Innovative Food Science and Emerging Technologies 33 (2016) 506–514

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Application of edible coatings to improve global quality of fortified pumpkin

Carolina E. Genevois, Marina F. de Escalada Pla, Silvia K. Flores *

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, (1428), CABA, Argentina
Member of the National Scientific and Technical Research Council (CONICET), Argentina

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 1 November 2015
Accepted 2 November 2015
Available online 1 December 2015

Keywords:
Fortified foods
Cucurbita moschata Duchesne ex Poiret
Edible coating
Color
Texture
Iron bioaccessibility

ABSTRACT

A refrigerated ready-to-eat food fortified with iron (Fe) and ascorbic acid (AA) was produced using pumpkin (*Cucurbita moschata* Duchesne ex Poiret) and applying a dry infusion process. It was observed that the presence of both Fe and AA in the vegetable matrix (control system) produced the browning of the product. The edible coatings application based on k-carrageenan or tapioca starch was proposed in order to improve the product stability. The AA degradation in the tissue was significantly reduced in the pumpkin with a starch-based coating. The result of an "in vitro" gastric and intestinal digestion assay indicated that when Fe was in the coating, Fe solubility at pH 2 was lower than control and tended to improve at pH 8. It was interpreted as a better accessibility of Fe at intestinal lumen level, and moreover, it could avoid gastric side effects. The products obtained were safe from microbiological view point and presented a satisfactory color and texture.

Industrial relevance: The formulation of food fortified with iron (Fe) represents a challenge from nutritional as well as technological view point because the reactivity of this mineral with other food matrix nutrients. This work proposes the elaboration of a vegetal refrigerated food, ready to eat, fortified with Fe and ascorbic acid (AA). The pumpkin was selected as raw material due its high consume and availability, proper nutritional characteristics and low cost. The dry infusion technique applied is sustainable, economic and with a minimal use of drinking water. In addition, biopolymer-based edible coatings were applied as an emerging technology for the carrying of micronutrients. It was demonstrated that when an edible coating was performed, the color and AA retention were improved and the Fe accessibility at pH of lumen intestinal trended to be higher. This study shows that the production of fortified pumpkin is simple and transferable to the food industry, and constituting a contribution from the food technology to the innovation of processes and formulation of a functional food fortified with Fe.

1. Introduction

The use of edible films and coatings in the food industry is a new topic of great interest due to their potential to increase the shelf life of foods. The possible action modes of edible coatings include delay of the moisture migration, slowing of the solutes or gas transport (O_2, CO_2), reduction of the migration of oils and fats, improvement of the mechanical properties, retention of volatile compounds, support of food additives (Flores, Famá, Rojas, Goyanes, & Gerschenson, 2007). Edible films can act additionally or cooperatively with other factors (Ramos, Miller, Brandão, Teixeira, & Silva, 2013) in improving the overall quality of the food, providing protection to the external microbial contamination, extending the shelf life of food and possibly improving the efficiency of packing materials. Besides presenting advantages such as biodegradability, ability to be ingested and low oxygen permeability (Han, 2000), these films can act as a barrier against external factors or as a carrier for the incorporation of food additives as antimicrobials, antioxidants, flavors, colors, which can improve the antimicrobial protection, appearance, texture and taste during storage (Cuppett, 1994). It has been reported that the application of edible coatings in fruits and vegetables improved retention of color and flavor components during storage, extended product life, retarded moisture and firmness loss, and product senescence (Ciolacu, Nicolau, & Hoorfar, 2014; Dhall, 2013; Rojas-Graü, Soliva-Fortuny, & Martin-Belloso, 2009). Tamer and Çopur (2010) applied a chitosan-based coating on the surface of strawberries, carrot, mango, cantaloupe, pineapple and mushroom and reported a growth inhibitory effect of microorganisms and an improved stability. Garcia et al. (2008) reported that the application of a cassava starch-based films containing potassium sorbate reduced significantly the activity of microorganisms in samples of pumpkin (*Curcumis moschata*, Duchesne). Recently, progress has been made in the use of films and coatings to incorporate edible nutrients or bioactive compounds. Mei and Zhao (2003) evaluated the feasibility of applying edible films based on milk proteins to incorporate high concentrations of calcium and vitamin

* Corresponding author at: Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, (1428), CABA, Argentina. Tel./fax: +54 11 45 76 33 66.
E-mail address: sflores@di.fcen.uba.ar (S.K. Flores).

http://dx.doi.org/10.1016/j.ifset.2015.11.001
1466-8564/

E, concluding that the functionality of the film forming matrix was affected by high concentrations of nutrients.

Commonly available fortified foods include non-structured and formulated foods. In contrast, impregnation or vacuum impregnation allows the introduction of physiologically active compounds to vegetal tissues without disrupting their cellular structure, but inducing changes in their behavior during further processing. In this sense, some authors (Alzamora et al., 2005; Barrera, Betoret, & Fito, 2004; Fito et al., 2001; Martín-Diana et al., 2007; Oms-Oliu et al., 2010) analyzed the feasibility of vacuum impregnation treatment in the development of products fortified with calcium and iron (Fe) from fresh fruits and vegetables. Recently, a pumpkin fortification process was optimized minimizing the color detriment due to the combined addition of AA and Fe into vegetal matrix. The pumpkin impregnated with 0.216 g/kg of Fe and 0.80 g/kg of AA resulted with a final color more similar to the control (pumpkin without fortification). In that opportunity, authors reported that, in general, air drying produced color detriment on pumpkin fortified with AA and Fe. It was also established that edible tapioca starch coating exerted a protective effect in terms of the color of pumpkin pieces during drying. The color variation due to drying process applied (ΔE) was 9.05 ± 0.07, while when product was coated, (ΔE) was 1.5 ± 0.4 (Genevois, Flores, & de Escalada Pla, 2014). From these results, it would be also interesting to evaluate different coatings strategies, such as using low gelation temperature polysaccharides, in order to diminish rate of detriment reactions like browning or AA lost. In this sense, k-carrageenan can constitute edible films by lowering the temperature and, on the other hand, pregelatinized tapioca starch results useful to formulate coatings at low temperatures.

The objective of the present work was to formulate a refrigerated ready-to-eat food based on *Cucurbita moschata* Duchesne ex Poiret fortified with Fe and AA. Application of edible coatings was also proposed in order to improve the product stability during processing and storage.

2. Material and methods

2.1. Chemicals

Food grade sucrose, glucose (Anedra, Argentina) and k-carrageenan (Degussa, Argentina) or pregelatinized tapioca starch (Lorenz Companhía, Brazil) were employed. The additives: $FeSO_4.7H_2O$ (Merck, Argentina), potassium sorbate (Sigma, USA), L-(+)-ascorbic acid (Merck, Argentina), citric acid and glycerol (Sintorgan, Argentina) and other chemicals used were of analytical grade.

2.2. Preparation of the pumpkin fortified with Fe and AA

2.2.1. Dry infusion process of pumpkin cylinders

Pumpkin (*Cucurbita moschata* Duchesne ex Poiret) obtained in a local supermarket was carefully washed and rinsed with distilled water. Then, 120 cylinders (≈960 g) of 29 mm diameter and 15 mm thickness were cut from the mesocarp using a stainless steel cork borer. The cylinders were blanched with water vapor for 8 min, rapidly cooled for 1 min by immersion in water at 0 °C and then submitted to a dry infusion (Alzamora, Guerrero, Nieto, & Vidales, 2004) according to Genevois et al. (2014) with some modifications. Briefly, pumpkin cylinders were placed in a plastic bowl and sprinkled with powdered glucose (33 g/100 g pumpkin) and sucrose (23 g/100 g pumpkin). Water from vegetal tissue began to flow from the pumpkin cylinder to the surrounding. In that moment, citric acid (0.15 g/100 g pumpkin), potassium sorbate (0.104 g/100 g pumpkin) and AA (2.21 g/100 g pumpkin) were added to the liquid solution and the orbital agitation started up. Citric acid was added in order to decrease pH values below 5, since sorbate is more effective, as an antimicrobial, in this range of pH (Damodaran, Parkin, & Fennema, 2008). The dry infusion was carried out at 20 °C up to equilibrium on an orbital shaker (Vicking S.A., Argentina) at 35 rpm.The described conditions of infusion were selected taking into account previous assays. Equilibrium was reached at 72 h when pumpkin cylinders and the surrounding solution achieved the same a_w and pH values. Once the dry infusion was concluded, the cylinders were drained through a stainless steel strainer. The pumpkin cylinders obtained by the previous described dry infusion methodology were named F0 (80 cylinders).

It must be remarked that a control system (F3, 40 cylinders) was performed in similar conditions used for F0 but fortified with Fe by adding $FeSO_4\ 7H_2O$ (0.17 g/100 g pumpkin, equivalent to 34 mg Fe/100 g pumpkin) to the dry infusion media.

2.2.2. Coating process of pumpkin cylinders

Pumpkins without Fe in the infusion medium (F0) were divided into two batches (40 cylinders each one), one of them was coated by dipping in a solution based on pregelatinized tapioca starch (F1), and the other one was coated with a k-carrageenan solution (F2). The methodology used in the preparation of edible coatings was as follows: pregelatinized tapioca starch (6% *w*/*w*) or k-carrageenan (2% *w*/*w*) was dissolved in distilled water. Glycerol (1.5% *w*/*w*) as a plasticizer, potassium sorbate (0.025% *w*/*w*) as antimicrobial agent and $FeSO_4.7H_2O$ (0.5% *w*/*w*) were added to each biopolymer aqueous solution. The preparations were stirred at room temperature (20 °C) on a magnetic stirrer for 20 min until a proper homogenization. For starch-based coating, pumpkin pieces were immersed for 5 min, removed from the solution and dried in a convection chamber at 35 °C for 4 h. In the case of k-carrageenan, the coating solution was placed on a hot plate until a temperature of 67 °C was reached; then pumpkin cylinders were coated with gel of k-carrageenan and allowed to cool for coating constitution. The dry infusion process and the coating were performed at least twice.

2.2.3. Packaging and storage

Finally, each pumpkin system (F1, F2 and F3) was introduced into low-density polyethylene bags of 80 μm thickness, provided with an easy-to-close Ziploc® closing. The bags were filled with the corresponding 5 pieces (40 g) and stored in a chamber at 8 °C.

2.3. Product characterization

In order to analyze the changes during the processing and storage, the samples were taken from blanched pumpkin, equilibrated pumpkin after dry infusion or final coated product. In addition, samples throughout storage at 8 °C were characterized.

2.3.1. pH and a_w

Pumpkin cylinders were reduced to a puree with the aid of a homogenizer UltraTurrax (IKA, USA) at 6500 rpm for 20 s. The pH was determined with a pHmeter (Cole-Parmer, USA). Water activity (a_w) was measured with a hygrometer (Aqualab, USA) at 20 °C. Determinations were performed at least in duplicate on the samples from blanched pumpkin, equilibrated pumpkin after dry infusion or final coated product.

2.3.2. Moisture and soluble solids contents

Pumpkin samples were frozen and freeze dried (Christ, Germany) for 48 h at 1.1 Pa and 25 °C, to determine the water content. The percentage of soluble solids (°Brix) was determined with a refractometer with automatic temperature compensation (Atago, USA) in the juice extracted from pumpkin cylinders by pressing the sample with a spatula. Water loss (WL) and solid gain (SG) in the different systems were calculated according to the following equations (de Escalada Pla, Campos, & Gerchenson, 2009):

$$WL = \frac{M_t \times m_t - M_0 \times m_0}{M_0} \times 100,$$

$$SG = \frac{M_t \times ss_t - M_0 \times ss_0}{M_0} \times 100,$$

where M_t (g) is the average mass of pumpkin cylinders at time t, m_t is the moisture content of tissue at time t [g water/100 g pumpkin, wet basis], M_0 (g) is the cylinder mass average at initial time (before the dry infusion), m_0 is the initial water content of tissue [g water/100 g pumpkin, wet basis], ss_0 and ss_t are the soluble solid contents in tissue at initial time and at time t [°Brix, or g ss/100 g pumpkin, wet basis], respectively.

Measurements were performed in duplicate on the samples from blanched pumpkin, equilibrated pumpkin after dry infusion or final coated product.

2.3.3. Color

The color determination was made on the final products at initial time as well as after 9 days of storage at 8 °C. CIE L*a*b* color parameters [L*: lightness, a*: greenness–redness, b*: blueness–yellowness] were evaluated using a photocolorimeter (CM-Model 508d, Minolta, Japan), under illuminant D65 and with the observer at an angle of 2°. Averages of the measurements performed in three pumpkin cylinders are reported. From these parameters, chroma (Chr) value was also calculated. This parameter describes color intensity (Olivera et al., 2008) and was calculated as

$$Chr = \sqrt{(a^*)^2 + (b^*)^2}.$$

2.3.4. Texture characterization

Texture characterization was performed on final products according to de Escalada Pla et al. (2009). Briefly, each pumpkin tissue cylinder was subjected to a compression test to fracture in a Universal Press (Instron, USA) equipped with a 100 N load cell and a parallel compression plate of 30 mm in diameter. The crosshead speed was set in 5 mm/min. Compression force (N) and displacement (mm) required to fracture were recorded. The stress (Pa) was calculated as the ratio of the force (N)/cylinder cross-section (m^2) and the strain (%) was determined as displacement (mm)/initial height of cylinder (mm) × 100. Stress curves as a function of strain were plotted. The firmness (Pa) of the samples was calculated as the ratio of the stress at break (Pa)/strain at break (%). Six replicates were tested for each system, and the average of results is reported.

2.3.5. Optical microscopy

The surface and the cross-section of the final products were observed with an optical microscope Zeiss Axioskop 2 Plus (Zeiss, Germany) at 100× and 200× magnifications.

2.4. Nutritional characteristics

2.4.1. Determination of L-(+)-ascorbic acid

AA was determined according to De'Nobili et al. (2013). Briefly, twenty grams of samples were weighed and then broken in the presence of 20 g of oxalic acid solution of 1% (*w*/*v*) with the aid of a homogenizer (UltraTurrax, Germany). The homogenate was diluted to 100 mL with oxalic acid solution 1% (*w*/*v*). The suspension was homogenized by stirring and then centrifuged at 5000 rpm for 10 min at 6 °C (Eppendorf, Germany). Aliquots of the supernatant were taken for spectrophotometric determination of AA, upon reaction with 2,6-dichlorophenol–indophenol. The determinations were performed on the samples in triplicate after applying infusion and coating and throughout 18 days of storage at 8 °C.

The retention of AA content was calculated as the ratio between AA content during storage to initial AA content (AA/AA_0). Ascorbic acid retention was fitted to a pseudo first order kinetic (De'Nobili et al., 2013).

2.4.2. Determination "in vitro" of the iron bioaccessibility

The amount of the Fe *bioaccessibility* (soluble Fe after simulated digestion) was analyzed according to Nayak and Nair (2003) with slight modifications (de Escalada Pla et al., 2009; Gautam, Platel, & Srinivasan, 2010). Briefly, sample was hydrolyzed with pepsin (pH 2.0) at 37 °C for 90 min in order to simulate the gastric digestion, then pH was adjusted to 7.0–8.0 with $NaHCO_3$ 0.8 M and after that, pancreatin–bile salts solution was added and incubated at 37 °C for 90 min, to simulate the digestion in the duodenum. Then the digestion mixture was cooled in an ice bath and filtered through an ash-free filter paper (WhatmanNro.40, UK). The determination was performed in triplicate on products after 9 days of storage at 8 °C.

Iron content was then determined in the supernatants of the digestions and in the remaining residue after digestion at basic pH. Elemental Fe determination was performed by atomic absorption spectroscopy (Shimadzu AA 6800, Japan) at λ = 248.3 nm and using an air–acetylene flame according to an AOAC official method.

Iron bioaccessibility at pH 2 and at pH 8 was calculated as follows:

$$Bioaccessibility_{pH:2} = \frac{Fe_1 \cdot 100}{Fe_T}$$

$$Bioaccessibility_{pH:8} = \frac{Fe_2 \cdot 100}{Fe_T}$$

$$Fe_T = Fe_2 + Fe_{Residue}$$

where

Fe_1 is the Fe content determined on the filtering of the enzymatic digestions of tissue at pH 2,
Fe_2 is the Fe content determined on the filtering of the enzymatic digestions of tissue at pH 2 and subsequent digestion at pH 8.
$Fe_{Residue}$ is the Fe content determined on the insoluble residue that remained after enzymatic digestions.

Table 1
Physicochemical parameters of the pumpkin products obtained by dry infusion: without Fe (F0), with Fe–tapioca starch coating (F1), with Fe–k-carrageenan coating (F2) and with Fe but without coating (F3, control system).

Parameters	Dry infusion without Fe (F0)	Fe–tapioca starch coating (F1)	Fe–k-carrageenan coating (F2)	Dry infusion with Fe (F3, control)
pH	3.45± 0.01[a]	3.39 ± 0.09 [b]	3.3 ± 0.2 [b]	3.38 ± 0.01[b]
a_w	0.927 ± 0.003 [c]	0.879 ± 0.007 [d]	0.954 ± 0.002 [e]	0.931 ± 0.003 [c]
°Brix	41 ± 2 [f]	48.2 ± 0.2 [g]	31 ± 1 [h]	37.0 ± 0.5 [i]
Moisture (%)	37.7 ± 0.8 [j]	22.4 ± 0.8 [k]	46.1 ± 0.2 [l]	35.5 ± 0.5 [m]
SG (%)	19 ± 1 [n]	13.9 ± 0.1[o]	16.8 ± 0.8 [n]	20 ± 3 [n]
WL (%) [A]	60.3 ± 0.4 [p]	72.6 ± 0.3 [q]	49.3 ± 0.2 [r]	60.7 ± 0.3 [p]
mg AA/100 g pumpkin w.b.)[B]	956 ± 55 [s]	1336 ± 64 [u]	842 ± 67 [s]	1081 ± 34 [t]
mg Fe/100 g pumpkin w.b.)[B]	N.A.	54 ± 10 [v]	40 ± 8 [v]	37 ± 9 [v]

Equal letters in the same row indicate absence of significant differences between the systems ($p > 0.05$).
[A] Absolute values were reported.
[B] Initial content—w.b.: wet basis, N.A.: not applicable.

Table 2
Color parameters of the pumpkin products obtained by dry infusion: with Fe–tapioca starch coating (F1), with Fe–k-carrageenan coating (F2) and with Fe but without coating (F3, control system), at the first and ninth days of storage.

Color parameters		Fe–starch-based coating (F1)	Fe–k-carrageenan-based coating (F2)	Dry infusion with Fe (F3, control)
L*	1st day	47 ± 1^{ab}	55 ± 3^{c}	48.5 ± 0.6^{b}
	9th day	44 ± 2^{a}	53 ± 2^{c}	48.9 ± 0.7^{b}
a*	1st day	20 ± 1^{d}	14 ± 3^{e}	16 ± 3^{e}
	9th day	18.1 ± 0.8^{d}	12 ± 1^{e}	16 ± 2^{e}
b*	1st day	43 ± 2^{f}	47.8 ± 0.7^{g}	45 ± 2^{f}
	9th day	43 ± 2^{f}	51 ± 3^{g}	42.1 ± 0.4^{f}
Chr	1st day	48 ± 2^{h}	50 ± 1^{i}	47 ± 2^{h}
	9th day	46 ± 1^{h}	52 ± 3^{i}	45 ± 1^{h}

Equal letters in the same row or between 1st and 9th day indicate absence of significant differences between the systems ($p > 0.05$).

2.5. Microbiological quality

The microbiological assays were performed according to the methodology described in the Compendium of Methods for the Microbiological Examination of Foods (Vanderzant & Splittstoesser, 1992). To evaluate the microbiological safety of the product, pumpkin cylinders were aseptically mashed using a type Stomacher device and serial dilutions in peptone water (Biokar, France) were performed. The counts of microorganism were total aerobic mesophilic bacteria in plate count Agar (Biokar, France) incubating the plates at 37 °C for 72 h, lactic acid bacteria in Man, Rogosa, and Sharpe Agar (Biokar, France) incubating the plates at 37 °C for 72 h and fungi and yeasts in Sabouraud agar (Biokar, France) incubating the plates at 25 °C for 5 days. The enumerations were performed in triplicate on the final product packed in plastic bags at initial time as well as after 9 days of storage.

2.6. Statistical analysis

The results are reported as the average and their standard deviation (SD). The statistical analyses of results were performed by applying ANOVA (α: 0.05), followed by multiple comparisons evaluated by Tukey's significant difference test. Nonlinear regressions were analyzed taking into account the determination coefficient (r^2) and the Durbin–Watson (DW) parameter. The Statistica software (version 6, StatSoft, Inc.2001, USA) was used for statistical treatment of data.

3. Results and discussion

3.1. Characterization of fortified pumpkin after dry infusion and coating

3.1.1. pH, a_w, moisture, SG% and WL%

The initial pH and a_w of the pumpkin after blanching and before the infusion process were 6.14 ± 0.05 and 0.99 ± 0.03, respectively. The physicochemical parameters of pumpkin cylinders after dry infusion without Fe (F0) or with Fe (F3) and after the Fe fortified edible coating process (F1 and F2), are detailed in Table 1. Once dry infusion equilibrium was reached, pumpkin cylinders without Fe (F0) showed a pH value of 3.45 ± 0.01. While in the batch containing Fe in the infusion medium (F3), a pH value of 3.38 ± 0.01 was recorded, which was similar to the pH reached after coating by F1 and F2 systems. Therefore, the Fe presence during production reduced the pH ($p < 0.05$) of the product. Similar behavior was also observed previously for fortified pumpkin obtained by infusion process (de Escalada Pla et al., 2009). In addition, a decrease of pH was also observed by Gaucheron (2000) when skim milk was fortified with $FeCl_2$ and $FeCl_3$. Probably, the acid hydrolysis of $Fe(H_2O)_6^{2+}$ (or Fe^{2+} ions) contributed to the additional pH decrease in the containing Fe systems. a_w values indicated that pumpkin

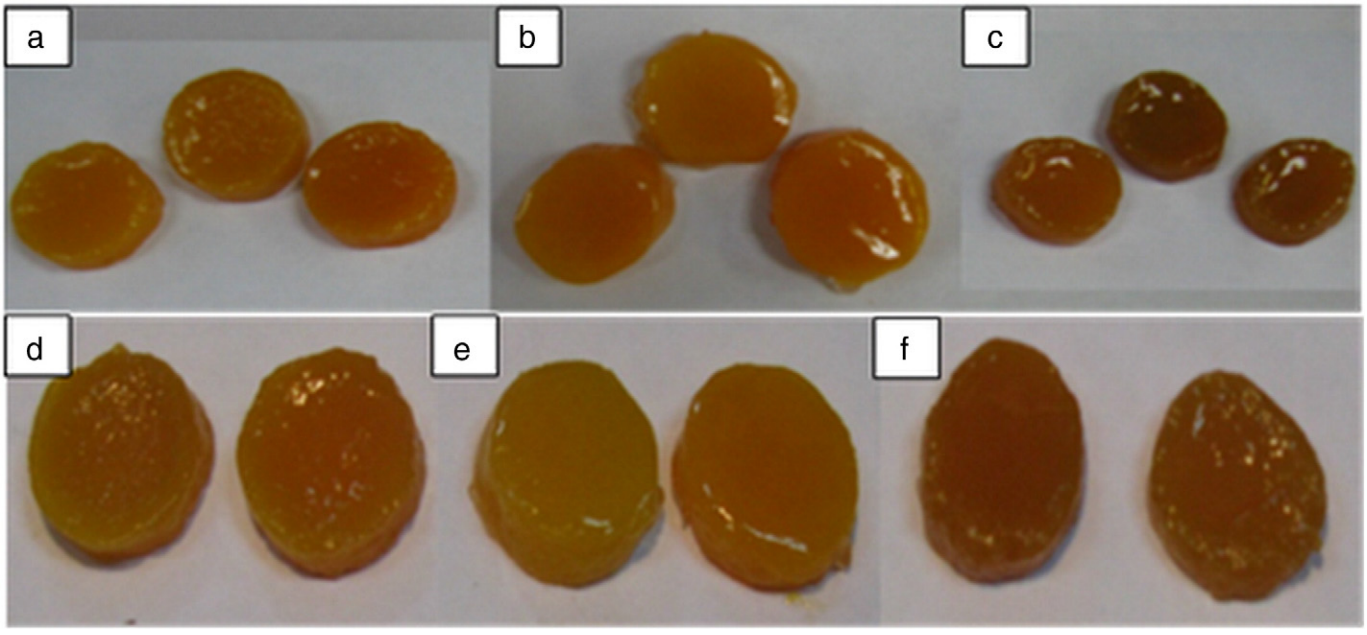


Fig. 1. Visual aspect of (a) pumpkin control system (F3), (b) pumpkin coated with Fe–k-carrageenan (F2), and (c) pumpkin coated with Fe–tapioca starch (F1), at the first day of storage at 8 °C. (d) pumpkin control system (F3), (e) Fe–k-carrageenan coating (F2), and (f) Fe–tapioca starch coating (F1), at the ninth day of storage at 8 °C.

Table 3
Texture parameters of the pumpkin products obtained by dry infusion: with Fe–tapioca starch coating (F1), with Fe–k-carrageenan coating (F2) and with Fe but without coating (F3, control system).

	Fe–starch-based coating (F1)	Fe–k-carrageenan-based coating (F2)	Dry infusion with Fe (F3, control)
Displacement at break × 10^3 (m)	3.9 ± 0.7[a]	3.3 ± 0.5[a]	3.4 ± 0.3[a]
Strain at break (%)	90 ± 20 [c]	45 ± 7 [b]	61 ± 5 [b]
Force at break (N)	40 ± 10 [d]	14 ± 4 [e]	23 ± 4 [e]
Stress at break × 10^{-3} (Pa)	110 ± 30 [f]	27 ± 8 [g]	51 ± 9 [g]
Firmness (Pa)	1200 ± 400 [h]	590 ± 80 [i]	840 ± 80 [h]
Starting height × 10^3 (m)	4.3 ± 0.3 [j]	7.2 ± 0.5 [k]	5.7 ± 0.3 [l]
Starting cross-section × 10^4 (m^2)	3.5 ± 0.4 [m]	5.3 ± 0.7 [n]	4.5 ± 0.2 [o]

Equal letters in the same row indicate absence of significant differences between the systems ($p > 0.05$). Starting height and cross-section were only added as additional information.

cylinders resulted in an intermediate or high moisture food and revealed significant differences between samples (Table 1). After osmotic dehydration, the a_w of F0 and F3 systems was reduced to a mean value of 0.929 ± 0.003, and non-significant differences were observed due to iron presence. It can be seen that the a_w value of pumpkin covered with tapioca starch was the lowest while a_w for k-carrageenan coating system was the highest one, as a consequence of the corresponding coating process applied for such samples. After dry infusion, samples without Fe (F0) showed an amount of soluble solids equivalent to 41 ± 2 °Brix. This value was slightly higher than the soluble solid content observed when Fe was added to the infusion formulation (F3, Table 1). The lowest concentration of soluble solids in the F2 formulation might be the result of the high moisture content of the product (Table 1) since this kind of coating was constituted by simple lowering of the temperature. However, the dry treatment applied to F1 system promoted a significant reduction of the moisture content and the subsequent increase of the soluble solids percentage. The SG% that occurred during the infusion process was also calculated resulting in 19 ± 1% in the system without Fe in the infusion media (F0), which was similar to the 20 ± 3% registered for control system (F3, Table 1). Regarding WL% parameter after dry infusion, it was around 60.5 ± 0.5% for systems without or with Fe (F0 and F3). However, WL% for F1 system was significantly higher than control, while F2 showed a lower WL% value ($p < 0.05$). These results for F1 were expected since it was necessary for an additional drying to establish the starch-based coating. Conversely, F2 system was constituted after cooling without any drying step, and therefore, F2 retained more water in the coating matrix.

3.1.2. Color evaluation

Table 2 shows the evolution of a*, b*, L* and Chr attributes of the final products at the first and ninth days of storage at 8 °C. As can be seen, cylinders coated with k-carrageenan (F2) were significantly lighter (L*) and had the highest Chr value ($p < 0.05$), while the system coated with starch (F1) showed slight differences with respect to the control system (F3) throughout the entire storage. The greatest value of Chr displayed by F2 was mainly due to its higher value of the b* as a result of a higher quantity of retained water in the coating structure. Conversely, pregelatinized tapioca starch coating was subjected to air drying treatment which could have influenced the browning of the pumpkin cylinders. The pigment concentration by reducing the amount of water available in the product, in addition to the AA degradation during process, may promote the browning of the product (de Escalada Pla et al., 2009). In the case of AA, the first step of its destruction is part of the non-enzymatic browning reactions chain (León & Rojas, 2007). Such irreversible degradation of AA can occur through hydrolysis simultaneously or competitively to AA oxidation when oxygen is present, producing 2-keto-L-gulonic acid which is a reactive molecule that suffers successive transformations that involve dehydrations and decarboxylations producing different browning active compounds (De'Nobili et al., 2013; Pérez, Flores, Marangoni, Gerschenson, & Rojas, 2009). Oxygen transfer from surrounding to the product was promoted during air drying because the convection system of the chamber and then browning increased in the F1 system. Nevertheless, L* value for F1 was only slightly lower when compared with that from control (F3) (Table 2, Fig. 1), possibly due to a protective effect of edible coating (Genevois et al., 2014). On the other hand, the carotenoids can be degraded, during the drying process, by exposure to heat and oxygen, with a consequent increase in cis-isomers (Lago-Vanzela, do Nascimento, Fontes, Mauro, & Kimura, 2013). In addition, no significant changes in color parameters were detected along 9 days of storage for all systems. In order to display the above-mentioned color changes, Fig. 1 shows pictures of the studied systems.

3.1.3. Texture evaluation

In order to compare the effect of different process and formulations on the textural properties of the final product, samples were submitted to compression test. Compression until fracture parameters (force and strain at fracture, as well as initial height and section) of the evaluated systems are shown in Table 3. Fig. 2 displays the stress versus strain representative plots for the studied systems. It can be observed a typical profile of pumpkin tissue submitted to heat treatment. Forces at rupture in the order of 20–40 N were also obtained previously with blanched pumpkin submitted to a subsequent osmotic treatment (de Escalada Pla et al., 2009), while raw pumpkin had presented failure force on their plots and forces at rupture one order higher with respect to cooked pumpkin (de Escalada Pla, Delbon, Rojas, & Gerschenson, 2006). Turgor

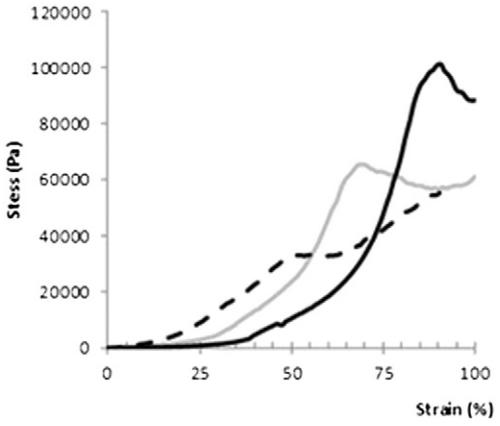
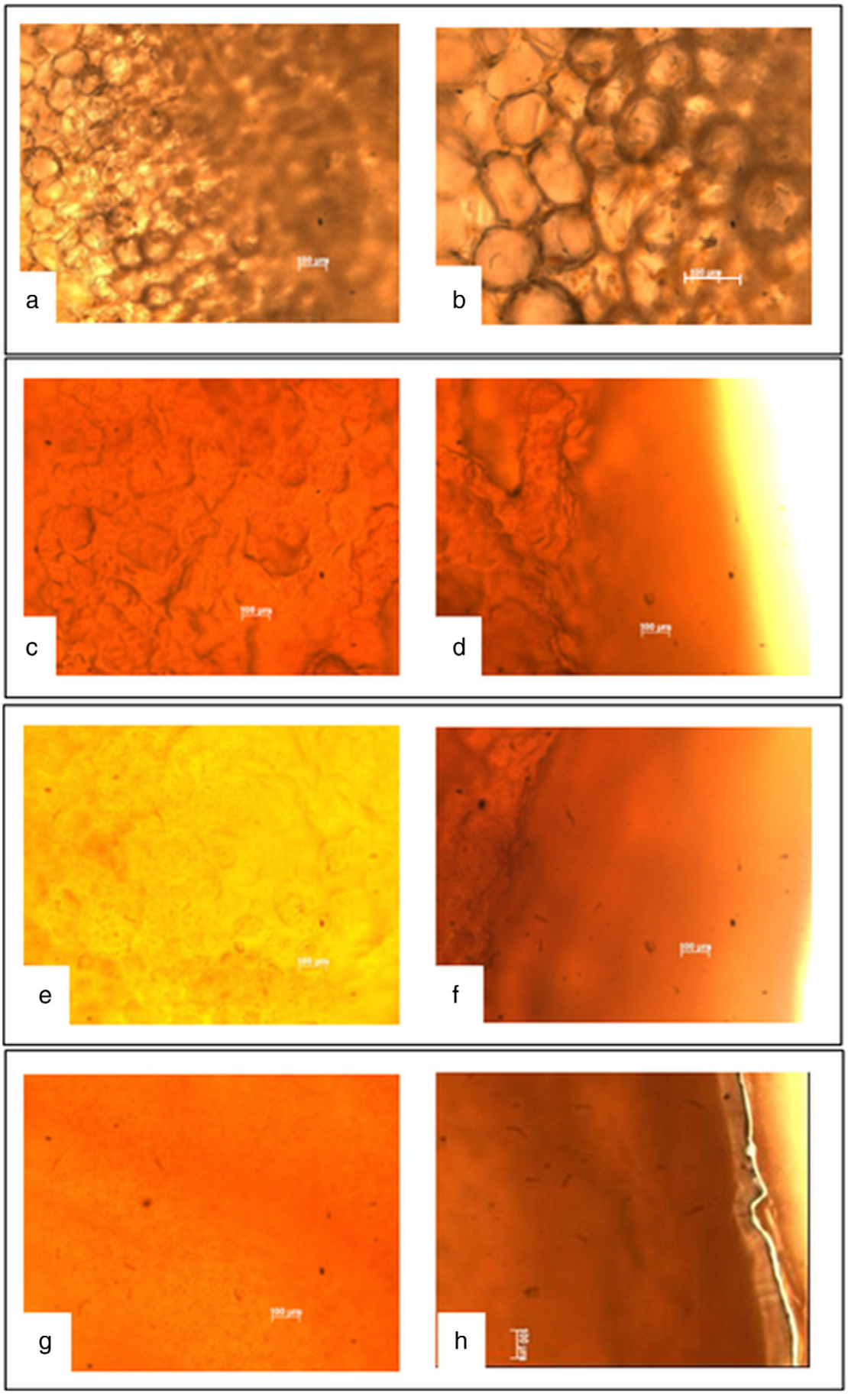


Fig. 2. Texture profile: representative stress versus strain curves of the pumpkin products obtained by dry infusion: with Fe–tapioca starch coating (F1), black line, with Fe–k-carrageenan coating (F2), dashed line and with Fe but without coating (F3, control system), gray line.

Fig. 3. Optical microscopy: (a) raw pumpkin tissue 100×, (b) raw pumpkin tissue 200×, (c) control system (F3) tissue surface 100×, (d) control system (F3) tissue cross-section 100×, (e) Fe–tapioca starch coating (F1) surface100×, (f) Fe–tapioca starch coating (F1) cross-section 100×, (g) Fe–k-carrageenan coating (F2) surface 100×, (h) Fe–k-carrageenan coating (F2) cross-section 100×.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lost and cell disruption occur due to treatment (Figs. 2 and 3). It can be seen that F1 (starch coating) required a greater force to rupture than F2 (k-carrageenan coating). It must be highlighted that the subsequent air drying process applied on F1 produced shrinkage and consequently a reduction in cylinders' cross-section. Therefore, highest stress ($p < 0.05$) was registered for F1 to achieve the breakdown peak. At the same time, this sample (F1) showed the largest degree of strain, then higher firmness (1200 ± 400 Pa) were obtained when compared with F2 ($p < 0.05$), and a similar trend (non-significant) was observed when compared with control (F3). The increased stress required for breaking the tissue may be due to higher solids concentration by further drying treatment which resulted in retracted tissue which requires higher force to deform the vegetal matrix. In a previous work, authors (de Escalada Pla et al., 2009) stated that WL produced tissue retraction, and so cells became closer, and determining firmness according osmotic equilibrium was reached. The system with k-carrageenan (F2) required the lower stress to achieve tissue breakdown peak when compared with F1. It was also possible to observe the rupture of coating in a first instance and then the tissue fracture with less stress (Fig. 2). This result could be explained considering the generation of a softer product as a result of the higher retention of water in the coating. Both the F2 and the F3 systems required a lower force and strain to get the tissue breakdown (Table 3).

3.1.4. Optical microscopy

Fig. 3a and b show the raw pumpkin tissue, where the structure of the cells can be seen clearly denoting cell wall integrity, separated by the intercellular fluid. Fig. 3c and d display the pumpkin subjected to blanching with subsequent dry infusion (control system, F3). Here, cell disintegration with a significant loss of intercellular space and intracellular fluid is notorious. Fig. 3e and f show the pumpkin tissue surface coated with starch-based coating where a disintegration of the structure with cell turgor loss is observed, in a similar way with that of the control system. It can be observed in Fig. 3f the interface between the vegetal matrix and the starch coating which seems well adhered. Unlike the starch-coated system, the k-carrageenan system shows a coating with a smooth and even surface that prevented the plant structure to be observed (Fig. 3g). Fig. 3h shows the pumpkin cylinder section where a separation among the vegetable matrix and k-carrageenan coating is clearly observed, suggesting a reduced adherence of the coating to the surface of the pumpkin. This observation also helps to explain the texture profile previously reported for F2.

3.2. Nutritional parameters

3.2.1. L-(+)-ascorbic acid content

The AA content of the different systems was determined during 18 days of storage at 8 °C. This period of time was appropriate to better accomplish the kinetic study. Fig. 4 shows the retention of AA content related to the initial AA content (Table 1) (AA/AA_0) during storage. It was observed an AA content reduction for all systems studied. Fortified pumpkin coated with a starch coating containing iron showed the highest ($p < 0.05$) AA retention. Particularly, at 18 days (432 h) of storage, the AA/AA_0 ratio for F1 was twice in comparison with that from F2 and F3.

Ascorbic acid retention was adequately fitted to a pseudo first-order kinetic (De'Nobili et al., 2013) with goodness (r^2) higher than 0.82 and DW higher than 1.7, which determines that no significant correlation was observed in the residues based on the order in which data appeared. (Fig. 4, Table 4).

Pumpkin coated with pregelatinized tapioca starch (F1) showed a lower AA loss rate ($p < 0.05$) in comparison with the F2 and F3 systems. This fact was related to the reduction in a_w obtained for F1 and, consequently, a decrease on the rate of AA degradation reaction by hydrolysis mechanism (De'Nobili et al., 2013). The batch coated with k-carrageenan (F2) and the control system (F3) revealed the greatest rate of AA loss over the evaluated period. In the case of F2, it was attributed to the higher a_w and moisture content of this system (Table 1), which accelerated the rate of AA degradations. In the case of F3, no differences were observed when comparing AA degradation rate with that from F2 (Table 4), even though a_w from F3 was considerably lower ($p < 0.05$) (Table 1). These results suggest a protective effect of the proposed edible coating on the fortified product. In order to determine if the observed effect was due to the edible coating per se or to the fact of separating Fe from the tissue, two new systems were assayed. The additional systems were prepared by fortifying the tissue with Fe and AA and then covering them with the respective edible coating formulated without Fe. After the storage, it was observed that the systems additionally assayed, without Fe in the coating, showed higher AA retention than F1 and F2. Systems with starch coating presented 40% more AA content while systems covered with k-carrageenan coating presented 60%, after 18 days of storage. It is well known the action of hydrophilic coatings as oxygen barriers (Espinel Villacrés, Flores, & Gerschenson, 2014; Ribeiro, Vicente, Teixeira, & Miranda, 2007) and such property can diminish the AA loss by oxidation. Possibly, the presence of iron in coating formulations and a higher water content might increase the oxygen-scavenging properties (Mahieu, Terrié, & Youssef, 2015) promoting the generation of reactive species that degrade AA during quantification. These results indicate that the edible coating "*per se*" protected the AA incorporated to vegetal matrix.

It is noteworthy that even considering such important AA loss registered for the F2 and F3 systems, a 50 g portion of the product after 18 days of storage still covers the total of the RDI for lactating women (Argentine Food Code).

3.2.2. Iron content and iron bioaccessibility

Non-significant differences were observed among the Fe content of the three fortified systems (F1, F2, and F3, Table 1) resulting in a mean value of 44 ± 9 mg Fe/100 g of the final product. This suggests that a 50 g portion covers 100% of the RDI for Fe in women (Argentine Food Code). It is important to remark that in the present work, exacerbated concentrations were evaluated in order to better detect iron and AA effects. However, it was noted that for consumers' acceptance, the mineral content should be adjusted.

The first stage toward bioavailability comprises mineral solubility in the intestinal tract, i.e. the so-called "bioaccessibility" (Cilla et al., 2011; Parada & Aguilera, 2007). In relation to bioaccessibility, it is important to consider the efficacy of mineral fortificants present in the food tested as influenced by the interplay of promoter factors, such as organic acids, and inhibitory factors, such as calcium, casein, and polyphenols (Cilla et al., 2011; Perales, Barberá, Lagarda, & Farré, 2006; Perales, Barberá, Lagarda, & Farré, 2007). Fig. 5 shows the percentage of bioaccessible iron at simulated gastric (acidic pH) and gut (basic pH) conditions, and also the percentage of Fe that remained in the insoluble residue. It was observed that systems containing Fe in the edible coatings tended to have a higher percentage of bioaccessible Fe at pH 8. Similarly, it was assessed the percentage of Fe in the insoluble residue after "*in vitro*" digestion, which represents the amount of Fe that is not used by the body and is eliminated by stool. The system coated with starch (F1) showed a value of 12% of the total Fe ingested and the system coated with k-carrageenan (F2) showed a value of 13%. For the control system (F3), the insoluble residue showed a slightly higher value, 18%. These results are relevant because it has been reported for other food matrices that most of Fe was insolubilized during its passage through the duodenum, and therefore, its bioaccessibility was reduced (Nayak & Nair, 2003). In the present work, more than 80% of the iron content in the fortified product turned bioaccessible, in all cases. Moreover, when iron was compartmentalized in the coating (F1 and F2), Fe solubility at pH 2 was lower than control ($p < 0.01$) and tended to improve at pH 8. It was interpreted as a better accessibility of this mineral at intestinal lumen level where the absorption occurs and at the same time could avoid one of the major problems caused by iron ingestion, as

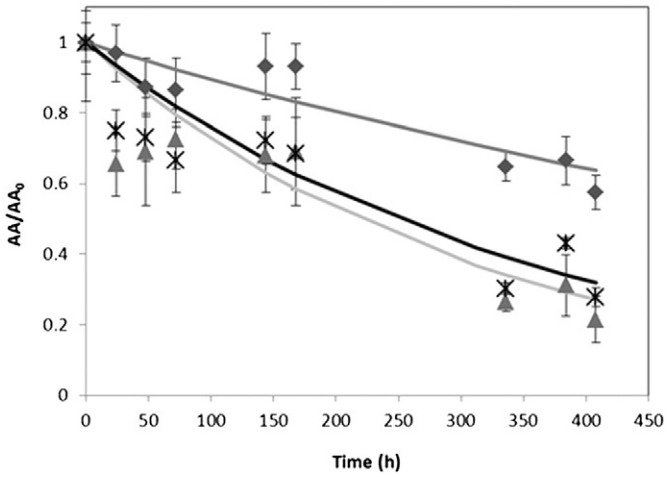

Fig. 4. . Experimental points of the retention of AA content related to the initial AA_0 content (AA/AA_0) during storage and the corresponding fitted lines of a pseudo first-order kinetic for pumpkin products obtained by dry infusion: with Fe–tapioca starch coating (F1: ▬ and ♦), with Fe–k-carrageenan coating (F2: ▬ and ▲), and with Fe but without coating (F3: control system: ▬ and ✱).

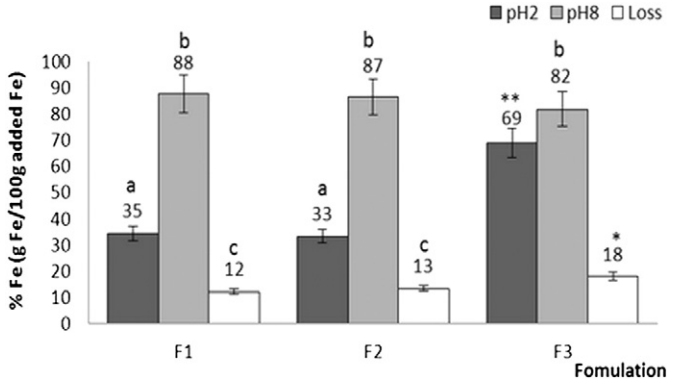

Fig. 5. Bioaccessibility of Fe expressed as the percentage of soluble Fe at gastric (pH 2) and gut (pH 8) simulated conditions, and the percentage of Fe that remained in the insoluble residue (loss) of the pumpkin products obtained by dry infusion: with Fe–tapioca starch coating (F1), with Fe–k-carrageenan coating (F2) and with Fe but without coating (F3, control system). Same letters in the columns of the same response means non-significant differences ($p > 0.05$). *Significant differences ($p < 0.05$). **Significant differences ($p < 0.01$).

gastrointestinal side effects like epigastric and abdominal pain, nausea and vomiting (Rockey, 2006).

3.3. Microbiological quality

In order to verify the safety of the proposed process, samples of the final product were submitted to microbiological analysis. The determination of the microbiological quality of the products was performed through the total count of aerobic mesophilic bacteria, lactic acid bacteria and fungi and yeast as indicators of the hygienic quality of the product and the process applied. In the present work, count results indicated that the mesophilic aerobic bacteria and lactic acid bacteria populations were around 10^3 CFU/g, while fungi and yeasts count was below 100 CFU/g. There were no significant differences between all systems evaluated for the corresponding microbial counts. It is important to remark that the total counts of mesophilic aerobic bacteria and fungi and yeast were lower than those ruled as maximum values (5×10^4 CFU/g and 10^3 CFU/g, respectively) in the Argentine Food Code for Dietary Foods for Special Dietary Uses (ready-to-eat products). These results indicate that hygienic processing together with the antimicrobial barriers applied (low pH, a_w reduction, potassium sorbate addition) contributed to the safety of the final product.

4. Conclusion

In the present research, a refrigerated ready-to-eat food fortified with Fe and AA was successfully prepared using pumpkin (*Cucurbita moschata* Duchesne ex Poiret) as raw material applying a dry infusion process. Final products fortified with AA and Fe resulted with adequate quality from color and microbiological view point under the conditions herein assayed. Water content as well as water activity of the final product was affected by the type of coating and the process applied. AA retention was also strongly affected by the a_w of the final product and application of edible coating improved AA stability. The Fe supported in the coatings tended to have a higher bioaccessibility at in vitro simulated lumen conditions. It is important to remark that mineral content must be adjusted for the required limits established by regulations.

Acknowledgments

This research work received financial support from the University of Buenos Aires (UBACyT 20020110200192 and 20020090200693), The National Agency of Scientific and Technological Promotion of Argentina (ANPCyT PICT-2005-38239) and The National Scientific and Technical Research Council of Argentina (CONICET, PIP11220090100531 and PIP20020100100700).

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Table 4
Fitted parameters of the AA degradation kinetic (pseudo 1st order) in the pumpkin products obtained by dry infusion: with Fe–tapioca starch coating (F1), with Fe–k-carrageenan coating (F2), and with Fe but without coating (F3, control system).

Fitted parameters	Fe–starch-based coating (F1)	Fe–k-carrageenan-based coating (F2)	Dry infusion with Fe (F3, control)
C_0 (mg AA/100 g pumpkin)	1330 ± 50^{a}	750 ± 60^{b}	970 ± 70^{c}
k (h^{-1})	0.0011 ± 0.0002^{d}	0.0032 ± 0.0007^{e}	0.0028 ± 0.0005^{e}
r^2	0.8417	0.8242	0.8311
DW	1.71	2.08	2.16

Fitting equation: $C = C_0\exp(-k^*t)$, where C is AA concentration (mg AA/100 g pumpkin), C_0 is the AA adjusted initial concentration (mg AA/100 g pumpkin).
k is the rate constant for AA degradation, t: time in hours.
r^2: determination coefficient, DW: Durbin–Watson parameter.
Values in the same row followed by the same letter are not significantly different ($p > 0.05$).

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