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Pumpkin (*Cucurbita moschata* Duchesne ex Poiret) mesocarp tissue as a food matrix for supplying iron in a food product

Marina F. de Escalada Pla, Carmen A. Campos, Lía N. Gerschenson, Ana M. Rojas *

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

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ABSTRACT

Pumpkin (*Cucurbita moschata* Duchesne ex Poiret) was evaluated as a matrix to supply iron (Fe^{2+}) absorption in a hurdle processed product which combined water activity (a_W) and pH depression as well as an antimicrobial addition. L-(+)-Ascorbic acid was added to the formulated system for Fe^{2+} and β-carotene stability; though it suffered an important degradation after 23 days of storage. There was a nonsignificant difference in firmness and in rate constants of color degradation between pumpkin cylinders containing iron and those without its addition. Sensorial evaluation showed that the presence of iron did not impair acceptability and produced no taste differences. All iron present was biologically available according to the results obtained from *in vitro* digestion with pepsin and pancreatin and biliary salts. Consequently, pumpkin mesocarp tissue is a good fiber-rich food matrix for iron support and a promising raw material for future functional food product developments.

1. Introduction

Nutritional iron deficiency is still common in young women and children in developing countries where monotonous plant-based diets provide low amounts of bioavailable iron (Zimmermann and Hurrell, 2007). Iron deficiency is also common in women and young children in industrialized countries. In the UK, 21% of female teenagers between 11 and 18 years old and 18% of women between 16 and 64 years old are iron deficient (Heath and Fairweather-Tait, 2002). The effectiveness of medicinal iron sulphate supplementation for reducing anemia is poor, since adherence to treatment is low because of the several well-known side effects observed (Moreira-Araújo et al., 2008). New methods, like biofortification, to enhance native iron content of plant-based staple foods are promising (Zimmermann and Hurrell, 2007). For the moment, iron fortification of food is probably the most practical, sustainable, and cost-effective long-term solution to control iron deficiencies (Laxminarayan et al., 2006).

The iron compound and type of fortification should be chosen on the basis of the fortification vehicle, iron requirements of the target population, and iron bioavailability of the local diet. The main barriers to successful iron fortification are the following: (1) finding an iron compound that is adequately absorbed but causes no sensory changes to the food vehicle. The most bioavailable iron compounds are soluble in water or into diluted acid solutions but often react with other food components to cause off-flavors, and color changes, fat oxidation or both (Zimmermann and Hurrell, 2007) and (2) overcoming the inhibitory effect on iron absorption that is produced by dietary components such as phytic acid and phenolic compounds and calcium (Hurrell, 2002).

The foods used as iron vehicles have to be widely consumed by the targeted group and easily accepted (Moreira-Araújo et al., 2008). The foods most often used for mass fortification are the staple cereal flours. Iron is only poorly absorbed from high-extraction flours because of the presence of phytate and other inhibitory factors (Hurrell et al., 2004). Milk products can also be considered as main vehicles to be fortified with iron. Nevertheless, other milk compounds like calcium, casein-whey protein and cacao, in the particular case of chocolate milk, produce a significant decrease in iron absorption. In addition, iron reactivity produces fat, vitamins and amino acid oxidation and so, off-flavor can appear and loss of nutritional value can therefore occur (AAP, 1999; Gaucheron, 2000; Salgueiro et al., 2002; Sher et al., 2001). Sugar is an additional alternative to be considered as an iron vehicle. However, when sugar is added to drinks or infusions, tannins and other inhibitory factors it can significantly reduce iron bioavailability. Salt fortification with iodine has demonstrated to be an effective strategy to combat its deficiency in the world. Iron fortification of salt, instead, has several technological inconveniencies. First of all, color change can appear, mainly with high moisture content. Another inconvenience is the difference between sodium salts and ferrous salts in granulation and density, a fact that produces heterogeneous powder mixes. On the other hand, in many

* Corresponding author. Address: Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina. Tel.: +54 11 4576 3366/3397; fax: +54 11 4576 3366.
E-mail address: arojas@di.fcen.uba.ar (A.M. Rojas).

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countries, salt consumption is decreasing because of health concerns; consequently iron fortification of salt does not seem to be a very good choice. Finally, seasonings with iron addition have demonstrated to be effective in diminishing the anemia index in those countries where they are widely consumed like India or other Asiatic countries (Hurrell, 1997; Salgueiro et al., 2002). The major challenges therefore are to increase iron content to nutritionally useful levels and to assure that the added iron is bioavailable (Zimmermann and Hurrell, 2007).

The use of food and vegetable materials impregnated with biological active components, like vitamins and minerals Ca^{2+} and Zn^{2+}, has been widely studied for a long time (Abbott et al., 2000; Gras et al., 2003; Khin et al., 2006; Zhao and Xie, 2004). More recently, the effect of Fe^{2+} on the kinetics of osmotic dehydration (Barrera et al., 2004) and on other characteristics of apple tissue (Betoret et al., 2005) has begun to be studied. Nevertheless, there is little information about the effect of iron addition on organoleptic (texture, color, flavor) and nutritional characteristics of preserved fruits and vegetables. In this sense, no specific data concerning pumpkin (*Cucurbita moschata* Duchesne ex Poiret) was found.

In a previous paper (de Escalada Pla et al., 2005) the composition of this kind of pumpkin was studied and neither lignin nor phenolic compounds that can inhibit iron absorption were found, a fact that allows one to consider this matrix as potentially interesting for fortification. Consequently, the aim of this work was to evaluate the performance of pumpkin (*Cucurbita moschata*, Duchesne ex. Poiret) mesocarp tissue for supporting Fe^{2+} added as $FeSO_4$ salt; in which, functional and physicochemical characteristics of a pumpkin based fortified food were studied.

2. Materials and methods

To assure food microbial stability throughout the research period, a combined technique was applied to preserve pumpkin. The barriers selected to be applied in the hurdle technology used (Leistner, 1995) were a mild heat treatment, a_w and pH lowering and addition of potassium sorbate as an antimicrobial. Glucose and sucrose were used as a_w depressors taking into account their a_w depressing potential and sweetening characteristics (Gliemmo et al., 2001; Whistler and Daniel, 1993). Citric acid was chosen to reduce the pH, because of its excellent flavor in relation to the vegetal product obtained (Lindsay, 1993); at the same time, low pHs assured the solubilization of iron-salts in the aqueous phase. Citric acid is also a complexating agent, though not sufficiently stable complexes are formed with Fe^{2+} to become an obstacle for physiological iron absorption (Burriel Martí et al., 1992; Lindsay, 1993).

L-(+)-Ascorbic acid was used in the preparation; it was able to act as a hydrogen donor, in synergism with Fe^{2+} as well as a scavenger for free radicals to preserve carotenes from degradation (Gaucheron, 2000; Yanishlieva and Malarova, 2001). At the same time, the presence of L-(+)-ascorbic acid is necessary for an adequate absorption of Fe^{2+} in the gastrointestinal tract when this ion is provided through a vegetarian diet (Olson and Hodges, 1987), as well as to preserve Fe^{2+} from oxidation through its complexation (Gorman and Clydesdale, 1983). Potassium sorbate was applied as an inhibitor of yeast and mould growth (Castro et al., 2002; Gliemmo et al., 2004; Praphailong and Fleet, 1997; Sofos, 2000).

Vanillin was used as a flavoring agent. Although Fitzgerald et al. (2003) reported that vanillin was also an effective inhibitor of yeast and mould growth, the concentration used in this paper was one order of magnitude below the minimum inhibitory concentration reported in literature when vanillin was used as an antimicrobial agent (Fitzgerald et al., 2003; Vasantha Rupasinghe et al., 2006).

2.1. Chemicals

Food grade glucose monohydrate and sucrose were used. All additives ($FeSO_4 \cdot 7H_2O$, Merck, Argentina; potassium sorbate, Sigma, SL, USA; L-(+)-ascorbic acid, Merck, Argentina; citric acid, Anedra, Argentina; vanillin, Sigma, USA) and chemicals used were of analytical grade. Fluorescein diacetate and calcofluor white were from Sigma (SL, USA) and specific for microscopy.

2.2. Sample preparation

Microbial stability was obtained through the combined use of: heat treatment for 8 min in saturated water vapor at normal pressure (100 °C), depression of water activity to 0.94 and pH to 4.00, as well as the addition of 0.0500% (w/w) of potassium sorbate.

The pumpkin (*Cucurbita moschata* Duchesne ex Poiret) was purchased at a local market. It was carefully washed and cut into cylinders of 10-mm length and 20-mm diameter employing a stainless steel cork borer. The cylinders were treated in saturated water vapor as described above and the vegetal tissue was also blanched and cooked. The pumpkin was rapidly cooled by immersion for 90 min in one of the following solutions:

Control solution for system B, containing 500 ppm of L-(+) ascorbic acid.

Fortified solution for system F, containing the same components of control solution as well as 500 ppm of Fe^{2+} incorporated with the addition of 2.49 g/l $FeSO_4 \cdot 7H_2O$.

In both cases, the temperature during immersion was 18 °C and the cooling solution: pumpkin ratio was 20:1.

After cooling, the pumpkin cylinders were introduced into low density polyethylene bags of 80 μm thickness, provided with an easy-to-close Ziploc™ system. Each bag was filled with 10 pumpkin cylinders (45 g) and 100.00 cm^3 (osmotic solution/pumpkin cylinder ratio ≈ 2.3) of a hypertonic osmotic covering solution. The composition of this glucose/sucrose-osmotic solution of a_w 0.847 and pH 3.0 is indicated below:

Glucose monohydrate	1320.0 g
Sucrose	1320.0 g
Distilled water	1680.0 kg
Potassium sorbate	4.40 g
L-(+)-Ascorbic acid	2.20 g
Vanillin	0.8800 g
Citric acid 50% P/P	≈6.00 ml

Bags were closed practically without headspace and stored at 25 °C ± 0.5 °C, in darkness.

2.3. Methods

Samples of recently cooled tissue as well as samples stored for 1, 3, 7, 16, 23, 30, 47 and 58 days at 25 °C, were all submitted to the following determinations:

pH and a_w: These physicochemical properties were measured at 25 °C for the osmotic solution as well as for the pumpkin cylinders, which were previously reduced to puree (OmniMixer, USA). Determinations were performed at least in duplicate, using a pH-meter, with a Ag°/AgCl-combined electrode (Cole-Parmer, USA), or with an hygrometer (Aqualab, USA), respectively.

Water and soluble solids: Samples were freeze dried for 48 h to determine water content. The percentage of soluble solids (Brix) was determined with a refractometer (Cole Parmer Instruments CP, USA) in osmotic solution as well as in the juice extracted from pumpkin cylinders by squeezing. Determinations were made in triplicate.