32 ^{g}
	1	14.80 ± 0.18 ^{a}	3.82 ± 0.02 ^{c}	0.37 ± 0.02 ^{de}	0.975	0.998	–
	3	15.40 ± 0.05 ^{c}	3.81 ± 0.03 ^{c}	0.39 ± 0.01 ^{e}	0.968	0.986	–
	6	15.64 ± 0.09 ^{d}	3.77 ± 0.01 ^{b}	0.43 ± 0.01 ^{f}	0.959	0.995	83.25 ± 0.44 ^{b}
HT	0	50.00 ± 0.03 ^{i}	4.81 ± 0.30 ^{e}	0.00 ± 0.00 ^{a}	–	–	94.62 ± 0.18 ^{f}
	1	45.16 ± 0.50 ^{f}	3.72 ± 0.06 ^{ab}	0.05 ± 0.01 ^{c}	–	–	–
	3	47.95 ± 0.06 ^{h}	3.70 ± 0.03 ^{ab}	0.04 ± 0.01 ^{bc}	–	–	–
	6	47.55 ± 0.27 ^{g}	3.62 ± 0.03 ^{a}	0.05 ± 0.01 ^{c}	–	–	88.40 ± 0.88 ^{c}
HT_{CaAA}	0	50.00 ± 0.01 ^{i}	3.73 ± 0.05 ^{b}	0.33 ± 0.01 ^{d}	1	1	93.22 ± 0.12 ^{d}
	1	46.40 ± 0.95 ^{f}	3.70 ± 0.06 ^{ab}	0.34 ± 0.01 ^{d}	0.929	0.988	–
	3	47.30 ± 0.10 ^{g}	3.71 ± 0.12 ^{ab}	0.35 ± 0.01 ^{d}	0.922	0.992	–
	6	48.06 ± 0.05 ^{h}	3.68 ± 0.05 ^{ab}	0.35 ± 0.01 ^{d}	0.900	0.983	87.40 ± 0.62 ^{c}

*Means values in the same column with the same letter do not differ significantly ($p > 0.05$).
* SS = soluble solids content; CA = citric acid content; AA/AA_0 = changes in content of ascorbic acid; Ca/Ca_0 = changes in calcium content; LT = light transmittance.

4. Conclusions

Atmospheric impregnation methodology of pineapple using sucrose solution with added calcium lactate and ascorbic acid was suitable for producing different pineapple products of high nutritional value. Sucrose concentration of syrup and process time affected the characteristics of pineapple enriched with ascorbic acid and calcium. So, pineapple tissue with high ascorbic acid and calcium content but firmness, water and sucrose content similar to fresh fruit was obtained through isotonic treatment. On the other hand, pineapple with high ascorbic acid content and moderate calcium content, but greater firmness and higher sucrose content than fresh fruit, from hypertonic treatment was obtained.

The impregnation process of pineapple slices with calcium and ascorbic acid was more effective using isotonic solution than hypertonic solution. Changes of mechanical properties and calcium content indicated the increasing in tissue firmness was not linked to calcium uptake but to osmotic dehydration process. Also, water loss and sucrose gain during osmotic dehydration of pineapple were not affected by nutrients incorporation.

Additionally, could be feasible to reuse the impregnation solution since non-meaningful changes occurred in ascorbic acid, calcium and sucrose content of the syrup during 6 h of impregnation treatment.

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