Olive fruits and vacuum impregnation, an interesting combination for dietetic iron enrichment

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Abstract In this study vacuum impregnation (VI) was employed for the iron enrichment of olive fruits, which are very interesting as food vehicle for VI mineral supplementation for the porosity of their pulp. NaFeEDTA was chosen for olives fortification since it prevents iron from binding with compounds that could hinder it from being efficiently absorbed and since it causes few organoleptic problems. In order to improve the efficiency of the VI process, several parameters of the whole process were studied by design of experiment techniques. First of all D-optimal design was employed for a preliminary screening of the most significant process variables and showed that the concentration of VI solution was by far the most significant process variable, though its time in contact with olives was also significant. A factorial design was then applied to the remaining variables and it showed that the speed of the addition of VI solution was also significant. Finally, the application of a face centered composite design to the three selected variables allowed to detect processing conditions leading to final iron contents of 1.5–3 mg/g, corresponding to an introduction of 10–15 mg Fe with four or five fortified olive fruits. No effect on olive taste was observed at these concentrations. The results showed that olive fruits were the most interesting vehicles for the supplementation of both iron and other minerals.

Keywords Iron · Enrichment · Olive fruits · Vacuum impregnation · Design of experiment

Introduction

The vacuum impregnation (VI) process of a porous food product involves exchanging the internal gas or liquid occluded in its pores with an external liquid phase under the action of pressure changes (Fito and Pastor 1994; Fito 1994). The operation is carried out in two steps: at first, a closed tank containing the food is connected to a vacuum pump so that a vacuum pressure p_1 is imposed to the product for a time t_1 . In the second step, the process is completed by restoring atmospheric pressure and by immersing the food in the impregnating solution for a relaxation time t_2 (Zhao and Xie 2004). In the first step, the internal gas expands and takes with it the native liquid, which outflows from the porous structure, in the second step the external solution capillary flows into the food under the thrust of the restored pressure. The decisive action of the pressure gradient between the pores and the external surroundings allows to classify VI among hydrodynamics mechanism (HDM), but the whole process, including also the deformation-relaxation step, is more complex and affected by several variables (Fito et al. 1996). In the last twenty years an important study dealing with the theoretical aspects of the VI process was published (Fito and Pastor 1994) and several studies were performed with the aim of studying the effects of the process variables on the physical properties of the employed food (Zhao and Xie 2004).

The possibility of introducing into the void phase of porous foods a solution containing water and various chemical components makes the VI process particularly interesting for the production of foods with high nutritional

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Table 1 The experimental matrix selected by D-optimal design, the experimental plan (in brackets) and the obtained responses Y

EXP	X ₁ ^a Vac. Press. (MPa)	X ₂ Vac. Time (min)	X ₃ Fe Conc. (mg/100 mL)	X ₄ Speed of the addition (mL/s)	X ₅ Contact time (min)	X ₆ Osmotic Pressure	Y mg Fe/g olives
1	−1 (0.03)	−1 (20)	+1 (50)	+1 (~15)	+1 (30)	[1 0] (N.C. ^b)	1.65
2	−1 (0.03)	+1 (30)	−1 (10)	−1 (~1.5)	+1 (30)	[1 0] (N.C.)	0.33
3	+1 (0.05)	−1 (20)	−1 (10)	+1 (~15)	−1 (10)	[1 0] (N.C.)	0.20
4	−1 (0.03)	−1 (20)	−1 (10)	+1 (~15)	+1 (30)	[0 1] (Iso-G)	0.27
5	+1 (0.05)	1 (30)	+1 (50)	−1 (~1.5)	−1 (10)	[0 1] (Iso-G)	1.41
6	−1 (0.03)	−1 (20)	+1 (50)	−1 (~1.5)	−1 (10)	[0 0] (Iso-NaCl)	0.96
7	−1 (0.03)	1 (30)	−1 (10)	+1 (~15)	−1 (10)	[0 0] (Iso-NaCl)	0.21
8	+1 (0.05)	−1 (20)	−1 (10)	−1 (~1.5)	+1 (30)	[0 0] (Iso-NaCl)	0.26
9	+1 (0.05)	1 (30)	+1 (50)	+1 (~15)	+1 (30)	[0 0] (Iso-NaCl)	1.52
1 bis	−1 (0.03)	−1 (20)	+1 (50)	+1 (~15)	+1 (30)	[1 0] (N.C.)	1.60
3 bis	+1 (0.05)	−1 (20)	−1 (10)	+1 (~15)	−1 (10)	[1 0] (N.C.)	0.21
6 bis	−1 (0.03)	−1 (20)	+1 (50)	−1 (~1.5)	−1 (10)	[0 0] (Iso-NaCl)	1.10

X₁, vacuum pressure; X₂, vacuum time before addition of the VI solution; X₃, iron concentration in the VI solution; X₄, speed of the addition of VI solution; X₅, contact time of olive sample with VI solution; X₆, osmotic pressure of the VI solution; N.C., not controlled, Iso-G, isotonic by glucose; Iso-NaCl, isotonic by NaCl

and functional properties. Recently Betoret et al. (2011) included VI applications in an interesting paper dealing with the development of functional foods and pointed out that the VI process allows the inclusion of desirable solutes into the porous structure of foods without modifying their integrity. In this study, several references are reported about the fortification of different foods, both of animal and vegetable origin, with calcium, zinc and other minerals, vitamin E, probiotics. Moreover, the innovative perspective of using VI even for the protection of active food compounds was considered (Betoret et al. 2011).

Iron deficiency is one of the most frequent nutritional deficiencies. WHO-FAO (2001) defined Recommended Daily Intakes (RDIs) of 9–14 and 24–29 mg/day for over 18 man and woman, respectively, where the ranges are related to iron bioavailability. Even higher intakes are recommended during pregnancy. Plant-based diets, enhances the risk of deficiency because of the low levels-low bioavailability of iron in food of vegetable origin. Thus, iron fortification of food is one of the most important goals in the area of food enrichment and several studies were developed with this purpose (Martínez-Navarrete et al. 2002). Food fortification can successfully improve the iron nutritional status of a group of population, i.e. vegans, on a long-term perspective, but some challenges must be overcome, such as a suitable bioavailability of the added iron and its possible adverse effect on food color, flavor and stability. Solubility is important due to its supposed influence on bioavailability, but most water-soluble ferrous salts are highly colored and also have a metallic taste (Hurrell 1997). Moreover, the effect of iron in promoting fat oxidation has to be considered. NaFeEDTA appears, at

present, the most appropriate iron source, but its unique drawback might be its price. Nevertheless NaFeEDTA does not need the simultaneous addition of adsorption enhancers, such as ascorbic acid, and a further compensative saving can be obtained from the need of less sophisticated packaging materials (Hurrell 1997).

Although cereal flours, cereal based-foods and other vegetables have been frequently used for iron fortification (Martínez-Navarrete et al. 2002; Betoret et al. 2011), olives could be a very interesting food vehicle of iron in Mediterranean countries, where these fruits are largely employed in traditional local dishes and as a finger food. Therefore, the production of enriched table olives could be also an interesting business for table olive manufacturers.

Thus in this study NaFeEDTA and VI were employed for the enrichment of olive fruits, which, to our knowledge, had

Fig. 1 The VI impregnation equipment

Table 2 The experimental matrix of the factorial design, the experimental plan (in brackets) and the obtained responses Y

EXP	X ₁ ^a Vac. Press. (MPa)	X ₂ Vac. Time (min)	X ₄ Speed of the addition (mL/s)	Y mg Fe/g olives
1	−1 (0.03)	−1 (20)	−1 (~1.5)	2.11
2	+1 (0.05)	−1 (20)	−1 (~1.5)	2.06
3	−1 (0.03)	+1 (30)	−1 (~1.5)	2.43
4	+1 (0.05)	+1 (30)	−1 (~1.5)	2.24
5	−1 (0.03)	−1 (20)	+1 (~15)	1.93
6	+1 (0.05)	−1 (20)	+1 (~15)	1.68
7	−1 (0.03)	+1 (30)	+1 (~15)	1.74
8	+1 (0.05)	+1 (30)	+1 (~15)	1.64
9	0 (0.04)	0 (25)	0 (~8)	1.78
10	0 (0.04)	0 (25)	0 (~8)	1.97

X₁, Vacuum pressure; X₂, Vacuum time before addition of the VI solution, X₄, Speed of the addition of VI solution

never been used as food vehicle for mineral supplementation. These fruits are certainly very interesting as VI substrate for the porosity of their pulp, which ensures the entrance of oxygen and the exchange of gases between the internal tissues and the external environment. Several parameters of the VI process were considered and optimized by design of experiment (DoE) techniques. The amounts of iron in fortified olives were detected by UV–Vis spectroscopy.

Materials and methods

Materials

Chemicals were obtained from Sigma Chemicals (St. Louis, MO). All water used in the preparation of reagents was MilliQ[®] grade. All the employed glassware was washed, soaked in 1 M HCl for at least 4 h, and rinsed with milliQ[®] water.

Stoned pickled green table olives (cv. *Hojiblanca*, size 500 fruits/kg) were purchased on the local market. The covering liquid contained water, NaCl, lactic acid (acidulant) and L-ascorbic acid (antioxidant) and was discarded. Each fruit was pulled out of the brine, gently tapped on filter paper, and then longitudinally split into two halves by a surgical scalpel. A 10 g (exactly weighed) sample of half olives was used for each VI experiment.

Methods

Vacuum impregnation

The VI equipment consisted of a 15 L laboratory polymethylmetacrylate vacuum chamber adapted for the vacuum treatment (Fig. 1): it was custom designed starting from Sanplatec Vacuum Dessicator MB Type [Sanplatec

Corp., Japan (Kitaku Osaka, Japan)]. Its final design was developed from the scheme reported (Guillemin et al., 2006). The internal pressure was controlled by a vacuum pump (Vacuubrand MZ2C) and a vacuum regulator installed on the desiccator.

In order to prepare the NaFeEDTA impregnating solutions, amounts from 0.7 to 5.5 g of NaFeEDTA (exactly weighed) were dissolved in H₂O in 1 L volumetric flasks.

About 10 g of half olives, exactly weighed, were disposed into a 600 mL becker at ambient temperature. For each test, the becker was then placed into the VI chamber and held under vacuum for a fixed time before admitting, by depression, the external impregnation solution through the top valve of the vacuum chamber into the becker. After the impregnation time set for each test, impregnated half olives were drained and washed twice by MilliQ[®] water; finally, olives were gently tapped on filter paper and then collected in a round bottomed polypropylene test tube for the next step.

Determination of the iron content of olives

Preparation of chromogen solution

Preliminary the chromogen solution was prepared by dissolving 36 mg of 1,10-phenanthroline in 10–15 mL of water in a 100 mL volumetric flask. The solution was sonicated and then immersed in a boiling water bath. After cooling, the solution was brought to volume with 3 M sodium acetate and stored refrigerated in the dark to minimize light catalyzed deterioration.

Preparation of the iron reducing solution

In order to prepare an Fe³⁺ reducing solution, 7.5 g of hydroxylamine monohydrochloride were dissolved in few

Table 3 The CCD experimental matrix, the experimental plan (in brackets) and the obtained responses Y

EXP.	X ₃ ^a Fe Conc. (mg/100 mL)	X ₄ Speed of the addition (mL/s)	X ₅ Contact time (min)	Y mg Fe/g olives
1	−1 (25)	−1 (~1.5)	−1 (20)	0.86
2	+1 (75)	−1 (~1.5)	−1 (20)	2.62
3	−1 (25)	+1 (~15)	−1 (20)	0.72
4	+1 (75)	+1 (~15)	−1 (20)	2.12
5	−1 (25)	−1 (~1.5)	+1 (40)	0.97
6	+1 (75)	−1 (~1.5)	+1 (40)	2.91
7	−1 (25)	+1 (~15)	+1 (40)	0.96
8	+1 (75)	+1 (~15)	+1 (40)	2.67
9	−1 (25)	0 (~8)	30 (0)	0.94
10	+1 (75)	0 (~8)	30 (0)	3.16
11	0 (50)	−1 (~1.5)	30 (0)	1.94
12	0 (50)	+1 (~15)	30 (0)	1.64
13	0 (50)	0 (~8)	−1 (20)	1.61
14	0 (50)	0 (~8)	+1 (40)	1.90
15	0 (50)	0 (~8)	30 (0)	1.78
16	0 (50)	0 (~8)	30 (0)	1.67
17	0 (50)	0 (~8)	30 (0)	1.68

X₃, Iron concentration in the VI solution; X₄, Speed of the addition of VI solution; X₅, Contact time of olive sample with VI solution

mL of H₂O in a 100 mL volumetric flask, before adding 15 ml of HCl 37%. The solution was then brought to volume with water.

Preparation of iron standard solutions

Iron standard solutions were prepared in duplicate with an iron concentration of 10, 5, 2.5, 2, 1, and 0.5 mg/100 mL, starting from a NaFeEDTA solution containing 50 mg Fe/100 mL. A 1 mL aliquot of each iron standard was mixed with 5 ml of the chromogen solution. After standing at room temperature for 15 min, absorbance was measured at 510 nm by an Agilent 8543 UV–visible spectroscopy system and the standard curve was constructed using linear regression.

Iron extraction and determination

Finally, 50 mL of MilliQ[®] water were added to the fortified olives. After homogenization by Ultra-Turrax T25 for 12 min (8000 rpm speed), the slurry was centrifuged at 3500 rpm for 30 min. Five mL of the clear solution (S₁) were then mixed with 10 mL of the hydroxylamine monohydrochloride solution in a 30 mL glass capped tube, agitating by vortex for 30 s and solution S₂ was obtained. 1 mL of S₂ was then added to 5 mL of the chromogen solution and gently mixed. After standing 15 min in the dark at ambient temperature, the absorbance at 510 nm was

measured against a blank of 5 mL sodium acetate + 1 mL MilliQ[®] water, since in these conditions the absorbance of sample solution S₂ was <0.01. The iron concentration was obtained by applying the regression equation previously determined.

Design of experiments

Design of experiment was employed to single out the variables that significantly affected the amounts of retained iron, evaluate their possible interactions and obtain levels of iron fortification that could be interesting for the industrial production of enriched olives. It was performed by an R-based chemometric software (Group of Chemometrics of the Italian Chemical Society 2016).

Results and discussion

Although iron is frequently determined in food by standard flame atomic adsorption spectrometry (EFSA 2010), the determination by UV–VIS spectroscopy is simpler, faster, and cheaper and is within everybody's reach. In 2001 an UV–VIS method employing bathophenanthroline disulfonic acid (BPDS) as Fe²⁺ complexing agent was validated by comparison with standard atomic adsorption method for the detection of iron in fortified foods (Kosse et al. 2001). Since BPDS is not easily commercially available and 1,10-

Fig. 2 The coefficients of the models obtained by the D optimal (a) and the 2^3 factorial (b) designs (X_1 , vacuum pressure; X_2 , vacuum time before addition of the NaFeEDTA solutions; X_3 , Fe

concentration in the VI solution; X_4 , speed of addition of VI solution; X_5 , contact time of olives with the VI solution; X_6 , osmotic pressure of the VI solution)

phenantroline has also been largely employed for the Fe^{2+} colorimetric evaluation, in this study the proposed method was slightly modified and 1,10-phenantroline was employed instead of BPDS. Moreover, the reported protein precipitation with trichloroacetic acid was not necessary for olive fruits, so that the whole analytical method was even faster and simpler. After reduction of Fe^{3+} to Fe^{2+} by reaction with an excess of hydroxylamine hydrochloride (Kosse et al. 2001), the reaction of Fe^{2+} with 1,10-phenantroline allowed the evaluation of the total Fe^{2+} content by measuring the absorbance at 510 nm. The total iron content of the olives employed for the impregnation was 0.9–1.2 mg/100 g.

Several ferrous and ferric compounds have been used for the fortification of different foods: Hurrell classified them into four groups and one of them was the group of protected iron compounds, such as hemoglobin and FeEDTA complexes, such as NaFeEDTA. An important advantage of NaFeEDTA over other iron fortification compounds is that it prevents iron from binding with phytic and oxalic acids, polyphenols or other compounds that could hinder iron from being efficiently absorbed from vegetables. Moreover, iron combined in NaFeEDTA causes fewer organoleptic problems than other water-soluble iron compounds and it does not promote lipid oxidation, with the consistent formation of oxidized rancid products (Martínez-Navarrete et al. 2002; Hurrell 1997). Starting from the assumption that “Iron from ferric sodium EDTA is 2–3 times more bioavailable than from other mineral sources” and that there is no safety concern for it up to an exposure to EDTA above 1.9 mg EDTA/kg bw/day (EFSA 2010), Design of Experiment was employed to enhance the amounts of retained iron in impregnated olives. The total iron amount (mg Fe/olives) was used as response.

Based on literature data and on preliminary tests, seven process variables that might affect the efficiency of VI were selected and investigated by a screening design, with the goal of removing the non relevant ones. Five variables (vacuum pressure, vacuum time before addition, iron concentration in the VI solution, contact time of the olive sample with the VI solution and speed of the addition of the VI solution) were quantitative variables and were studied at two levels. The two remaining variables (osmotic pressure of the VI solution and employed isotonic agent) were collapsed in a single qualitative variable at three levels (not controlled, controlled by glucose, and controlled by NaCl) (Table 2). The presence of this variable made impossible to use a standard design, such as a Plackett Burman design or a Fractional Factorial design.

In this screening step, only the linear terms were investigated, and the corresponding model was the following:

$$1. Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4 + b_5 X_5 + b_{6A} X_{6A} + b_{6B} X_{6B}$$

It has to be noticed that variable 6, being a qualitative variable at three levels, requires two terms. Therefore, the best subset of experiments to be performed must be defined by using a D-Optimal design.

The whole experimental domain (5 variables at 2 levels and 1 variable at 3 levels) is made by 96 experiments ($2^5 \times 3^1$), among which D-Optimal Design selected a subset of 9 experiments sufficient to obtain relevant information about the effects of the studied variables.

The obtained model, whose coefficients are graphically shown in Fig. 2a, is

$$2. Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 (***) + b_4 X_4 + b_5 X_5 (*) + b_{6A} X_{6A} + b_{6B} X_{6B}$$

Fig. 3 The coefficients of the model obtained by face Central Composite Design (X_3 , Fe concentration in the VI solution; X_4 , speed of addition of VI solution; X_5 , contact time of olives with the VI solution) and the Response Surface Plot on the plane of variables X_3 and X_5

The experimental matrix and the experimental plan, i.e. the conditions of the 9 selected experiments, are reported in Table 1, together with the amount of total iron detected in olives at the end of each experiments. Three randomly selected experiments (1, 3 and 6) were replicated, in order to estimate the experimental variability. The maximum total amount of retained iron attained in this design was 1.7 mg/g olives, thus allowing the introduction of about 10 mg iron with about six olives.

Variable 6 (osmotic pressure) is non significant; thus the most convenient level, i.e. the absence of an isotonic agent (which, by the way, was the level at which the best results were obtained), was selected for the next designs. Only variables 3 (iron concentration) and 5 (contact time) significantly affected the amount of iron in impregnated olives, but the large effect of variable 3 could mask the effect of the other variables.

In order to better investigate the possible effect of variables 1, 2, and 4 on the VI process, the concentration of the impregnating VI solution and its contact time with fruits were set at the higher values previously explored (50 mg Fe/100 mL and 30 min) and a 2^3 factorial design was performed. Eight experiments plus two replicates of the central point were carried out in a randomized order (Table 2), which allowed the estimation of the coefficients of the following model

$$3. \quad Y = b_0 + b_1X_1 + b_2X_2 + b_4X_4 + b_{12}X_1X_2 + b_{14}X_1X_4 + b_{24}X_2X_4$$

The plot of the coefficients (Fig. 2b) shows that also the speed of VI addition (X_4) is significant.

Finally, the three significant variables (X_3 , X_4 and X_5) were further explored by a faced Central Composite Design (CCD), which allowed studying their interactions and the quadratic terms. The postulated model was

$$4. \quad Y = b_0 + b_3X_3 + b_4X_4 + b_5X_5 + b_{34}X_3X_4 + b_{35}X_3X_5 + b_{45}X_4X_5 + b_{33}X_3^2 + b_{44}X_4^2 + b_{55}X_5^2$$

where X_3 is the concentration of VI solution, X_4 is the speed of VI solution addition, and X_5 is its contact time with olive fruits. Since the concentration and the contact time were shown to have a positive effect on the iron enrichment, their upper levels were increased to 75 mgFe/100 mL and 40 min, respectively (Table 3). As far as the non significant variables are concerned, they were set at 0.03 MPa (X_1) and 20 min (X_2), without controlling the osmotic pressure of the impregnating solution (X_6). The results of the CCD (Fig. 3a) confirmed that the concentration of the VI solution was by far the most significant variable, although also contact time was significant. The maximum total amount of retained iron attained in the experiments of this design was 3.2 mg/g, which is certainly higher than what is frequently employed in fortified food products (Hurrell 1997). It is important to underline that olives are generally consumed in small servings and these Fe amounts would allow the introduction of 10–15 mg Fe with three to five olives. No effect on olive taste was observed at these concentrations.

From the response surface on the plane of variables X_3 and X_5 (Fig. 3b) it is possible to single out the cheapest conditions leading to a desired iron content when NaFeEDTA and vacuum impregnation are employed for

olives enrichment. For example, considering the cost of the management of the waste impregnating solution, it could be advisable to employ a minimal volume of a concentrated solution, but at least 50 mL/10–12 g are required to cover up 10–12 g olives. On the other hand, the preparation of a more concentrated solution would certainly involve a higher waste of NaFeEDTA. Considering the response surface plots and a target of about 2 mg Fe/g olive (i.e. 20–24 mg/10–12 g olives), 50 mL of the solution containing 50 mg Fe/100 mL are probably not enough to fulfill the target, since the required amount of Fe are too close to the amount dissolved in 50 mL. Thus, employing 50 mL of a solution with a slightly higher Fe concentration, i.e. close to 60 mg/100 mL, appears more appropriate, can be coupled with low contact time (22–23 min) and does not change the amount of the waste solution.

Conclusions

The systematic approach to the study of the VI process of olive fruits with NaFeEDTA has allowed to single out processing conditions suitable for the development of a new interesting functional food. The obtained results allow to consider olive fruits among the most interesting vehicles for the supplementation of iron and probably other minerals, and to plan the production of enriched table olives on a large scale. The influence of NaFeEDTA on the absorption of valuable micronutrients should be further investigated, in spite of the fact that the results of several studies suggested that the absorption and metabolism of zinc, copper, calcium, manganese, and magnesium are not impaired at the NaFeEDTA levels discussed above.

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