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## Use of response surface methodology to optimise vacuum impregnation of $\beta$ -carotene from *Daucus carota* in *Pachyrhizus erosus*

Julio E. González-Pérez,<sup>ID</sup> Oscar Jiménez-González,<sup>ID</sup> Nelly Ramírez-Corona<sup>ID</sup>  
and Aurelio López-Malo<sup>ID</sup>\*

Consuming carotenoid-rich foods prevent and reduce certain types of cancer and cardiovascular and degenerative diseases. In this work, response surface methodology (RSM) was used to maximise  $\beta$ -carotene (from *Daucus carota* juice) vacuum impregnation into *Pachyrhizus erosus* (PEC) cylinders (1 mm diameter by 2 mm length). The impregnation was carried out at 40 °C, an absolute pressure of 51 mm Hg, and a 10 g carrot juice/fresh product ratio. The factors considered were the immersion time ( $t$  = 20, 30, and 40 min) and the osmotic agent concentration ( $C$  = 20, 35, and 50 °Brix). The polynomial equations obtained to predict solute gain (SG), water loss (WL), total carotenoids (TCs), total soluble solids (TSSs), and water activity ( $a_w$ ) of the product had  $R^2 > 0.846$ . Increasing the osmotic agent concentration increased SG, TC, and TSS values and decreased WL and  $a_w$  values. From the obtained polynomial equations of the analysed responses, a quadratic effect was observed in the processing time due to the saturation of solutes on the product surface. The optimum conditions were  $t$  = 31 min and 50 °Brix, resulting in an impregnated PEC with a value of 269  $\mu\text{g}$  of  $\beta$ -carotene/100 g dry base, 19.3 °Brix, and  $a_w$  = 0.946. It was possible to incorporate carotenoids in PEC by optimising the vacuum impregnation conditions; however, a drying treatment is necessary to increase the shelf life of the obtained product.

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## 1. Introduction

*Pachyrhizus erosus* is known as Mexican yam, Mexican turnip, yam bean, or “jicama”.<sup>1</sup> It is rich in fibre, vitamins (C and B6), and minerals (K, Ca, and P, among others). However, due to its moisture content ranging from 80 to 85% (w.b.), it requires storage in a cool and dry place to avoid spoilage.<sup>1–3</sup> Therefore, drying processes have been used to increase its shelf life. Some quality problems of dried jicama are browning (enzymatic browning), flavour (sweetness) losses, and nutritional value reduction (regarding vitamins, minerals, and antioxidant content).<sup>4</sup>

Food fortification has been a strategy to combat malnutrition due to a lack of micronutrients such as iron, zinc, and vitamin A,<sup>5</sup> which are responsible for various ailments. These include physical deficiencies (congenital disabilities) or cognitive deficiencies (reduced or undeveloped cognitive ability and reduced productivity).<sup>5,6</sup> In this context, food fortification is the addition of micronutrients to increase nutritional value. The term biofortification involves using micronutrients, such as vitamins or minerals, to improve nutritional value through

agronomic practices, conventional plant breeding, or modern biotechnology.<sup>7</sup> In this attempt, Kruger *et al.*<sup>8</sup> mentioned that food-to-food addition could be a strategy to achieve this objective. In addition, food enrichment and fortification have been implemented as a drying pre-treatment to compensate for the nutrient loss or to incorporate new nutrients into foods.<sup>9,10</sup>

An important source of these compounds is the juice from fruits and vegetables, which has high content of these nutrients. In particular, orange carrots contain  $\alpha$  and  $\beta$ -carotene,<sup>11</sup> which have a  $\beta$ -ring and  $\epsilon$ -ring at the beginning and end of a long chain of alternating double bonds. This chain is responsible for the orange, red, and yellow colours.<sup>12</sup> The presence of  $\beta$ -carotene has been related to disease prevention caused by oxidative stress (some cancer types, ageing, vascular atheroma, *etc.*) because they are vitamin A precursors.<sup>13</sup> Therefore, it is important to increase carotenoid content to improve nutritional value and human health.<sup>14</sup>

Impregnation with concentrated juices is currently used because it avoids adding refined sugar and increases bioactive compounds.<sup>15–17</sup> However, this technique requires long impregnation times. Moreover, in some cases, the compounds do not penetrate the product centre, causing a barrier of compounds outside and avoiding the total water outflow.<sup>16,18</sup> In contrast, vacuum impregnation has been proposed to introduce liquids into the porous matrix,<sup>19</sup> improving nutritional

Departamento de Ingeniería Química, Alimentos y Ambiental, Universidad de las Américas Puebla, Ex hacienda de Santa Catarina Mártir, San Andrés Cholula, Puebla, C.P. 72810, Mexico. E-mail: aurelio.lopezm@udlap.mx

characteristics and visual/sensory aspects<sup>20</sup> inherent to juices, such as colour, acidity, astringency, and sweetness, among others.

So far, only reagent-grade carotenoids have been used for impregnation, such as diluted  $\beta$ -carotene in water<sup>13</sup> or sugar solutions,<sup>21</sup> that are incorporated into the food matrix using vacuum pressure. In another study, apples were dehydrated and subsequently immersed in a solution of  $\beta$ -carotene and water to stain the tissue and incorporate carotenoids with a coloured solution.<sup>22</sup>

The use of response surface methodology (RSM) as an optimisation tool has been widely adopted in the food industry to increase the efficiency of food processes. Factorial,<sup>23,24</sup> Box–Behnken,<sup>16</sup> and orthogonal<sup>25</sup> experimental designs have been used to maximise the incorporation of sucrose solutions or bioactive compounds through the optimal combination of process conditions such as temperature, vacuum pressure or concentration of osmotic solution.<sup>16,20,26</sup>

This study aimed to evaluate the effect of vacuum pressure, processing time, and concentration of carrot juice concentrate on the quality of jicama impregnation using response surface methodology. The responses assessed were mass transfer parameters (water loss and solute gain), water activity ( $a_w$ ), total soluble solids (TSSs), colour, and total carotenoids (TCs). Also, the impregnation of carotenoids in jicama (*P. erosus*) was optimised.

## 2. Materials and methods

### 2.1. Materials

Fresh jicamas (*P. erosus*) ( $a_w = 0.979 \pm 0.005$ ;  $6.6 \pm 0.3$  °Brix, and pH =  $6.30 \pm 0.03$ ) were purchased at a local market (San Andrés Cholula, Puebla, Mexico) and processed on the same day. The jicamas were selected for their uniform shape and size (200 g per sample). Then, they were washed, sanitised with a peracetic acid solution (by immersion in a  $100 \text{ mg L}^{-1}$  solution for 10 min), peeled, and cut into cylinders (1 mm diameter  $\times$  2 mm length) using a handmade sharpened metal tube. Each cylinder weighed  $1.241 \pm 0.007$  g.

Osmotic solutions of 20, 30, 40 and 50 °Brix were prepared by diluting carrot juice concentrate (*Daucus carota*,  $70.0 \pm 0.2$  °Brix and pH =  $5.36 \pm 0.07$ ) with distilled water. It was generously donated by Kerr by Ingredion (Ingredion Mexico S.A. de C.V.).

### 2.2. Physicochemical analysis

Moisture was determined by drying the samples for 24 h at  $105^\circ\text{C}$  in an oven to constant weight according to AOAC International<sup>16,27</sup> method 950.466. Eqn (1) and (2) were considered to report the moisture fraction on a wet basis (w.b.) and dry basis (d.b.), respectively. Determinations of total soluble solids (TSS, in °Brix) of homogenised samples were performed, with a digital refractometer (Atago Co., Pocket PAL-RI, Tokyo, Japan).<sup>28</sup> Water activity ( $a_w$ ) was determined at  $25.0 \pm 0.5^\circ\text{C}$  using a hygrometer (AquaLab, 4TEV, USA).

$$M_k = \frac{\text{g water in sample}}{\text{g fresh sample}} = \text{w.b.}, \quad k = 0 \text{ or VI} \quad (1)$$

$$\frac{\text{w.b.}}{1 - \text{w.b.}} = \text{d.b.} \quad (2)$$

where  $M_k$  is the moisture fraction of the sample  $k$ ,  $k = 0$  is the fresh sample, and  $k = \text{VI}$  is the impregnated sample.

Real porosity of fresh jicama ( $\epsilon_r$ ) was calculated with eqn (3), using apparent and real densities (in  $\text{kg m}^{-3}$ ).<sup>24</sup> It was estimated in triplicate.

$$\epsilon_r = \frac{\text{real density} - \text{apparent density}}{\text{apparent density}} \quad (3)$$

Apparent density was determined using Archimedes' principle, which gives the relationship between the weight of a jicama cylinder and the volume displacement of *n*-heptane when introducing the sample.<sup>29</sup> Jicama cylinders were ground to puree, and the real density was measured with a pycnometer.<sup>24</sup> Fresh and impregnated jicama cylinders were homogenised with a mortar and pestle for pH determination. The pH value of 10 g of homogenised samples and 10 mL of carrot juice solutions at  $20.0 \pm 1.0^\circ\text{C}$  was measured by direct immersion using a pH meter (Orion Research, 420A, IL, USA).

### 2.3. Impregnation treatment

Vacuum impregnation procedures were performed in a desiccator (150 mm internal diameter by 275 mm height) at  $40^\circ\text{C}$  (to avoid the juice from boiling) and at 51 mm Hg of absolute pressure. The system's temperature was controlled using a thermos-regulated bath (Büchi, B-300 Base, Flawil, Switzerland). And the pressure was controlled with a vacuum pump (Büchi, V-300, Flawil, Switzerland). One jicama cylinder in an upright orientation (with its circular face perpendicular to the bottom of the system) was immersed into glass tubes ( $20 \times 150$  mm) with the osmotic solutions. A 10 g osmotic solution/g jicama ratio was considered to avoid excessive dilution of osmotic solution concentrations during the experiment. Also, this ratio was adequate to guarantee contact between the product and the osmotic solution. The treatment was performed at different immersion times (20, 30, and 40 min). This time corresponds to two periods: the first was the impregnation under vacuum (10, 20, and 30 min), and the second was the relaxation period (10 min) at atmospheric pressure (598 mm Hg).<sup>8</sup> The jicama cylinders were removed from the osmotic solution, weighed, and blended for different determinations. One impregnated cylinder was used for each determination, and all the experiments were performed in triplicate.

### 2.4. Mass transfer properties

Water loss (WL) and solute gain (SG) were determined with eqn (4) and (5), respectively,<sup>10,17</sup>

$$\text{WL} = \frac{m_{\text{S0}}M_0 - m_{\text{VI}}M_{\text{VI}}}{m_{\text{S0}}} \quad (4)$$

$$\text{SG} = \frac{m_{\text{VI}}(1 - M_{\text{VI}}) - m_{\text{S0}}(1 - M_0)}{m_{\text{S0}}} \quad (5)$$



where WL = water loss (g water/g fresh product), SG = solute gain (g solutes/g fresh product),  $m_{s0}$  = fresh product weight (g),  $m_{vi}$  = impregnated product weight (g), and  $M_0$  and  $M_{vi}$  are the moisture fraction (wet basis) of fresh and impregnated products, respectively.

## 2.5. Colour characteristics

A colour-meter (Konica Minolta CR-400, Osaka, Japan) was used to measure the colour parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) of impregnated jicama and juice. The measurement was performed in reflectance mode on the CIELab\* scale, illuminating C, and with an observer angle of 2°.  $L^*$ ,  $a^*$  and  $b^*$  values were used to calculate the yellow index (YI), the total colour difference ( $\Delta E$ ), chroma ( $C^*$ ), and hue (Hue°) using eqn (6)–(9), respectively.<sup>30–32</sup>

$$YI = 90.909 \left( \frac{b^*}{L^*} \right) \quad (6)$$

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (7)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (8)$$

$$\text{Hue } (^\circ) = \tan^{-1} \left( \frac{b^*}{a^*} \right) \quad (9)$$

where  $L^*$ ,  $a^*$ , and  $b^*$  are the sample values.  $L_0^*$ ,  $a_0^*$  and  $b_0^*$  are the colour parameters for fresh jicama or carrot juice.

## 2.6. Total carotenoids

The methodology reported by Desobry *et al.*<sup>33</sup> with some modifications was used to determine total carotenoids.<sup>34</sup> For carotene extraction,  $2.50 \pm 0.14$  g of impregnated samples and 5 mL of acetone (99.5%) were homogenised with a mortar. The solution was then macerated in the dark for 10 min; the extracts were centrifuged for 10 min at  $3900 \times g$  at 5 °C and filtered through Whatman filter paper No. 4. Solvent extractions were performed until colour exhaustion was reached with a total volume of 35 mL acetone. The absorbance of extraction was read at 450 nm using a UV-visible spectrophotometer (Shimadzu, UV-1900i, Tokyo, Japan). Total carotenoids were calculated according to Beer's law described in eqn (10) and expressed as mg  $\beta$ -carotene/100 g product d.b.

$$\text{Abs} = \varepsilon \times C \times l \quad (10)$$

where Abs is the absorbance at 450 nm,  $\varepsilon$  is the molar extinction coefficient of  $\beta$ -carotene in acetone ( $134 \times 10^3$  L mol<sup>-1</sup> cm<sup>-1</sup>).

## 2.7. Rheological parameters

The apparent viscosity (in mPa s) of carrot juice at different concentrations (20–50 °Brix) and 40 °C was determined by the application of linearly increasing shear rate values using a Brookfield DV-III Ultra Programmable Rheometer (Brookfield Engineering Laboratories, Stoughton, M.A., USA) fitted with a No. 2 spindle. A volume of 10 mL of osmotic solution was added to the sample cup and maintained at  $40 \pm 0.2$  °C with

a recirculating water bath (PolyScience, ADO7R, USA). Shear rate values ranged from 2.4 to 40 s<sup>-1</sup>. The rheogram of fluid dynamic viscosity vs. the shear rate was fitted to the Ostwald de Waele power law model (viscosity =  $k[\text{shear rate}]^{n-1}$ , where  $k$  represents the consistency index and is an analogue of the apparent viscosity (Pa s<sup>n</sup>) to analyse the fluid behaviour; if  $n = 1$  the fluid was Newtonian,  $n > 1$  dilatant fluid or  $n < 1$  pseudoplastic.<sup>35</sup>

## 2.8. Statistical analysis

Response surface methodology (RSM) was applied to analyse the effect of immersion time ( $t$ ) and osmotic agent concentration ( $C$ ), since both variables modify the quality and mass transfer of foods impregnated with juice concentrate.<sup>8,36,37</sup> Experimental conditions were set up according to a  $3 \times 4$  factorial design. Independent variables were coded at each level according to eqn (11). Independent variables of the design (actual and coded) are listed in Table 1. A total of 12 experiments with their replicates were performed. Some parameters of impregnated jicama such as WL, SG,  $a_w$ , TSSs (°Brix), colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$  and  $\Delta E$ ), and total carotenoids were analysed.

$$x_i = \frac{W_i - W_c}{\Delta W_i}, \quad i = 1, 2, 3, \dots, j \quad (11)$$

where  $W_i$  = the coded value of the independent variable,  $x_i$  = the dimensionless value of  $W_i$ ,  $W_c = W_i$  at the central point and  $\Delta W_i$  = step change of the  $i$  variable.

Eqn (12) was used to fit each experimental value with a second-order polynomial model. ANOVA was used to analyse the statistical significance of the models. A statistical test was performed with the Minitab v.18 Statistical Software (Minitab Inc., State College, PA, USA). The “backward elimination procedure” of non-significant terms was used for the model fitting. A  $p \leq 0.05$  value was used to establish significant differences within means.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (12)$$

Table 1 Actual and coded values of the  $3 \times 4$  factorial design for jicama impregnation<sup>a</sup>

Run	Immersion time $t$ (min)	Carrot juice concentration $C$ (°Brix)
1	30 (0)	20 (−1)
2	30 (0)	30 (−0.333)
3	30 (0)	40 (0.333)
4	30 (0)	50 (1)
5	40 (1)	20 (−1)
6	40 (1)	30 (−0.333)
7	40 (1)	40 (0.333)
8	40 (1)	50 (1)
9	20 (−1)	20 (−1)
10	20 (−1)	30 (−0.333)
11	20 (−1)	40 (0.333)
12	20 (−1)	50 (1)

<sup>a</sup> Numbers in parenthesis are coded values.



The estimated responses  $Y$  were mass transfer (WL, SG and WR), physicochemical ( $a_w$  and TSS), colour ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $\Delta E^*$  and YI), and nutraceutical (TC) parameters.  $X_i$  ( $i = 1$ , immersion time ( $t$ , in min) and  $i = 2$ , osmotic agent concentration ( $C$ , in °Brix)) represents the independent variables;  $\beta_0$  is the intercept coefficient,  $\beta_1$ , and  $\beta_2$  are the linear coefficients;  $\beta_{11}$ , and  $\beta_{22}$  are the quadratic coefficients and  $\beta_{12}$  is the interaction coefficient.

A Pearson's correlation was established using Minitab v.18 Statistical Software to establish a relationship between TCs and  $\Delta E$ .

The TC impregnated in jicama was optimised by maximising this value. Finally, an impregnation experiment was conducted under the predicted process conditions to validate the obtained model.

### 3. Results and discussions

Tables 2 and 3 show the mean of experimental responses of mass transfer,  $a_w$ , total soluble solids, carotenoid impregnation in jicama, and colour parameters for the 12 runs of jicama

vacuum impregnation according to a  $3 \times 4$  factorial design in Table 1.

A second-order polynomial equation fitted to each response variable was used to analyse the significant effects of the process variables. Polynomial models obtained after eliminating the non-significant terms ( $p \geq 0.05$ ) are shown in Table 4. According to the ANOVA, all the non-coded models (except for solute gain, SG) were sufficiently accurate in predicting the responses and presented a non-significant lack of fit ( $p \geq 0.05$ ). The polynomial model equation properly fitted the experimental data, with coefficients of determination values of  $R^2 > 0.8$  for  $M_k$ , WR, WL,  $a_w$ , TSSs, TCs,  $\Delta E$ , and YI. On the other hand, the polynomial model equations for  $L^*$ ,  $a^*$  and  $b^*$  colour parameters have  $R^2 > 0.6$ , which indicates that they are not suitable for predicting these experimental values.

In general, the second-order polynomial equations showed that the parameters related to WR, WL,  $a_w$ , TSSs and TCs were significantly affected mainly by linear coefficients (time and concentration), quadratic coefficients of time and concentration, and the time–concentration interaction ( $p \leq 0.05$ ). The

**Table 2** Mass transfer parameters (weight reduction = WR, moisture fraction =  $M_k$ , water loss = WL, and solid gain = SG), water activity ( $a_w$ ), total soluble solids (TSSs), and total carotenoids (TCs) of impregnated jicama cylinders with solutions of different concentrations<sup>a</sup>

Run	$M_k$ (g g <sup>-1</sup> w.b.)	WR (g g <sup>-1</sup> )	WL (g g <sup>-1</sup> )	SG (g g <sup>-1</sup> )	TSSs (°Brix)	$a_w$	TCs (mg $\beta$ -carotene/100 g d.b.)
1	0.855 ± 0.001	0.276 ± 0.016	0.303 ± 0.014	0.027 ± 0.002	9.479 ± 0.199	0.977 ± 0.008	0.09 ± 0.007
2	0.796 ± 0.009	0.428 ± 0.009	0.467 ± 0.008	0.039 ± 0.002	15.289 ± 0.025	0.968 ± 0.002	0.17 ± 0.006
3	0.708 ± 0.004	0.570 ± 0.019	0.618 ± 0.013	0.047 ± 0.006	21.890 ± 0.41	0.955 ± 0.003	0.27 ± 0.001
4	0.700 ± 0.001	0.613 ± 0.016	0.651 ± 0.01	0.038 ± 0.005	18.808 ± 0.142	0.940 ± 0.009	0.29 ± 0.003
5	0.851 ± 0.003	0.167 ± 0.014	0.196 ± 0.012	0.029 ± 0.002	9.043 ± 0.200	0.983 ± 0.002	0.05 ± 0.004
6	0.819 ± 0.002	0.345 ± 0.019	0.369 ± 0.016	0.024 ± 0.004	11.402 ± 0.195	0.984 ± 0.004	0.14 ± 0.003
7	0.811 ± 0.004	0.433 ± 0.010	0.445 ± 0.008	0.013 ± 0.002	14.309 ± 0.201	0.967 ± 0.009	0.22 ± 0.006
8	0.763 ± 0.009	0.521 ± 0.014	0.539 ± 0.011	0.019 ± 0.003	15.493 ± 0.030	0.954 ± 0.003	0.20 ± 0.001
9	0.856 ± 0.008	0.124 ± 0.009	0.172 ± 0.008	0.048 ± 0.001	8.727 ± 0.055	0.978 ± 0.002	0.09 ± 0.007
10	0.831 ± 0.005	0.268 ± 0.030	0.314 ± 0.025	0.046 ± 0.005	9.139 ± 0.036	0.975 ± 0.004	0.11 ± 0.002
11	0.803 ± 0.002	0.346 ± 0.009	0.397 ± 0.007	0.051 ± 0.002	13.012 ± 0.001	0.966 ± 0.001	0.14 ± 0.001
12	0.788 ± 0.003	0.418 ± 0.010	0.463 ± 0.008	0.045 ± 0.002	13.451 ± 0.002	0.959 ± 0.009	0.19 ± 0.007

<sup>a</sup> Mean ± standard deviation,  $N = 3$ .

**Table 3** Colour parameters of impregnated jicama cylinders with solutions of different concentrations of concentrated carrot juice<sup>a</sup>

Run	$L^*$	$a^*$	$b^*$	$\Delta E$	YI
1	58.55 ± 1.25	2.54 ± 0.46	21.09 ± 5.02	19.40 ± 3.07	32.66 ± 7.24
2	60.35 ± 1.91	3.75 ± 0.80	22.45 ± 0.47	19.28 ± 1.67	33.85 ± 1.82
3	56.37 ± 0.73	4.83 ± 0.58	22.63 ± 0.98	22.19 ± 1.64	36.53 ± 2.17
4	57.28 ± 0.73	6.57 ± 0.21	27.20 ± 0.67	25.46 ± 0.26	43.17 ± 0.59
5	58.51 ± 1.90	4.54 ± 0.71	18.99 ± 0.37	18.21 ± 1.27	29.52 ± 0.43
6	56.24 ± 1.27	3.14 ± 0.65	18.89 ± 1.13	19.46 ± 1.72	30.56 ± 2.42
7	57.34 ± 1.50	3.39 ± 1.53	20.84 ± 2.14	20.08 ± 0.58	33.00 ± 2.55
8	57.77 ± 1.41	6.05 ± 0.88	26.11 ± 1.50	24.23 ± 1.04	41.08 ± 1.83
9	64.66 ± 0.77	1.83 ± 0.75	18.20 ± 1.28	13.06 ± 1.49	25.60 ± 1.99
10	61.41 ± 1.32	2.86 ± 0.08	22.00 ± 1.81	18.19 ± 0.70	32.54 ± 2.00
11	56.62 ± 3.94	4.53 ± 1.68	21.66 ± 3.12	21.58 ± 2.47	32.59 ± 0.08
12	55.35 ± 1.10	6.23 ± 0.45	24.87 ± 1.14	24.81 ± 0.35	40.84 ± 1.12

<sup>a</sup> Mean ± standard deviation,  $N = 3$ .  $\Delta E$  is the total colour difference, with respect to fresh jicama,  $L_0^* = 71.40 \pm 3.98$ ,  $a_0^* = -0.75 \pm 1.43$ , and  $b_0^* = 7.34 \pm 5.89$ . YI is the yellow index.





Table 4 Non-coded regression coefficients of second-order polynomial models<sup>a</sup>

Model	$R^2$	Lack of fit
$M_k = 1.353 - 0.031t - 0.0035C + 0.0005t^2$	0.846	0.35
$WR = -1.471 + 0.087t + 0.024C - 0.0014t^2 - 0.0002C^2 + 9.6 \times 10^{-5}tC$	0.986	0.28
$WL = -1.453 + 0.088t + 0.025C - 0.0015t^2 - 0.0002C^2 + 7.6 \times 10^{-5}tC$	0.984	0.43
$SG = 0.075 - 0.001t$	0.733	ND
$a_{wM} = 1.043 - 0.0055t + 0.001C + 0.0001t^2 - 0.00002C^2 - 2 \times 10^{-5}tC$	0.798	0.23
$a_{wAO} = 1.045 - 0.0007t + 0.003C + 2 \times 10^{-5}tC$	0.997	0.11
$TSSs = -44.70 + 2.801t + 0.835C - 0.0455t^2 - 0.00836C^2 + 1 \times 10^{-9}tC$	0.856	0.58
$TCs = -0.58 + 0.034t + 0.01C - 6 \times 10^{-4}t^2 - 0.0001C^2 + 1 \times 10^{-4}tC$	0.891	0.33
$L^* = 82.89 - 0.655t - 0.613C + 0.0158tC$	0.620	0.16
$a^* = -0.74 + 0.198t - 0.047C + 0.0044C^2 - 0.0051tC$	0.730	0.18
$b^* = -0.74 + 1.250t + 0.508C - 0.0152t^2 + 0.0085tC$	0.600	0.28
$\Delta E = -10.99 + 1.250t + 0.508C - 0.0152t^2 + 0.0085tC$	0.807	0.19
$YI = -5.16 + 1.848t + 0.397C - 0.0307t^2$	0.799	0.26

<sup>a</sup> Parameters in bold have a significant ( $p \leq 0.05$ ) effect on response.  $M_k$ , moisture fraction ( $\text{g g}^{-1}$ ); WR, weight reduction ( $\text{g g}^{-1}$ ); WL, water loss ( $\text{g g}^{-1}$ ); SG, solute gain ( $\text{g g}^{-1}$ );  $a_{wM}$  water activity of the sample;  $a_{wAO}$  water activity of osmotic solution; TSSs, total soluble solids ( $^{\circ}\text{Brix}$ ) and TCs, total carotenoids ( $\text{mg } \beta\text{-carotene}/100 \text{ g d.b.}$ );  $L^*$ , lightness;  $a^*$ , red to green;  $b^*$ , yellow to blue;  $\Delta E$ , total colour difference with respect to fresh jicama; YI, yellow index. ND: not determined.

models for  $b^*$  and  $\Delta E$  were not significantly affected ( $p \geq 0.05$ ) by quadratic coefficients of concentration. In the case of the model for  $a^*$ , the quadratic coefficients of time had no significant effect ( $p \geq 0.05$ ). On the other hand,  $M_k$  and YI were not significantly affected ( $p \geq 0.05$ ) by quadratic coefficients of concentration of the osmotic agent or the time–concentration interaction. SG was only affected by the time of the process.

### 3.1. Influence of process variables on water activity and mass transfer parameters

The pH of the impregnated samples does not show significant differences ( $p > 0.05$ ) between each experimental condition ( $\text{pH} = 6.00 \pm 0.02$ ). The  $a_w$  value of fresh jicama was  $0.982 \pm 0.005$  ( $25.01 \pm 0.10 ^{\circ}\text{C}$ ). Initial  $a_w$  values of osmotic solutions were  $0.972 \pm 0.003$ ,  $0.960 \pm 0.004$ ,  $0.940 \pm 0.006$ , and  $0.912 \pm 0.002$ , for 20, 30, 40, and 50  $^{\circ}\text{Brix}$  concentrations, respectively. After the process, the impregnated samples achieve an  $a_{wM}$  value between 0.933 and 0.987, corresponding to a percentage variation of around 5%. The relationship between the jicama WL and the water gain in the osmotic solution was determined by analysing the effects of time and concentration of the osmotic agent. The experimental data were fitted to a second-order polynomial equation for each  $a_w$ . For both models, the linear and interaction coefficients of  $t$ – $C$  were significant ( $p \leq 0.05$ ). The model allowed for the observation that when the  $a_{wM}$  decreases, the  $a_{wAO}$  increases.

Fig. 1 shows the effect of the  $t$ – $C$  interaction on  $a_{wM}$ . As shown, the higher the initial concentration of the osmotic agent, the lower the  $a_{wM}$  value that is reached. Some authors relate this behaviour to the osmotic pressure caused by the concentration gradient between the impregnated sample and the osmotic solution; the higher the gradient, the greater the mass transfer.<sup>10</sup> Xie & Zhao<sup>38</sup> found that during the impregnation of Royal Gala apples with corn syrup solutions, solutions at 50% reduced the final  $a_w$  ( $a_{w,\text{sample}} = 0.97$ ) more than those at 20% ( $a_{w,\text{sample}} = 0.98$ ). It is important to note that in some

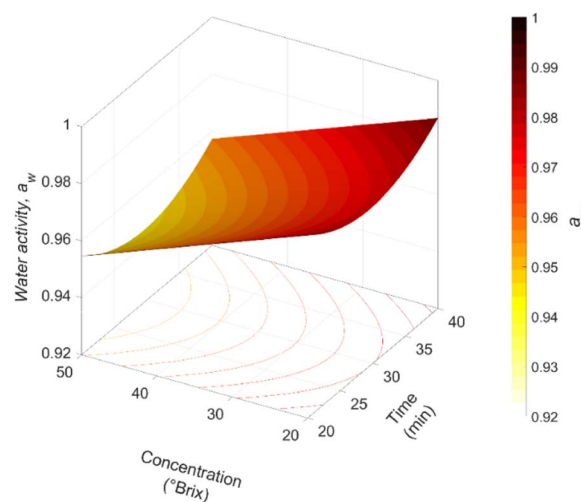


Fig. 1 Time–concentration interaction ( $t$ – $C$ ) effect on water activity ( $a_w = 1.043 - 0.0055t + 0.001C + 0.0001t^2 - 0.00002C^2 - 0.00002tC$ ) of jicama cylinders impregnated with carrot juice concentrate.

osmodehydrated and impregnated products, the  $a_w$  may remain high, so these techniques are used as pre-treatments for a following drying step that reduces the  $a_w$  ( $a_w < 0.6$ ) to have microbiologically stable products.

On the other hand, increasing the immersion time improves the reduction of  $a_{wM}$ . However, at processing times longer than 30 min, an increase in the  $a_{wM}$  is observed. This is because the longer the vacuum pressure was applied, the more water was lost from the jicama. This water is diluted more slowly in the juice because the system had no mechanical force to homogenise the system concentration. Therefore, when the system pressure is restored, most of the previously removed water is returned to the sample along with the osmotic solution. Since the water reincorporated into the food matrix was not as tightly bound as before, the water activity increased with respect to the



fresh product. This behaviour was similar to that reported by Castagnini *et al.*;<sup>19</sup> these authors found that the  $a_w$  of apples vacuum impregnated with cranberry juice did not show significant differences with respect to the fresh product despite having reached a moisture reduction of more than 2%. Also, the literature reports an increase in moisture compared to fresh samples during vacuum impregnation of mangoes with grape residue solution,<sup>15</sup> apple slices with quercetin solutions,<sup>39</sup> and pineapples with calcium solutions.<sup>40</sup> Such a result can be due to the hydrodynamic mechanism induced by the pressure drop.

The moisture fraction of the fresh sample was  $0.921 \pm 0.001 \text{ g g}^{-1}$ , and after the impregnation treatment, this value was reduced between 7 and 24% (Table 2). The obtained mass transfer parameters for water loss and solid gain ranged between  $0.172\text{--}0.65 \text{ g g}^{-1}$ , and  $0.013\text{--}0.51 \text{ g g}^{-1}$ , respectively. It can be observed that the water transfer rate was higher than the solute transfer rate. The gain of solutes was similar to that reported during the impregnation of apples with Victoria grape juice concentrate and watermelons with sucrose solutions.<sup>16,41</sup>

Fig. 2 shows the effect of the  $t$ - $C$  interaction on the mass transfer parameters obtained with the models in Table 4. As noticed, both WL and WR increase when the concentration of the osmotic agent increases. Similar behaviour is observed at processing times lower than 30 min. However, at higher times, a lower WL and WR are achieved. González-Pérez *et al.*<sup>16</sup> described a similar behaviour in SG; this occurred mainly because a solute layer was formed on the external part of the food matrix, which limited mass transfer. In the case of jicama, a solute layer was not formed, but a water barrier was formed on the product's surface, limiting the permeation of the osmotic solution. In addition, some studies have found that matrices with low real porosities (such as fresh mangoes,  $\epsilon_r = 0.01$ , and melons  $\epsilon_r = 0.07$ ) showed a reduction in solute transfer during vacuum impregnation with concentrated sucrose solutions, since it is more difficult to introduce a viscous osmotic solution into small pores<sup>17,42</sup> as those of jicama ( $\epsilon_r = 0.070 \pm 0.004$ ).

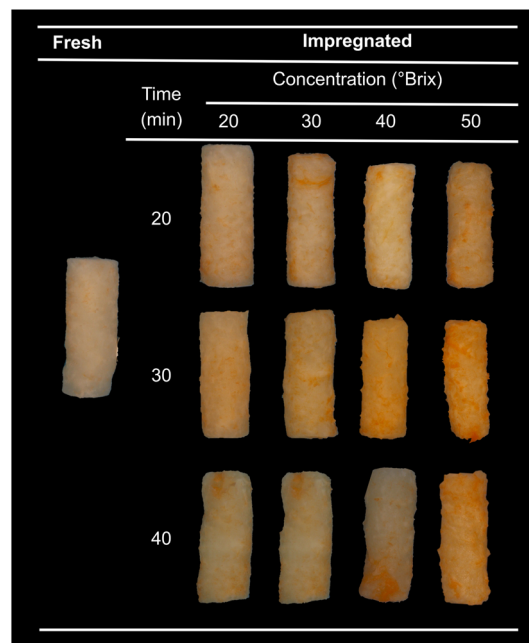


Fig. 3 General appearance of fresh and vacuum-impregnated jicama cylinders with carrot juice concentrate.

### 3.2. Influence of process variables on colour characteristics

A comparison of the general appearance and colour parameters of fresh and impregnated jicama with carrot juice concentrate is shown in Fig. 3 and Table 3. All the impregnated samples presented significant changes ( $p < 0.05$ ) in the total colour difference ( $\Delta E$ ), taking the fresh sample as a reference. The  $\Delta E$  of the impregnated samples was due to the colour of the osmotic solution. The total colour differences between the impregnated sample and the corresponding osmotic solutions were  $24.02\text{--}33.86$ ,  $26.4\text{--}31.15$ ,  $23.61\text{--}29.66$ , and  $18.72\text{--}20.92$  for 20 °Brix ( $L_0^* = 47.66 \pm 1.49$ ,  $a_0^* = 18.77 \pm 1.22$ , and  $b_0^* = 43.19 \pm 2.33$ ), 30 °

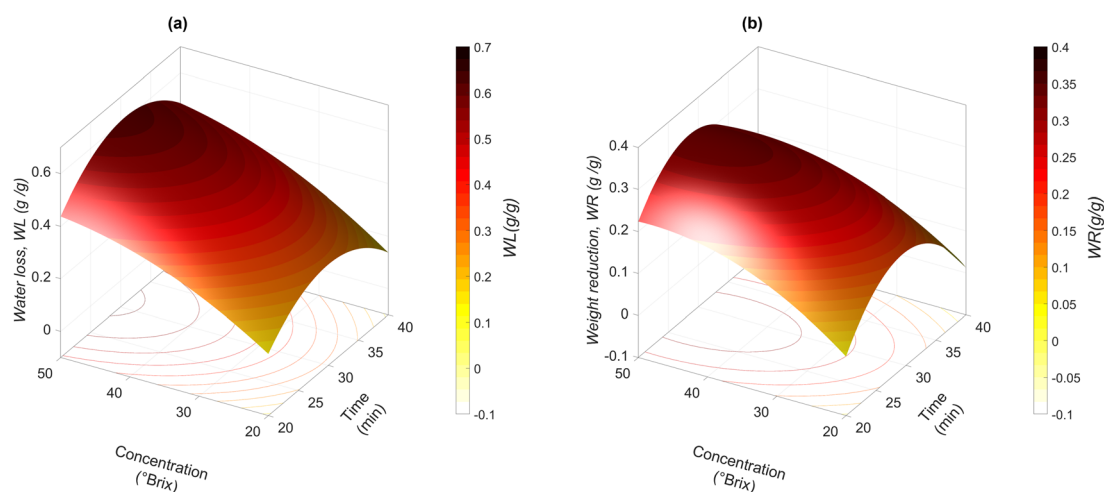


Fig. 2 Time-concentration interaction ( $t$ - $C$ ) effect on (a) water loss ( $WL = -1.453 + 0.088t + 0.025C - 0.0015t^2 - 0.00024C^2 + 0.000076tC$ ) and (b) weight reduction ( $WR = -1.471 + 0.087t + 0.024C - 0.0014t^2 - 0.0002C^2 + 0.000096tC$ ) of jicama cylinders impregnated with carrot juice concentrate.



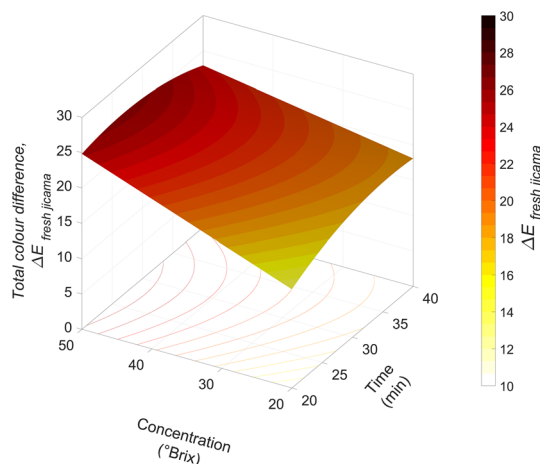


Fig. 4 Time–concentration interaction ( $t$ – $C$ ) effect on the total colour difference (with respect to fresh jicama,  $\Delta E = -10.99 + 1.250t + 0.508C - 0.0152t^2 + 0.0085tC$ ) of impregnated jicama using carrot juice concentrate.

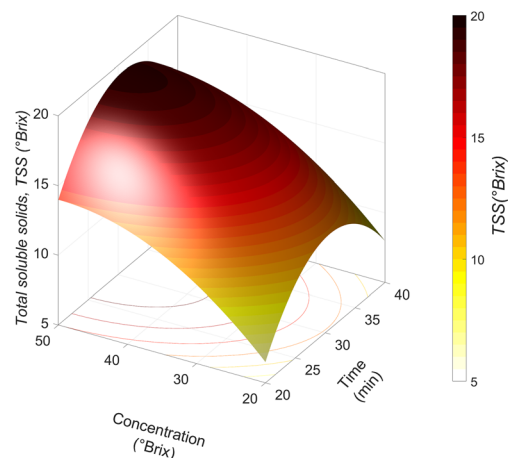


Fig. 5 Time–concentration interaction ( $t$ – $C$ ) effect on total soluble solids ( $TSSs = -44.70 + 2.801t + 0.835C - 0.04545t^2 - 0.00836C^2 + 0.0008tC$ ) of jicama cylinders impregnated with carrot juice concentrate.

Brix ( $L_0^* = 46.92 \pm 0.49$ ,  $a_0^* = 21.13 \pm 0.31$ , and  $b_0^* = 42.79 \pm 0.20$ ), 40 °Brix ( $L_0^* = 45.34 \pm 0.05$ ,  $a_0^* = 21.42 \pm 0.06$ , and  $b_0^* = 41.54 \pm 0.06$ ), and 50 °Brix ( $L_0^* = 44.52 \pm 0.83$ ,  $a_0^* = 20.13 \pm 1.09$ , and  $b_0^* = 39.23 \pm 0.28$ ), respectively. Therefore, the smaller the difference, the closer the colour was to that of the juice.

Fig. 4 shows the effect of the  $t$ – $C$  interaction on the colour variation. The total colour variation increased with an increasing concentration, showing a significant ( $p < 0.05$ ) increase at 30 min of processing. A significant ( $p < 0.05$ ) reduction of the mass transfer parameters was observed at longer times. This behaviour was similar to that reported during vacuum impregnation ( $P = 500$  mm Hg and  $t = 7$  min) of lycopene :  $\beta$ -carotene solution (ratio of 50 : 50, 40 : 60 and 25 : 75) in bananas, mangoes, peaches and papayas. These showed that colour variation increased when the  $\beta$ -carotene concentration increased.<sup>43</sup> The  $\Delta E$  was similar to the behaviour of mass transfer parameters (WL and WR). Some authors relate the impregnated sample's mass transfer parameters with the osmotic solution's colour parameters; the higher the WL or SG values, the greater the  $\Delta E$ .<sup>43,44</sup>

The YI of the carrot juice concentrate solutions was between 82.38 and 83.9. This value significantly increased as the juice concentration increased, indicating an increment in the carotenoids' concentration, which translated into the yellow colour intensity.<sup>43,45</sup> The impregnated samples had YI values of 25.6–43.17. According to the YI model in Table 4, the YI value increases significantly ( $p < 0.05$ ) when increasing the concentration and is related to the amount of the impregnated osmotic solution.

### 3.3. Influence of process variables on total soluble solids

TSSs of fresh jicama were  $6.6 \pm 0.3$  °Brix. After the impregnation process, the food matrix increased the amount of TSS by between 32 and 232% (8.72 and 21.89 °Brix).

According to the model in Table 4 and Fig. 5, increasing the osmotic solution concentration favours a TSS increment in the food matrix. However, at concentrations close to 50 °Brix of

carrot juice, a slight decrease in TSSs is observed; during the vacuum stage, the jicama loses a greater amount of water due to the concentration gradient (between the food matrix and the osmotic solution), which increases the osmotic pressure of the system. Likewise, as mentioned above, all the water removed forms a barrier on the food matrix; for low water content, this barrier can be dissolved with the juice, but as more water is eliminated at longer times, it becomes insufficient to dissolve it completely. Therefore, when atmospheric pressure is restored, the water that failed to dilute the juice and the juice diluted with part of the removed water, are impregnated into the jicama tissue, resulting in a product with fewer TSSs (compared to the experiments not affected by a significant dilution of the osmotic solution). This behaviour depends on the type of osmotic agent since it has been found that in grape juice concentrate and fructose corn syrup, the increase in TSS incorporation by vacuum is favoured by increasing the time and concentration of the osmotic agent.<sup>16,38</sup> However, our findings indicate that at high concentrations of the osmotic agent, TSS impregnation began to decrease due to the viscosity of the osmotic agent.

In this work at concentrations of 20 and 30 °Brix of carrot juice, the solutions behave as Newtonian fluids, keeping the fluid viscosity during the whole process at  $28.32 \pm 0.46$  and  $45.12 \pm 0.104$  mPa s, respectively. However, at concentrations of 40 and 50 °Brix, the osmotic solutions behaved as dilatant fluids ( $n > 1$  power law) with viscosity variations of 76.64–78.56 and 142–146.2 mPa s, respectively. These results would explain why the incorporation of the osmotic solution decreases when the atmospheric pressure is re-established since a dilatant fluid increases its viscosity as the strain rate increases (caused by force generated when the pressure is re-established).

### 3.4. Influence of process variables on total carotenoids

Impregnated jicama TCs were 0.05–29 mg  $\beta$ -carotene/100 g d.b. Carrot juice concentrate had 154.66 mg  $\beta$ -carotene/100 g d.b. The average recommended daily carotenoid intake



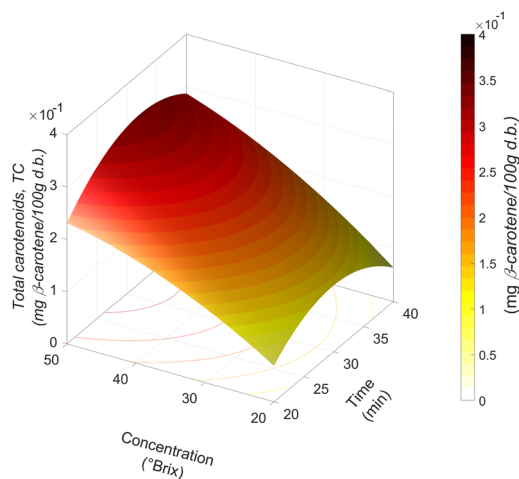


Fig. 6 Time–concentration interaction ( $t$ – $C$ ) effect on total carotenoids (TCs =  $-0.58 + 0.034t + 0.01C - 6 \times 10^{-4}t^2 - 0.0001C^2 + 1 \times 10^{-4}tC$ ) of jicama cylinders impregnated with carrot juice concentrate.

requirements for adults worldwide vary from 0.81 to 22 mg  $\beta$ -carotene per day.<sup>46,47</sup> Therefore, under the maximum impregnation conditions, a portion of 100 g of the obtained product may contribute a considerable percentage to the minimum daily requirement of  $\beta$ -carotene.

According to the polynomial model for predicting TCs (Fig. 6), the effect of  $t$ – $C$  interaction is remarkably similar to that of TSS impregnation since the higher the concentration of the osmotic solution, the higher the amount of TCs in the food matrix. Likewise, a reduction of TC impregnation is observed at times higher than 30 min. In most of the studies on the impregnation of  $\beta$ -carotene in fruits, TCs increased with an increasing solution concentration, as in the vacuum impregnation of bananas, papayas, peaches and mangoes with solutions of  $\beta$ -carotene and lutein in water or apples in sucrose solutions with  $\beta$ -carotene powder.<sup>21,43</sup>

### 3.5. Model validation

Optimisation of the impregnation process was carried out by maximising the TCs or  $\Delta E$ . In each case, the same optimum process conditions were found. To validate this, a Pearson's

correlation was used to analyse the existence of a relationship between the two. The analysis revealed a strongly ( $r^2 = 0.6645$ ) significant positive correlation ( $p < 0.01$ ). This result establishes that higher values of TCs lead to higher  $\Delta E$ , indicating higher juice incorporation.

Impregnation experiments were conducted under the optimum process conditions for model validation. The optimum process parameters were at  $t = 31$  min and  $C = 50^\circ$  Brix carrot juice. The experimental values (mean of the three experiments) are shown in Table 5. It was observed that the estimated values of each response are consistent with the fitted data. Therefore, the models are adequate to assess the behaviour of the impregnation process. The amount of TCs reached provides  $\sim 30\%$  of the minimum recommended percentage of daily carotenoid intake requirements for adults.<sup>46</sup> The  $a_w$  value of impregnated tissue under optimum conditions is very high. This excess water must be removed by a drying process to avoid spoilage and prolong its shelf life. Some studies have analysed the shelf life of vacuum-impregnated products, such as melons with calcium lactate. However, microbiological deterioration of the product was observed after 5 days.<sup>48</sup> On the other hand, the use of drying after an impregnation process increases the shelf life of kiwifruit vacuum impregnated with  $60^\circ$  Brix sucrose solutions up to 9 months.<sup>49</sup> Thus, the vacuum impregnation process is suitable as pretreatment to fortify the food matrix tissue with some solutes of interest, but it requires a drying step.

The colour parameters for the sample under the optimum conditions were  $L^* = 56.44 \pm 0.71$ ,  $a^* = 6.39 \pm 0.18$ , and  $b^* = 26.47 \pm 0.52$ ; these correspond to values of yellow/slightly orangey ( $\text{Hue}^\circ = 76.4 \pm 0.12$  and  $\text{Chrome} = 27.2 \pm 0.7$ ).  $\text{Hue}^\circ$  values are like those reported for orange sweet potatoes.<sup>31</sup>

Some authors have reported that some carotenoid-rich fruits and vegetables have yellow and orange flesh.<sup>32,33</sup> Compared with the fresh sample, all chromatids had a change giving a net colour difference  $\Delta E_{\text{jicama}} = 25.29 \pm 0.25$  ( $L_0^* = 71.4 \pm 3.9$ ,  $a_0^* = -0.746 \pm 0.2$ ,  $b_0^* = 7.34 \pm 5.89$ ,  $\text{Hue}_0^\circ = 98.8 \pm 0.04$ , and  $\text{Chrome}_0 = 11.0 \pm 0.02$ ). The colour of the impregnated sample was mainly due to the colour of the osmotic agent because the net colour difference between the impregnated sample and  $50^\circ$  Brix juice was less than that of the fresh jicama  $18.58 \pm 0.24$

Table 5 Predicted and experimental values of response under optimum process conditions ( $t = 31$  min and  $50^\circ$  Brix) for vacuum impregnation of jicama with carrot juice

Response	Predicted value (CI 95%)	Experimental value
$M_k$ (g g <sup>-1</sup> w.b.)	0.7133 (0.698/0.728)	0.710 $\pm$ 0.0001
$a_{wM}$	0.943 (0.939/0.948)	0.941 $\pm$ 0.002
WL (g g <sup>-1</sup> )	0.650 (0.634/0.694)	0.661 $\pm$ 0.010
WR (g g <sup>-1</sup> )	0.617 (0.602/0.632)	0.616 $\pm$ 0.011
TSSs ( $^\circ$ Brix)	19.2 (17.9/20.6)	19.00 $\pm$ 0.02
TCs (mg $\beta$ -carotene/100 g d.b.)	0.269 (0.248/0.290)	0.271 $\pm$ 0.007
$L^*$	56.423 (55.327/57.520)	56.44 $\pm$ 0.71
$a^*$	6.211 (5.600/6.822)	6.39 $\pm$ 0.18
$b^*$	26.376 (24.787/27.964)	26.47 $\pm$ 0.52
$\Delta E$	25.389 (24.142/26.637)	25.32 $\pm$ 0.26
YI	42.490 (40.264/44.716)	42.63 $\pm$ 0.30





(juice chromatids showed  $L_0^* = 77.3 \pm 0.02$ ,  $a_0^* = -2.52 \pm 0.18$ ,  $b_0^* = 7.75 \pm 0.35$ , Hue° =  $64.4 \pm 0.20$ , and Chrome =  $41.8 \pm 1.1$ ).

## 4. Conclusions

In conclusion, the overall findings demonstrate the potential of vacuum impregnation to incorporate bioactive compounds, such as carotenoids, into jicama effectively. The increase of the osmotic agent concentration favoured the increase of mass transfer parameters (WL and WR), the reduction of  $a_w$ , and the impregnation of TSSs,  $\Delta E$ , YI, and TCs. At long immersion times, the water removed during the vacuum pressure stage reduced the juice impregnation during the pressure build-up stage, which increased the  $a_{wM}$  and  $M_k$  and reduced the WL, WR, TSS,  $\Delta E$ , YI, and TC values. Optimisation of the impregnation process parameters using response surface methodology to maximise TCs or  $\Delta E$  resulted in finding optimal processing conditions. These findings highlight the potential of vacuum impregnation and response surface methodology in improving the nutritional quality of jicama and other food products. It is recommended for future studies to perform a drying treatment to reduce the  $a_w$  of a product in order to increase its shelf life.

## Author contributions

Julio E. González-Pérez: conceptualization, data curation, formal analysis, investigation, writing – original draft; Oscar Jiménez-González: writing – review & editing, methodology; Nelly Ramírez-Corona: formal analysis, writing – review & editing; Aurelio López-Malo: conceptualization, resources, writing – review & editing.

## Conflicts of interest

No conflict of interest related to this work.

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