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EFFECT OF IRON AND ASCORBIC ACID ADDITION ON DRY INFUSION PROCESS AND FINAL COLOR OF PUMPKIN TISSUE.

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Abstract

In the present study, pumpkin (*Cucurbita moschata* Duchesne ex Poiret) was used as raw material to produce sweet food fortified with iron (Fe) and ascorbic acid (AA). A dry infusion process with a subsequent air drying was applied. Response surface methodology was performed in order to analyze the effect of Fe and AA incorporation into the formulation on: water loss (WL) and solid gain (SG) during the dry infusion process, color changes (ΔE) and the dehydration percentage during subsequent air drying process. The results showed that the presence of Fe and/or AA promoted SG and WL during the dry infusion and also, weight changes during the air drying process (PP). An increase of the color changes was also observed. In turn, it was possible to obtain predictive equations for the parameters studied. The application of edible coating based on tapioca starch on pumpkin product was also tested showing a protective effect from the pumpkin color view point.

Key words: *Cucurbita moschata* Duchesne ex Poiret, functional foods, iron fortification, edible coating.

Abbreviations

Iron	Fe
Ascorbic acid	AA
Water loss	WL
Solid gain	SG
Color changes	ΔE
Weight changes due to air drying process	PP
Micronutrient malnutrition	MM
World Health Organization	WHO
Central composite design	CCD
Revolutions per minute	rpm
Recommended Daily Intake	RDI
Recommended Dietary Allowance	RDA
Non-enzymatic browning	NEB

1. INTRODUCTION

The micronutrient malnutrition (MM) is widespread over the world, but developing regions are the most affected. From a public health point of view, MM is a concern not only for the large number of people affected, but also because it remains a risk factor for many diseases (Ashwell, 2004). Iron (Fe) deficiency is considered the most prevalent of the MM, showing a continuous increase in its prevalence, representing the main nutritional deficiency problem in terms of magnitude and spatial distribution (Allen, Benoist, Dary & Hurrell, 2006; Souto de Olivera, 2009). At present, it is estimated that 2 billion people, or over 30% of the world population, are anemic, mainly due to Fe deficiency and this situation is further magnified in low-income areas with a high incidence of infectious diseases that contribute to the high prevalence of anemia according to World Health Organization (WHO, 2013). Both Fe deficiency and anemia, even in its moderate form, have serious health consequences for the population, including stunted growth and cognitive development (WHO, 2013; Zimmermann & Hurrell, 2007).

By the moment, food fortification with Fe is considered the strategy most sustainable and cost-effective against iron deficiency (Laxmi Narayan, Mills, & Berman, 2006; Tripathi & Platel, 2013). Nevertheless, there are some technological difficulties to be solved like changes and unpleasant sensory characteristics of the food matrix due to this fortification. The Fe compounds that are very soluble in water, for example ferrous sulfate, provide Fe of high bioavailability and, therefore, would be the primary choice in food fortification. However, in this type of compounds, Fe is highly reactive, causing oxidation of fats, vitamins and several amino acids in the food that is fortified (Boccio & Monteiro, 2004; Gaucheron, 2000) and, consequently, undesirable color and flavor changes in the food matrix could appear. Rao and Kawamura (2008) reported that the major technological problems caused by soluble salts of Fe in the production of food and beverages are the color and flavor alterations.

At the same time, there are dietary compounds which positively affect the Fe absorption, as is the case of ascorbic acid (AA). The presence of this hydrosoluble vitamin at the intestinal level promotes absorption of non-heminic Fe by means of its reduction to ferrous ion (Fe^{+2}). In foods, the AA acts as a reducing agent keeping the Fe in its soluble reduced form (de Escalada Pla, Campos, & Gerschenson, 2009; Souto de Olivera, 2009), and also acts as an antioxidant through the free radicals neutralization at the cellular level (Rojas, 1995). Some studies have also shown that vitamin A and, even more the β-carotene, significantly increase the bioavailability of Fe (Binaghi, Greco, López, Ronayne, & Valencia, 2005).

The policy adopted by some countries was to select as a carrier, those foods widely consumed by the risk groups. Vegetable and fruit matrices have widely been used to support vitamins and minerals like Ca^{2+} and Zn^{2+}, applying impregnation or vacuum impregnation technology for their enrichment (Gras, Vidal, Betoret, Chiralt, & Fito, 2003). This processing has been proposed by Zhao and Xie (2004) as a pre-treatment before the final drying step with the purpose of achieving two goals: decreasing moisture content before final air drying to save energy and incorporating functional solutes, such as nutrients, antimicrobial, antioxidant, and anti-browning agents to improve product quality. The impregnation processes of fruits and vegetables with hypertonic solutions were widely studied and well reported (Gras et al., 2003; Moreno et al., 2012; Spiazi & Mascheroni, 1997; Zhao & Xie, 2004). Dry infusion was recommended as a practical tool for small producers as fruit preservation process that could be performed in rural areas (Alzamora, Guerrero, Nieto & Vidales, 2003).

Edible coatings can have an additive or synergistic effect with other stress factors in the task of improving the overall quality of foods. The application of coatings on fruits and vegetables improved color and flavor retention during storage, extending the shelf life of the product, retarding moisture and/or firmness loss and product senescence (Campos, Gerschenson & Flores, 2011).

Pumpkin *Cucurbita moschata* is one of the most consumed vegetables in Argentina. Furthermore, an increasing interest in this vegetable has also been reported in other countries (Gwanama, Botha, & Labuschagne, 2008). Tissue from this kind of pumpkin was characterized previously (de Escalada Pla, Ponce, Wider, Stortz, Rojas, & Gerschenson, 2005; de Escalada Pla, Delbon, Rojas, & Gerschenson, 2006; de Escalada Pla, Ponce, Stortz, Gerschenson, & Rojas, 2007). More recently, the adequacy of pumpkin mesocarp tissue as a food matrix for Fe supply was reported (de Escalada Pla et al., 2009). The iron was incorporated after blanching and during the cooling step. Then, a hypertonic osmotic covering solution was added to storage bags.

The aim of the present work was to study: 1) the possibility of fortifying *Cucurbita moschata* Duchesne ex Poiret tissues with iron through a process of dry infusion, thus avoiding the use of huge amounts of hypertonic osmotic solutions; 2) the effect of the joint presence of Fe and AA on process parameters, physical and quality characteristics in the final product; and 3) the application of an edible coating based on tapioca starch for protecting pumpkin tissue from possible color detriments due to Fe/AA contents during the process and food storage.

2. Material and methods

2.1 Chemicals

Food grade sucrose and tapioca starch 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

Pumpkin (*Cucurbita moschata* Duchesne ex Poiret) obtained in a local supermarket was carefully washed and rinsed with distilled water. Then, cylinders of 15 mm diameter and 10 mm thickness were cut from the mesocarp using a stainless steel cork borer. The cylinders were blanched with water vapor for 8 minutes and then rapidly cooled for 1 minute by immersion in water at 0℃. F inally, they were impregnated with sucrose (900 g/kg of pumpkin), citric acid (1.5 g/kg of pumpkin) and potassium sorbate (1.9 g/kg) following a dry infusion process described by Alzamora et al. (2003). Briefly, pumpkin cylinders were placed in a plastic bowl and sprinkled with powdered sucrose. Water from vegetal tissue began to flow from the pumpkin cylinder to the surrounding sucrose concentrate. In that moment, citric acid, potassium sorbate, AA and Fe salt 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 and sorbic acid as an antimicrobial are more effective in this range of pH (Lindsay, 1996). In order to evaluate the effect of AA and Fe during the preparation process and on the final color quality, different amounts of AA and $FeSO_4.7H_2O$ were added to the systems according to a central composite design (CCD) of two factors (independent variables) and five levels (Table 1). Pumpkin used in all the systems came from a same single lot of raw product.

The dry infusion was carried out at 20℃ up to equilibrium on an orbital shaker (Vicking S.A., Argentina) at 35 revolutions per minute (rpm) to assure good contact of tissue and the impregnating system. Equilibrium was reached at 72 hours 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 and dried under forced air convection at 40℃ for 3 hours, in order to achieve a water activity (a_w) value below 0.85 (Fontana A., 2008).

Finally, the pumpkin cylinders were introduced into low density polyethylene bags of 80 µm thickness, provided with a Ziploc® type closure. Each bag was filled with 5 mesocarp pieces (10 g) and stored in a chamber at 18-20℃.

2.3 Preparation of the pumpkin fortified and coated

From the results obtained with CCD (see item 3.2), one formulation was chosen and one additional batch was performed. A dry infusion process, as previously described, was carried out and after draining, the cylinders were separated into two parts. One part was dipped into a solution of gelatinized starch in order to generate an edible coating on pumpkin cylinders, and the other part, pumpkin without coating was also prepared for comparing purposes in subsequent testing assays. Impregnated pumpkins with or without coating application were submitted to a drying process with force air convection at 40℃ for 3 hours in order to achieve the following purposes: (1) to constitute the coating, in the case of coated cylinders and (2) to obtain an additional reduction of a_w in both cases (Fontana A., 2008).

The edible coating was prepared with native tapioca starch (50 g/kg), glycerol (20 g/kg) as a plasticizer and potassium sorbate (1 g/kg) as an antimicrobial agent. Samples were packed and stored as previously explained.

2.4 Product characterization

In order to analyze the changes during the processing and storage of tissue, the samples were taken from blanched pumpkin, equilibrated pumpkin after dry infusion; and dried tissue after forced air convection drying. Also, samples of the final product after 9 days of storage at 18-20ºC were evaluated.

The following properties were measured:

♦ 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 seconds. The pH was determined with a pH meter (Cole-Parmer, USA).

Water activity (a_w) was measured with a hygrometer (Aqualab, USA) at 20ºC.

♦ Moisture and soluble solids contents:

Pumpkin samples were frozen and freeze dried (Christ, Germany) for 48 hours under vacuum (≈1.1 Pascal) and 25ºC, to determine the water content.

The percentage of soluble solids (ºBrix) 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, during the dry infusion step, were calculated according to the following equations (de Escalada Pla et al., 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 [ºBrix, or g s s/100 g pumpkin, wet basis], respectively. Measurements were performed in duplicate for each system and the average value is reported.

The water loss during the subsequent air drying process (PP) in wet basis was calculated as: $PP = \frac{Pi - Pf}{Pi} \times 100$

Pi: mass of the sample before convective drying.

Pf: mass of sample after convective drying.

♦ Color

Before and after drying, color parameters were evaluated using a photocolorimeter (Minolta, Japan) in the CIE L*a*b* space [L*: lightness, a*: greenness - redness, b*: blueness - yellowness] under illuminant D65 and with the observer at an angle of two degrees. From these parameters, color difference (ΔE) was calculated according to:

$$\Delta E = \sqrt{(L^* - L^*_{ref})^2 + (a^* - a^*_{ref})^2 + (b^* - b^*_{ref})^2}$$

Where reference values (L^*_{ref}, a^*_{ref} and b^*_{ref}) correspond to the control system, impregnated with sucrose in the presence of citric acid and potassium sorbate but without addition of Fe and AA in the dry infusion media (system C). In the case of the edible coating effect, the color difference was calculated taking as a reference the fortified cylinders after infusion and before coating and drying.

The value of Chroma parameter was also calculated. This parameter describes color intensity (Olivera et al., 2008) and was calculated as Chroma = $(a^{*2} + b^{*2})^{(1/2)}$. The averages of three measurements are reported.

2.5 Experimental design and statistical analysis

In order to evaluate the influence of AA and Fe during dry infusion and drying process as well as on final color quality, a CCD with two factors (independent variables) and at five levels (Table 1) was performed. The selection criterion for the lowest levels was, in the case of Fe, to cover 20% of the Recommended Daily Intake (RDI) with a 100 g portion, and in the case of AA, to cover 100% of the Recommended Dietary Allowance (RDA), according to the Argentine Food Code (2012) in its article 1363. The highest levels used were chosen to cover 100% of the RDA in the case of Fe, and in the case of AA was considered the level of no observed adverse effects value, with a maximum of 1000 mg. The central point (0;0) was performed in triplicate. Table 1 shows all experimental runs.

Dependent variables WL, SG, PP and ΔE were fitted using a second degree polynomial equation and a multiple regression procedure:

$$\psi = B_0 + B_1 x_1 + B_2 x_2 + B_{11} x_1^{\ 2} + B_{22} x_2^{\ 2} + B_{12} x_1 x_2$$

Where, ψ is the dependent variable analyzed; x_1 and x_2 are independent (Fe and AA contents) variables that affected ψ value; B_0 is the value of the fitted response at the center point of the design, (x_1 = 0 and x_2 = 0); B_1 and B_2 are the linear coefficients; B_{11} and B_{22} are the quadratic coefficients and B_{12} is the cross coefficient between factors. This equation permitted to evaluate the effects of linear, quadratic and interaction terms of independent variables on selected dependent variables. The analysis of variance (ANOVA) was conducted to assess the adequacy of the model by calculation of the F value for the regression and the determination coefficient (R^2), as well as to evaluate the significance of the equation coefficients. Three dimensional plots were generated (response surfaces) by fixing investigated variables to the center value of CCD.

On the other hand, in order to identify a Fe:AA ratio that minimizes undesirable color changes, the experimental values of the Chroma parameter were analyzed by the "Analysis of a central composite experiment (surface response)" module.

For color comparative purposes, an additional unfortified system was prepared under the same conditions as reference.

In addition, the significant differences among results were established by analysis of variance (ANOVA) with a significance level of 0.05 and applying a *post hoc* test, the Least Significant Difference (LSD) test. The results are reported based on their mean and standard deviation. Statistica software (version 6, StatSoft, Inc. 2001, USA) was used for the analysis of the design and generation of the response surfaces and also for statistical treatment of data.

3. Results and discussion

3.1 Characteristics of the impregnated and dried product

At the end of dry infusion, the a_w of pumpkin was in the range of 0.91 and 0.93, while the initial a_w, after blanching and before infusion, was ~ 1.0. Once equilibrated, samples also showed pH values in the range of 3.4 to 4.6. Neither AA nor Fe exerted significant effects on a_w of final product. Nevertheless, the pH decreased, as expected, when the AA concentration increased ($p<0.05$), as can be seen in Table 2.

Table 2, shows the values of WL and SG measured on tissues submitted to dry infusion for different contents of Fe and AA. Furthermore, PP values of the impregnated pumpkin after air drying are also reported. In order to analyze the effect of Fe and AA contents on the dry infusion and drying process, data were fitted using a second degree polynomial equation. The best fit equation and corresponding plots of the linear, quadratic and interactive effects of Fe and AA on SG, WL, and PP are shown in Figure 1, panel a, b and c respectively.

It could be seen that SG occurred during the dry infusion process and varied in the range of 8.8% - 19.7% (Table 2). Figure 1a, for SG, shows the response surface and the corresponding equation. The linear terms of Fe and AA were significant as well as the quadratic term of the factor AA (Figure 1a). It would mean that an addition of Fe or AA promotes the incorporation of solids inside the pumpkin tissue. However, the presence of both additives simultaneously presents an antagonistic effect because the interaction term was negative.

It could be seen that the WL was varied between 69% and 72.6% (Table 2). In this case, linear coefficients were both positive, indicating that the presence of Fe or AA promotes osmotic dehydration in the pumpkin vegetable matrix and it is expected that the addition of Fe to the formulation exerts the greatest influence on the value of WL, since the linear coefficient of Fe factor was positive and with a greater magnitude (Figure 1b). Once again, the presence of both additives simultaneously shows an antagonistic effect because the interaction term was negative. According to de Escalada Pla et al. (2009), Fe presence in pumpkin tissue favored the water loss during an impregnation process with hypertonic solution. Similar results were also reported by Barrera C., Betoret N. and Fito P. (2004) with vacuum impregnation of apple tissue fortified with calcium or Fe.

Subsequent air drying lowered the water activity about 15%. The final a_w ranged between 0.77 and 0.82. The weight changes due to the air drying process (PP) were approximately 21.9 to 26.9% (Table 2). The predictive equation (Figure 1c) indicated that the linear terms, the quadratic term for Fe and the interaction term were significant. A positive effect was observed through linear coefficients, indicating that the presence of Fe or AA promoted the air dehydration process. Significant negative coefficients for quadratic term of Fe and the interaction term were also observed, indicating a curvature of the surface.

3.2 Color evaluation

In Table 3, color attributes can be observed for the final product obtained from the different treatments. The color difference (ΔE) was determined taking control systems (unfortified) as reference. Response surface for ΔE and the corresponding equation are shown in Figure 1d. It could be observed that the addition of Fe or AA generated a darkened color of the pumpkin compared to the control system (Table 3). The second order equation obtained indicates that the linear and quadratic terms are significant, being the former positive and the latter ones, negative. The interaction term was not significant, suggesting that each factor exerts an independent effect on the color change (Figure 1d).

In order to assess the color development in the systems studied, a picture of them is shown in Figure 2. The control system (C), without fortification was also included for comparison purposes. It can be observed that system 6 showed the smallest color alterations due to the fortification and process applied. Based on these observations, differences in L* and in the Chroma parameter due to the final step of process were also analyzed. Table 3 shows L* and Chroma values for impregnated pumpkin, before and after the air drying process. In general, a reduction of L* and Chroma values after air drying, could be observed. This effect was not evidenced in system 6, neither in the control system, where no significant changes due to air drying, were observed for L* and neither for Chroma.

The addition of Fe or AA significant reduced L* and Chroma values in comparison with the control system. For all the systems studied, L* ranged between 31 and 38 and the Chroma presented values from 16.2 to 27 (Table 3). On the other hand, Chroma was the parameter most significantly affected by the drying process. It might be concluded that the color difference observed was mainly related to chromatic coordinates: a* and b* changes. The first step of AA destruction is part of the non-enzymatic browning (NEB) reaction chain (Rojas & Gerschenson, 2001; León & Rojas, 2007). Degradation of AA through hydrolysis can occur simultaneously to AA oxidation when oxygen is present, producing 2-keto-L-gulonic acid. It can then be considered that at least two irreversible parallel or competitive reactions proceed: the AA hydrolysis and the AA oxidation (De'Nobili, Curto, Delfino, Soria, Fissore, & Rojas, 2013). Some researchers reported that hydrolytic instability of AA could be responsible for NEB and the decrease in edible film lightness with storage (De'Nobili et al., 2013; Pérez, De'Nobili, Rizzo, Gerschenson, Descalzo, & Rojas, 2013). On the other hand, iron in the reduced state is an active prooxidant, and ascorbate, which could act as a hydrogen donor, in synergism with iron, serves as an effective chelator (Rosenthal, Rosen, & Bernstein, 1993). However, Hegenauer, Saltman, & Ludwig (1979) indicated that the conversion of ascorbate to dehydroascorbate and of dehydroascorbate to 2-keto-L-gulonate occurs rapidly even in unsupplemented milk. Thus, iron supplementation may not affect materially the vitamin C content of stored milk (Gaucheron, 2000). During the drying process, the carotenoids can be degraded by exposure to heat and oxygen, with a consequent increase in cis-isomers (Lago-Vanzela, do Nascimento, Fontes, Mauro, & Kimura, 2013). Probably, iron contents catalyzed this degradation, altering pumpkin color. Lightness and Chroma changes observed herein seemed to be related to independent mechanisms, one associated with AA destruction and the other with carotenoid oxidation. However, it could be interesting to determine the AA and Fe contents that minimize these effects.

The Chroma parameter was then analyzed in order to detect the Fe and AA concentration that let us obtain a Chroma value similar to that of the control system. Response surface obtained for the Chroma value is shown in Figure 3.

It must be remarked that all the coefficients of the corresponding second degree polynomial were significant (p <0.05), except for the coefficient of interaction.

In order to define a formulation that allows one to obtain an adequate color, the Chroma value from the control system (pumpkin without fortification) was taken as the target value. From equation of prediction, a formulation with 0.3475 g Fe/kg pumpkin and 0.8745 g AA/kg pumpkin was obtained. It must be remarked that concentration used on system 6 of CCD, was the most similar to that obtained according to optimization criteria. Nevertheless, the statistically recommended formulation was performed and the Chroma of the final product was evaluated recording a value of 29.6 ± 0.6 which is not significantly different (p<0.05) from the target value selected (System C, Table 3).

3.3 Characteristics of the pumpkin fortified and coated

Based on the formulation proposed, an additional batch was performed and one part of it was covered with a starch based coating. The other part of the batch was maintained without coating. All samples were tested evaluating their color (Table 4).

Pumpkin cylinders were weighed before and after the edible coating application. Consequently, it could be estimated that ~ 1g average of starch gel was deposited on the surface of each pumpkin cylinder during the dipping process.

During the drying process, a water loss of ~ 30% was registered, reaching the final product with an a_w value of ~ 0.8. Drying also affected the color of product as can be observed through the ΔE value at the beginning of storage, mainly on system without coating (Table 4). As can be observed, in Table 4, coating significant reduce product color changes due to the drying process.

Moreover, the processing applied significantly (p<0.05) reduced L* values for both systems, nevertheless, the uncoated system presented a higher reduction. With reference to the Chroma values, no significant differences were observed for system coated while a significant ($p<0.001$) reduction was recorded for uncoated one, due to air drying (Table 4). This suggests a protecting action of the starch coating used during the air drying process from the point of view of the color. Flores (2006) reported a low oxygen permeability of tapioca starch coatings, and this property could in part explain their capacity to protect pumpkin color specially avoiding AA and ferrous iron oxidation. Lago-Vanzela et al. (2013) assayed edible coatings from native and modified starches on pumpkin during drying and reported that dehydrated coated products had a better color and a significantly higher retention of trans-α-carotene and trans-β-carotene than products that did not receive coating. They claimed that the good carotenoid retention determined in the samples covered with modified cassava starch suggested that the coating worked as an efficient barrier against oxygen (Lago-Vanzela et al., 2013).

Table 4 also shows values obtained after 9 days of storage. In this case, it could be observed that for both samples, coated and uncoated, the Chroma value did not change significantly after nine days of storage at 18-20℃.

4. Conclusions

A dry infusion process could be used successfully to incorporate Fe and AA into pumpkin tissue. It was found that the addition of Fe or AA promoted osmotic dehydration in pumpkin and water loss during the subsequent air drying process.

The presence of Fe or AA intensified color differences of the systems when compared with the control system (unfortified) and this was mainly detected through the Chroma evaluation. The dry infusion with Fe and AA with subsequent air drying significantly decreased the value of the Chroma parameter of the pumpkin matrix with respect to the value for the unfortified system with the exception of product obtained through the impregnation in a formulation containing 0.216 g/kg of Fe and 0.80 g/kg of AA (system

6), for which the color after the drying process was similar to the one observed for the control system. From preliminary data herein reported, it might be suggested that edible tapioca starch coating exerted a protective effect in terms of the color of pumpkin cylinders during drying.

The present study provides important information for the design and processing of a pumpkin product fortified with Fe and AA which can enlarge the existing background for the optimization of the production and stability of new functional foods. As a perspective, a comparison of these results with a test of the consumers' acceptance could be interesting to perform.

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Captions to figures.

Figure 1. Pumpkin fortification with iron (Fe) and ascorbic acid (AA): Response surface for variables of dry infusion process a) Solid Gain (SG), b) Water Loss (WL), c) Weight changes during the air drying process (PP) and d) color changes (ΔE) respect to control system (without fortification). The best fitted second degree polynomials are:

$SG = 80.45\ Fe + 0.885\ AA + 0.0463\ AA^2 – 8.3802\ Fe\ AA$ (R^2: 0.9843, F: 110)

$WL = 307.57\ Fe + 6.26\ AA – 27.55\ Fe\ AA$ (R^2: 0.9908, F: 286)

$PP = 161.839\ Fe + 1.474\ AA – 179.523\ Fe^2 – 6.746\ Fe\ AA$ (R^2: 0.9960, F: 433)

$\Delta E = 79.438\ Fe + 1.267\ AA – 181.002\ Fe^2 – 0.041\ AA^2$ (R^2: 0.9848, F: 113)

Coefficients with significant effect are shown, R^2: determination coefficient, F: Fisher´s test value.

Figure 2. Pumpkin fortified with iron an ascorbic acid by dry infusion, after air drying. Numbers corresponds to systems from central composite design. Control system (C), without fortification, is also included.

Figure 3. Pumpkin fortified with iron (Fe) and ascorbic acid (AA) by dry infusion and air dried: surface response and the best fitted second degree polynomial for Chroma = $(a^{*2} + b^{*2})^{(1/2)}$:

$Chroma = 40.26 – 1.663\ AA – 105.59\ Fe + 0.0605\ AA^2 + 237.74\ Fe^2$

(R^2=0.755, lack of fit p = 0,119).

Captions for Tables.

Table 1. Treatments performed according central composite design for optimization of pumpkin fortification with iron (Fe) and ascorbic acid (AA). The control system (C) is also included.

Table 2: Pumpkin fortified with iron (Fe) and ascorbic acid (AA): measured values of water loss (WL), solid gain (SG), pH after dry infusion process and weight changes during the air drying (PP).

Table 3. Color difference (ΔE) of pumpkin fortified with iron (Fe) and ascorbic acid (AA) respect to control system (C) and color parameters: lightness (L*) and chroma before and after air drying process.

Table 4. Chroma and lightness (L*) parameters of fortified pumpkin with iron and ascorbic acid, coated and uncoated. Difference of color (ΔE) respect to impregnated pumpkin before coating and drying.

Table 1. Treatments performed according central composite design for optimization of pumpkin fortification with iron (Fe) and ascorbic acid (AA). The control system (C) is also included.

	Coded		Uncoded	
System	**Fe[1]**	**AA[1]**	**Fe[2]**	**AA[2]**
1	1	1	0.288	15.2
2	1	-1	0.288	5.6
3	-1	1	0.144	15.2
4	-1	-1	0.144	5.6
5	0	0	0.216	10.4
6	0	-2	0.216	0.8
7	0	2	0.216	20
8	-2	0	0.072	10.4
9	2	0	0.360	10.4
10	0	0	0.216	10.4
11	0	0	0.216	10.4
C	NA	NA	NA	NA

[1]Coded levels for Fe and AA
[2]Real values for Fe and AA (g/kg pumpkin)
NA: not added

Table 2: Pumpkin fortified with iron (Fe) and ascorbic acid (AA): measured values of water loss (WL), solid gain (SG), pH after dry infusion process and weight changes during the air drying (PP).

System	Fe[1]	AA[1]	WL[2]	SG	pH	PP
1	0.288	15.2	72.6±0.1^{a}	8.77±0.05^{a}	3.415±0.007^{a}	25.6±0.1^{a}
2	0.288	5.6	69.0±0.4^{b}	18.2±0.1^{b}	3.760±0.001^{b}	26.6±0.2^{b,c}
3	0.144	15.2	69.3±0.1^{b,c}	14.78±0.06^{c}	3.445±0.007^{a}	26.6±0.2^{b,c}
4	0.144	5.6	69.8±0.1^{b,c}	14.18±0.05^{d,f}	3.885±0.007^{e}	26.3±0.2^{b,c}
5	0.216	10.4	69.9±0.4^{b,c,e}	13.1±0.1^{e}	3.61±0.01^{c}	26.9±0.3^{b}
6	0.216	0.8	69.7±0.3^{b,c}	14.45±0.09^{f}	4.295±0.007^{f}	25.7±0.1^{a}
7	0.216	20	70.2±0.4^{c,d,e}	19.7±0.2^{g}	3.35±0.01^{g}	25.2±0.1^{a}
8	0.072	10.4	71.0±0.1^{d,e,f}	14.06±0.08^{d}	3.73±0.04^{b}	21.9±0.1^{d}
9	0.360	10.4	69.2±0.6^{b}	11.8±0.1^{h}	3.55±0.04^{d}	26.5±0.2^{b,c}
10	0.216	10.4	70.8±0.2^{e,f}	11.86±0.07^{h}	3.59±0.02^{c,d}	26.8±0.2^{b,c}
11	0.216	10.4	71.5±0.3^{f}	12.39±0.08^{i}	3.57±0.01^{c,d}	26.3±0.2^{b,c}
C	NA	NA	64.1±0.2	13.1±0.1^{e}	4.59±0.02	35.6±0.2

[1] Contents of Fe and AA (g/kg pumpkin).
[2]Absolute values were reported.
Mean and standard deviation (n = 3) are reported.
Different letters in the same column indicate significant differences ($p < 0.05$).

Table 3. Color difference (ΔE) of pumpkin fortified with iron (Fe) and ascorbic acid (AA) respect to control system (C) and color parameters: lightness (L*) and chroma before and after air drying process.

System	Fe[1]	AA[1]	ΔE	L^*_{After}	L^*_{Before}	$Chroma_{After}$	$Chroma_{Before}$
1	0.288	15.2	15.5±2.0 [a]	32.9±0.8 [a]	36.2±0.7	21±2 [a]	27.0±0.4
2	0.288	5.6	9.2±4.1 [b]	38±3 [b,A]	38.91±0.04[A]	25±3 [a,b]	30.2±0.5
3	0.144	15.2	17.4±0.2 [a]	33.3±0.3 [a,c]	36.3±0.4	17.72±0.06 [a]	27±1
4	0.144	5.6	14.4±0.2 [a,b]	34.6±0.8 [a,c,B]	36.5±0.4[B]	21.0±0.8 [a]	28±2
5	0.216	10.4	18.5±0.3 [a]	31.7±0.8 [a]	38.8±0.3	17.7±0.5 [a]	30.9±0.8
6	0.216	0.8	10.5±0.6 [b]	36.61±0.02 [b,c,C]	36.53±0.03[C]	27±3 [b,Z]	26±1[Z]
7	0.216	20	18.20±0.05 [a]	31±1 [a]	38.3±0.7	19±2 [a]	32±4
8	0.072	10.4	12.0±0.1 [a,b]	35±1 [a,b]	37.3±0.1	24.8±0.8 [a,b,Y]	28.50±0.05[Y]
9	0.360	10.4	16.5±0.7 [a]	33±1 [a]	36.03±0.06	19.91±0.07 [a]	28±3
10	0.216	10.4	19.6±0.5 [a]	31.26±0.08 [a]	38±1	16.2±0.6 [a]	30±6
11	0.216	10.4	17.9±1.2 [a]	32.6±0.2 [a]	41.9±0.6	18±2 [a]	39.0±0.2
C	0	0	NA	43.6±0.9 [D]	42.4±0.1[D]	31.1±0.8[X]	32±2[X]

[1] Contents of Fe and AA (g/kg pumpkin)
NA: not applicable.
L^*_{After} and $Chroma_{After}$ correspond to lightness and chroma after air drying.
L^*_{Before} and $Chroma_{Before}$, correspond to lightness and chroma before air drying.
Mean and standard deviation (n = 3) are reported.
Same letters within a column indicate non significant differences among systems (p<0.05).
Same capital letters within file indicate non significant differences due to air drying process for a same system (p<0.05).

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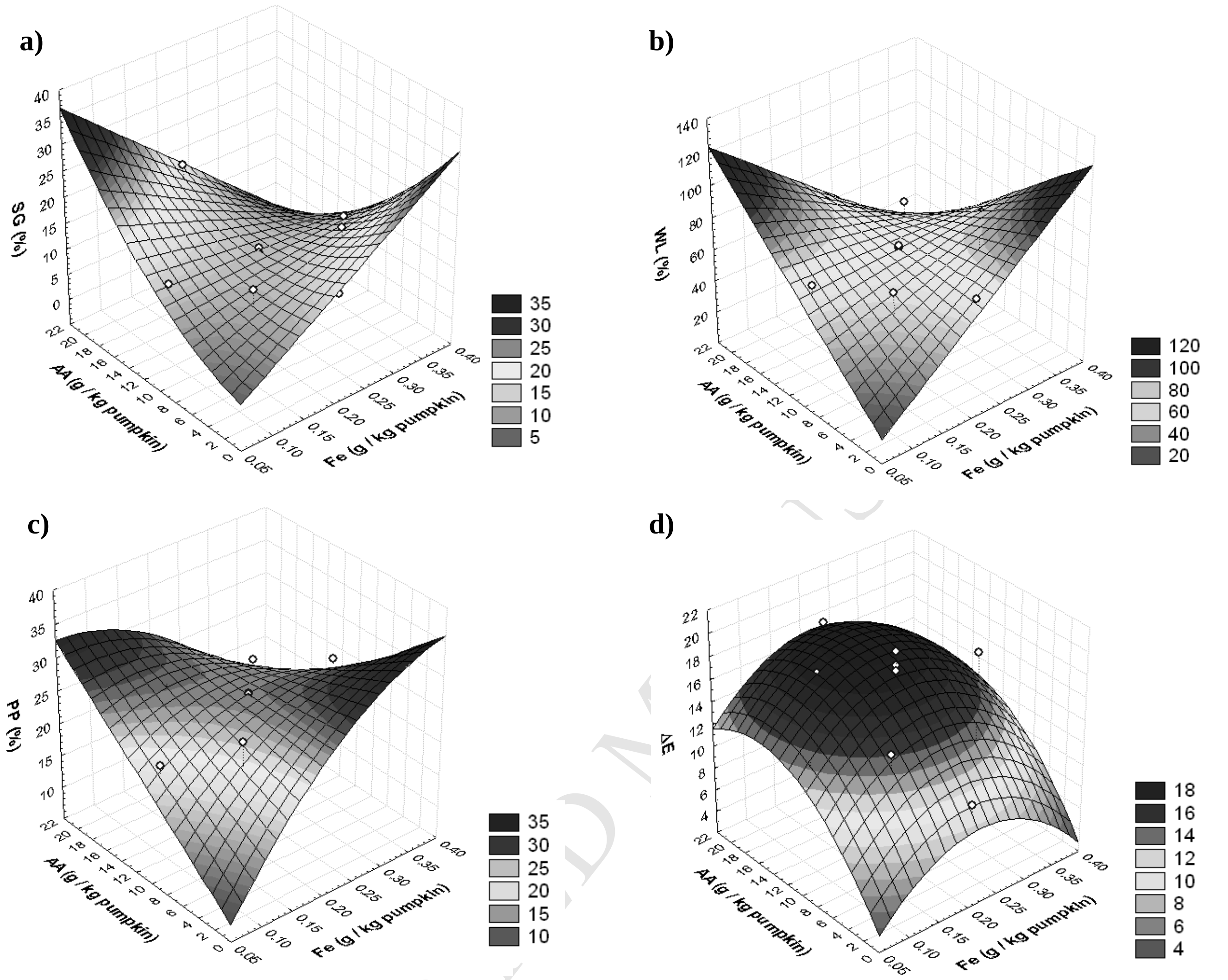
Table 4. Chroma and lightness (L*) parameters of fortified pumpkin with iron and ascorbic acid, coated and uncoated. Difference of color (ΔE) respect to impregnated pumpkin before coating and drying.

	Before drying (Ref)	After drying			
		Uncoated		Coated	
		d_0	d_9	d_0	d_9
Chroma	35.1±0.8^{a}	29.6±0.6^{b***}	28.09±0.07^{b***}	34 ±2^{a}	33.5±0.3^{a}
L*	40.5±0.5^{k}	34.20±0.08^{m***}	37.0±0.4^{l}	36.2±0.9^{l}	40.2±0.9^{k}
ΔE	NA	9.05±0.07	7.8±0.2	1.5±0.4^{z}	1.7±0.2^{z}

Mean and standard deviation (n = 3) are reported.
Same letters within a file indicate non significant differences ($p<0.05$; *** $p<0,001$).
Ref: impregnated system before coating and drying.
d_0: system at beginning of storage
d_9: system after 9 days of storage



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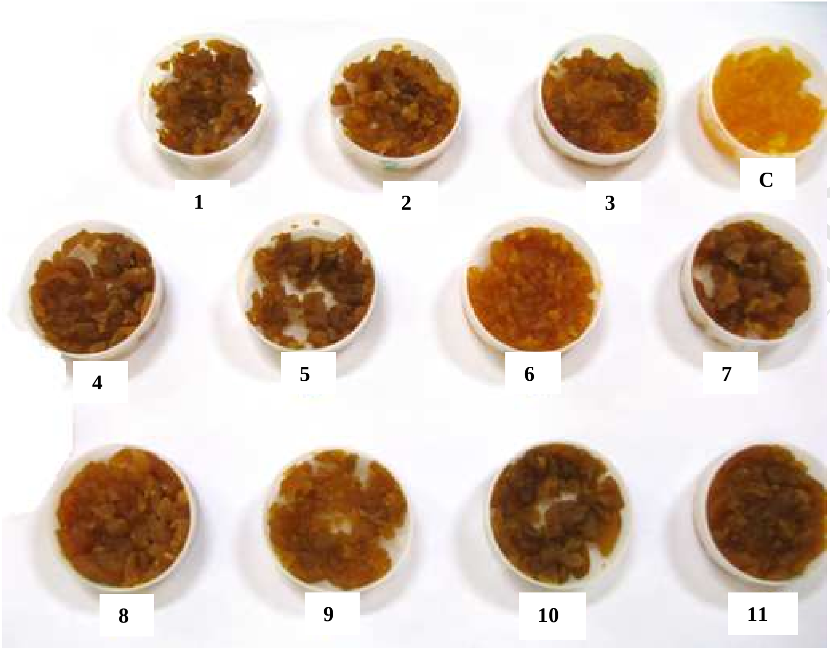
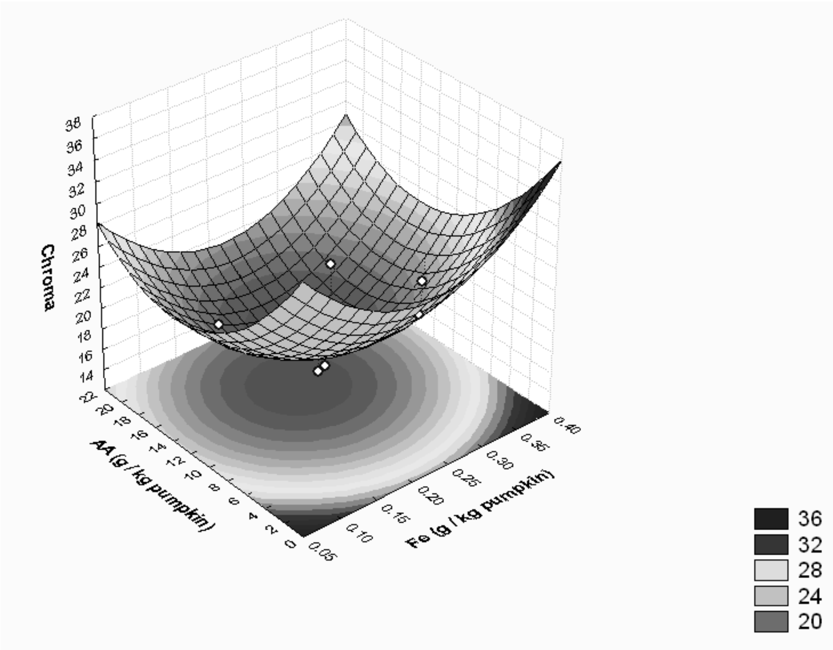


Figure 2. Pumpkin fortified with iron an ascorbic acid by dry infusion, after air drying. Numbers corresponds to systems from central composite design. Control system (C), without fortification, is also included.



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(R^2=0.755, lack of fit p = 0,119).

HIGHLIGHTS

- Pumpkin fortified with iron and ascorbic acid was developed.
- Dry infusion previous to air drying process allowed fortification of pumpkin.
- Iron / ascorbic acid ratio that minimize pumpkin color changes was determined.
- Edible coating based on tapioca starch protects pumpkin from color change.