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Original article

Optimisation of minimal processing variables to preserve the functional quality and colour of carrot juice by means of the response surface methodology

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Summary Combined processes based on acidification (pH: 4.5, 5.0, 5.5) and mild thermal treatments (*T*: 56, 58, 60°C) at different exposure times (*t*: 2, 4, 6 min) were optimised using the response surface methodology to improve the functional quality of carrot juice. The effects on α- and β-carotenes, total antioxidant activity (TAA) and colour parameters were assessed. All combinations exhibited higher α- and β-carotenes than untreated juice due to an increase on the extractability during processing. *T* was the most influential factor increasing carotenes as *T* increased. Conversely, TAA was more affected by pH. The maximum TAA was observed at pH 4.5 at 56°C. Moreover, samples with the lowest pH were the most luminous with highest a* and b*. The combination of pH 4.5 at 60°C, 4 min simultaneously showed high carotenes and TAA, resulting a good alternative to improve the functional quality and colour of carrot juice.

Keywords Antioxidant activity, carotenoids, functional properties, minimal processing.

Introduction

Carrots are a unique vegetable crop rich in most of the natural antioxidants including carotenoids, phenolics, vitamin C and tocopherol (Sharma *et al*., 2012). The α- and β-carotenes are precursors for vitamin A (retinol), required for a variety of biological processes including vision health, cell development, immune system and reproductive health (Ross, 2016). Moreover, the antioxidant power of β-carotene protects against the free radicals by acting as a strong quencher of singlet molecular oxygen and peroxyl radical scavenger. Carotenes have been implicated in the inhibition of cancer cells in animal models and in human, and they also have a role enhancing the immune function (Tapiero *et al*., 2004).

Carrot juice is a low-acid food (pH 6.0–6.5) that requires severe heat treatment (115–121°C) for protection from food spoilage; however, conventional heating treatments could result in poor sensory and nutritional quality. Thermal sterilisation has been demonstrated to cause partial conversion of the all-trans-β-carotenes to their cis-isomers which have different chemical and biological properties, thus differing in their antioxidant activity and bioavailability (Marx *et al*., 2003). Therefore, a good alternative is the application of minimal preservation processes based on the hurdle concept, which intelligently combines multiple preservation factors, reducing the intensity of each hurdle and optimising of the overall food quality (Leistner, 2000).

Despite the fact that pasteurisation using emerging technologies such as high hydrostatic pressure achieves high nutritional retention, its implementation involves high costs in equipment and installation (Fernandes, 2012). Therefore, it is demanding to find pasteurisation strategies more suitable for small and medium enterprises.

The aim of this study was to apply minimal processing to improve the functional quality of carrot juice using conventional technologies that could be afforded by small juice producers. In this sense, the impact of some combinations of mild thermal treatments (56, 58 and 60°C) at different exposure times (2, 4 and 6 min) combined with acidification (pH: 4.5, 5.0 and 5.5) was assessed on carotenoids, antioxidant activity and colour of carrot juice. The minimal processing variables were optimised using the response surface methodology.

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Materials and methods

Juice extraction

Carrots (*Daucus carota*) were purchased from a local market (Santiago, Chile) and sanitised by immersion in a chlorine solution (sodium hypochlorite 100 mg L^{-1}) for 5 min. Once rinsed in water and superficially dried in air (using a laminar flow cabinet for 10 min), the top, bottom and any dirt spot of the carrots were cut and discharged. The juice (pH 6.4, 11.3 ± 0.4°Brix) was extracted with a semi-industrial juicer (ZJ 145 CTA, Panyu, China) and immediately processed.

Combined treatments

Mild thermal treatments combined with acidification of carrot juice were carried out in a batch type bench scale system. Firstly, the pH of fresh extracted carrot juice (100 mL) was adjusted (pH 4.5, 5.0 or 5.5) with citric acid 30%p/v (Merck, Darmstadt, Germany). Then, the mild thermal treatment was conducted as reported by Char *et al*. (2009) using a glass jacketed vessel connected to a thermostatically controlled water bath with recirculation (JeioTech, Seoul, South Korea) which temperature was fixed to attain 56, 58 or 60 ± 0.1°C in the juice. The system was continually agitated with a magnetic stirrer, and the temperature was monitored by thermocouples and a data logger (Cole Palmer, Chicago, IL). The time needed to reach 57°C or 60°C in the juice was 2–2.5 min or 3.0–3.5 min, respectively. When the juice reached the desired temperature, the count of treatment time began (2, 4 and 6 min). After the treatment, the juice was immediately cooled in an ice bath, dispensed in dark Eppendorf tubes and stored at −18°C until used for the physical and chemical determinations.

Physical and chemical determinations

α- and β-carotene contents

The determination of α- and β-carotenes (α-C and β-C) was conducted using a chromatographic method following the methodology proposed by Wright & Kader (1997) with some modifications. Briefly, triplicates of each sample were extracted in a mix of hexane and methanol (1:1). After agitation and centrifugation (730 ***g***, 10 min, 4°C), the hexane phase was taken and the solvent was evaporated to dryness under nitrogen. The procedure was repeated three times. All the steps were performed under dimmed light. Identification and quantification of carotenoids was performed in a chromatographer equipped with DAD (Merck-Hitachi, L- 7455, Tokyo, Japan) and a C18 (3.9 × 300 mm) column (Nova-Pak, Waters, Milford, MA). The extracts were dissolved in 2 mL hexane for injection (20 μL). The mobile phase consisted of acetonitrile, methanol and methylene chloride 75:20:5, containing 0.1% butylated hydroxytoluene (PHT) and 0.05% trimethylamine. The flow rate was 1.5 mL min^{-1}, and the detection was performed at 450 nm. All reagents were HPLC grade and were purchased from Merck (Darmstadt, Germany). The standards were purchased from Sigma-Aldrich (Saint Louis, MO).

Total antioxidant activity

The antioxidant activity (TAA) was evaluated through a colorimetric method based on the free radical scavenging capacity of the sample evaluated with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical (Sigma-Aldrich, Saint Louis, MO) according to the methodology proposed by Gutiérrez *et al*. (2015). The calibration curve was obtained using Trolox (Sigma-Aldrich, Saint Louis, MO) as a standard, and the results were expressed as Trolox equivalents (μg TEq mL^{-1}).

Colour measurement

Colour of carrot juice was measured using a tristimulus colorimeter (Minolta CR-300, Ramsey, NJ, USA), with an 8 mm diameter of viewing aperture, D65 illuminant and 0° observer angle, previously calibrated in the CIE Lab colour space. Samples (5 mL) were placed in glass dishes (50 mm diameter) with black walls and filled up to 6 mm height. Colour parameters L* (lightness), a* (green–red component) and b* (blue–yellow component) values were recorded with white and black background. The hue angle, h: [$\tan^{-1}$ (b*/a*)], and chroma, C: [$(a^{*2} + b^{*2})^{1/2}$], were also calculated. Opacity, Op: L* (black background)/L* (white background), was also calculated to elucidate how the treatments affected the translucency of carrot juice.

Experimental design

Juice was processed by combinations of acidification with mild thermal treatments. Three levels of each independent variable (pH; temperature, *T*; and processing time, *t*) were chosen (Table S1). The treatment combinations were selected using a Box–Behnken type experimental design resulting in fifteen combined treatments, including three central points. Additionally, the untreated, pH, temperature and time controls were assessed. Response surface methodology (RSM) was used to study the effect of each independent variable on α- and β-carotene and TTA of treated carrot juice.

Statistical analysis

Results were expressed as mean ± standard deviation of three independent determinations. Analysis of

variance was performed for each polynomic equation corresponding to the response variables (α-carotene, β-carotene and TAA) ($P < 0.05$). The adjusted coefficient of determination (R^2_{adj}), Fisher's test and the lack of fit test were used to determine whether the selected polynomials were adequate to describe the observed data. Multivariate analysis of variance (MANOVA) was applied to detect differences in the colour parameters between juice samples. A principal component analysis (PCA) was applied to correlate the α-carotene, β-carotene, TAA and colour responses. The cophenetic correlation coefficient (CCC) was obtained as a measure of how faithfully the analysis preserves in the new space the original euclidean distances among data points. These analyses were conducted using the softwares Statgraphics Centurion XV.II® (StatPoint Technologies Inc., Warrenton, VA, USA) and InfoStat 2009 (InfoStat Group, Córdoba, Argentina).

Results and discussion

The physical and chemical characterisation of the raw carrot juice (untreated control) is presented in Table S2. The impact of different processing combinations was assessed on the main carotenoids with provitamin A activity (α-C and β-C), the total antioxidant activity and colour parameters of carrot juice.

α- and β-carotene contents

Untreated carrot juice (raw juice) exhibited 28.4 and 54.3 mg L^{-1} of α- and β-carotenes, respectively (Table S2), whereas combined treatments exhibited ranges from 26.4 to 47.8 and from 47.1 to 82.9 mg L^{-1} for α-C and β-C, respectively (data not shown). Therefore, an increase in the extractability of these compounds occurred during processing of carrot juice increasing the amount of carotenoids that were released from the food matrix, and thus, left accessible for reaction. These results are in agreement with numerous studies that have confirmed that mild thermal processing (50–90°C for few minutes) improved β-carotene extractability and bioaccessibility in carrots, carrot juice and purée compared to the untreated carrot product (Hiranvarachat *et al*., 2012; Zaccari *et al*., 2015). These studies also reported that mild thermal treatments combined with low intensities of other preservation factors increased the carotene content while avoiding the undesirable effects of high temperatures (e.g. texture defects, loss of flavour, off-flavours, loss of bioactive compounds and colour changes).

The effect of each independent variable of the combined treatments on carotene content was expressed by means of a second-order polynomial equation (Table 1). According to the *P*-values, processing temperature was the most significant variable influencing α-C and β-C contents ($P < 0.001$), followed by pH (Table 1). The proposed model was adequate to describe experimental data as satisfactory values of R^2_{adj} were obtained (85.4% and 90.1%). The α-C and β-C contents exhibited similar shape of surface response plots; therefore, only one of them (β-carotene content as influenced by T and pH at fixed times) is illustrated in Fig. 1. The increase in heat treatment temperature from 56 to 60°C enhanced carotene concentration, mostly at lower treating times (2–4 min). The pH axis exhibited a curvature due to the presence of the quadratic term showing higher carotene concentrations at pH 5.0. The maximum β-C and α-C contents (82.9 and 47.8 mg L^{-1}, respectively) were recorded at the highest T (60°C) for 2 min and pH 5.0. Conversely, when treatment time increased to 6 min, the temperature effect was reverted; thus, maximum carotene content was estimated at 56.8°C and pH 5.3 (Fig. 1).

These results are in agreement with those observed by Fikselová *et al*. (2008) who studied the influence of temperature conditions for β-carotene extraction from carrot. They observed that an increment of temperature up to 60°C positively influenced the extraction yield of carotenes. Moreover, Ma *et al*. (2015) also found that blanching treatments (with addition of 0.25% ascorbic acid at 86°C for 10 min) promoted the dissolution and stability of carotenoids (α-C, β-C and lutein) in carrot juice; conversely, sterilisation at higher temperatures (85, 100 and 121°C) increased the loss of carotenoids.

Carotenoids are released from the food material mainly because of cell disruption, which occurs during food preparation, processing and mastication.

Table 1 Analysis of variance and regression coefficients of the polynomial equation for each response variable: α- and β-carotene (α-C, β-C) and total antioxidant activity (TAA)

Model	β-C	α-C	TAA
Intercept	73.25 ± 1.88****	44.06 ± 0.96****	64.96 ± 1.07****
pH	3.13 ± 1.38*	2.99 ± 0.71**	−4.48 ± 1.00**
T	8.47 ± 1.38***	4.43 ± 0.71***	−3.56 ± 1.00**
t	−1.89 ± 1.38*	−0.92 ± 0.71**	2.39 ± 1.00*
pH^2	−9.17 ± 2.03**	−6.03 ± 1.04***	13.47 ± 1.46****
T^2	–	–	–
t^2	−4.83 ± 2.03*	−3.96 ± 1.04**	–
pH × T	−7.16 ± 1.95**	−4.35 ± 1.00**	1.90 ± 1.41*
pH × t	–	–	–
T × t	−4.75 ± 1.95*	−3.25 ± 1.00*	–
R^2_{adj}	85.36	90.06	89.56

****Significant at 0.01% level.
***significant at 0.1% level.
**significant at 1% level.
*significant at 5% level.

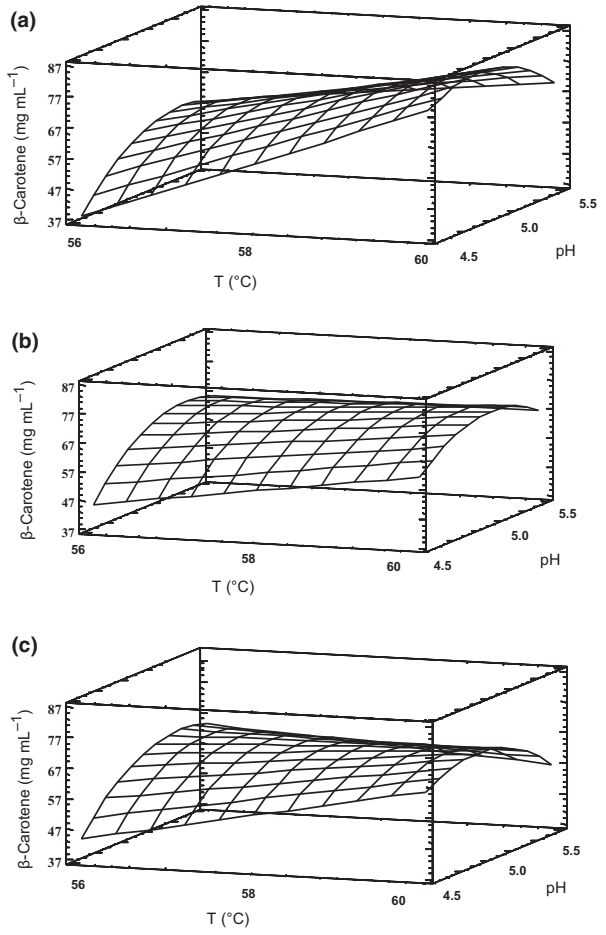

Figure 1 Combined effect of acidification and mild thermal treatments on β-carotene of carrot juice processed for (a) 2, (b) 4 and (c) 6 min.

Numerous factors can affect the bioaccessibility of carotenoids such as species of carotenoids, linkages at molecular level, amount of carotenoid, effectors, being the food matrix one of the most important factors. In carrots, β-carotene is located in the chromoplasts (organelles surrounded by a double-bilayer membrane) inside the plant cells (also surrounded by a cell membrane and a cell wall), where it is often associated with proteins and/or residual membranes (Hornero-Méndez & Mínguez-Mosquera, 2007). Consequently, several barriers have to be broken before carotenes can be released from the carrot matrix and become accessible for reaction and absorption (Knockaert *et al.*, 2012). When juicing carrots, some of these barriers were disrupted; however, part of the carotenes were retained by the membranes and the evident higher carotene content (showed by some treatments compared to the untreated sample) may have occurred as a consequence of their increased release caused by the thermal degradation of membranes and denaturation of proteins.

Total antioxidant activity

The TAA for the untreated carrot juice was 73 μg TEq mL^{-1} (Table S2), whereas combined treatments exhibited a range of TAA from 57 to 89 μg TEq mL^{-1} (data not shown). The effect of each independent variable of the combined treatments on TAA was also expressed by means of a second-order polynomial equation (Table 1). The proposed model was adequate to describe experimental data as satisfactory values of R^2_{adj} were obtained. The variable pH^2 turned out to be the most significant variable ($P < 0.0001$) influencing TAA followed by the linear variables pH and T (Table 1). For all the combinations temperature-time assayed, the highest TAA content was achieved at pH 4.5. In fact, all samples adjusted to pH 4.5 (TAA from 77 to 89 μg TEq mL^{-1}) exhibited higher TAA than the untreated control juice. This effect could be verified in the response surfaces and the corresponding contour plots for TAA as a function of T and pH at fixed times (Fig. 2; Fig. S1). The increase in T caused a decrease in TAA while the pH axis exhibited a bell-shaped dependence with a maximum at pH 4.5 and a minimum at pH 5.0 (Fig. 2). The maximum TAA content was achieved at pH 4.5 and 56°C and the minimum at pH 5.0 and 60°C.

Carrots are the single major source of β-carotene (which have provitamin A activity as well as antioxidant activity) and also are a good source of several other hydrophilic phenolic antioxidants which are known for a wide range of health-promoting properties such as anticancer, anti-atherogenic, anti-inflammatory and antimicrobial (Grassmann *et al.*, 2007; Sun *et al.*, 2009). Wu *et al.* (2004) measured the total antioxidant activity of many fruits, vegetables and other types of food considering both the lipophilic and hydrophilic fractions by the oxygen radical absorbance capacity (ORAC) assay and also examined the total phenolic content to evaluate their contribution to total antioxidant activity. They found that the hydrophilic fraction (11.56 ± 1.79 μmol TEq g^{-1}) made up >95% of the total antioxidant activity in raw carrot and concluded that the phenolic compounds accounted for the major portion of the antioxidant activity in many plants including carrots. Moreover, Sun *et al.* (2009) also found that phenolics (phenolic acids and flavonoids) made a greater contribution to the total antioxidant activity than carotenoids in different coloured carrots.

Colour measurement

Average L*, a*, b* colour parameter values and significant differences detected by MANOVA corresponding

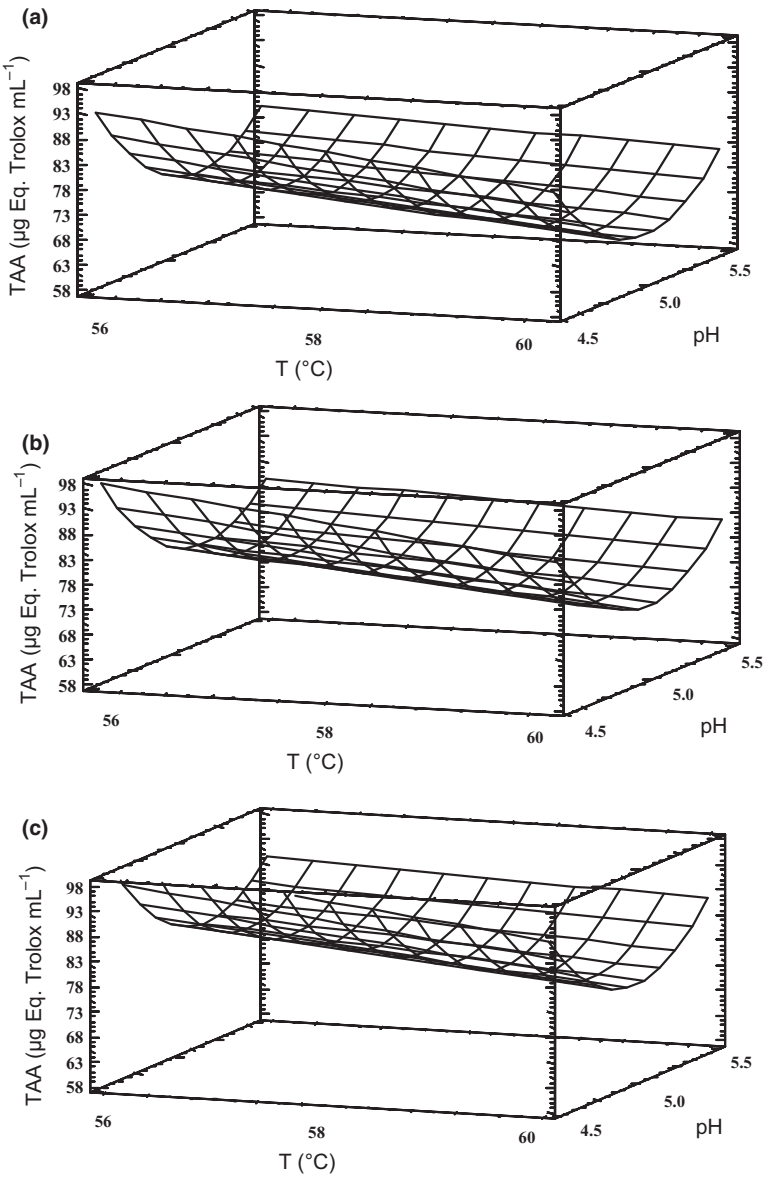

Figure 2 Combined effect of acidification and mild thermal treatment on total antioxidant activity (TAA) of carrot juice processed for (a) 2, (b) 4 and (c) 6 min.

to carrot juice treated by the combinations of acidification and mild thermal treatment are presented in Table 2. The positive a* and b* values indicated the red and yellow components of carrot colour, respectively. Overall, significant differences in L*, a*, b* values were observed between processed and control samples. The different temperature controls (C1, C2, C3) did not differ in their colour parameters, while pH control samples did (C4, C5, C6) (Table 2). In particular, pH control samples with higher citric acid content exhibited higher L*, a* and b* values (pH 4.5 > 5.0 > 5.5). Moreover, all samples processed by the combined treatments at pH 4.5 showed higher L*, a* and b* values than the untreated control (C7), regardless the treating temperature and time. The higher lightness and colour components a* and b* implied a better colour preservation of these treatments by preventing the enzymatic browning. Moreover, the higher a* and b* (red and yellow colour components, respectively) values observed might be also attributed to an increase on carotene concentration (orange natural pigments) as a result of its release from the cell chromoplasts caused by the combined treatments. Bermúdez-Aguirre & Barbosa-Cánovas (2013) studied colour changes of baby carrots immersed in citric acid solutions (0.5, 1.0 and 1.5%)

Table 2 Colour parameters and opacity corresponding to carrot juice treated by combinations of acidification and mild thermal treatment at different exposure times

Sample	pH	*T* (°C)	*t* (min)	L*	a*	b*	Op	SD†
1	5.0	58	4	33.15	7.88	12.49	1.02	k
2	5.5	56	4	29.56	8.51	12.7	0.98	l
3	5.5	58	6	31.01	6.46	11.59	0.98	m
4	5.0	60	2	30.88	8.22	13.86	0.96	j
5	5.5	60	4	30.89	6.49	11.26	1.04	n
6	5.0	56	6	32.54	7.72	12.49	0.98	p
7	4.5	60	4	31.41	10.82	15.61	0.99	o
8	4.5	58	2	31.35	10.51	15.4	0.99	r
9	4.5	58	6	32.37	11.54	15.19	0.97	i
10	4.5	56	4	31.76	10.97	14.52	0.94	d
11	5.0	60	6	31.66	8.93	13.87	0.97	c
12	5.0	58	4	30.92	9.1	13.73	1.03	b
13	5.5	58	2	30.32	7.55	12.25	0.92	q
14	5.0	58	4	31.55	8.9	13.57	0.99	a
15	5.0	56	2	30.23	9.56	14.97	0.95	h
C1	6.3	56	2	30.65	7.37	11.95	0.98	r
C2	6.3	58	2	30.56	7.77	11.86	1.00	r
C3	6.3	60	2	30.43	7.93	12.49	1.01	r
C4	4.5	25	6	31.59	11.08	16.41	0.98	g
C5	5	25	6	30.9	12.4	16.5	0.99	f
C6	5.5	25	6	27.61	7.85	12.46	1.00	e
C7	6.3	25	6	29.46	9.14	14.78	0.90	q

†Different letters indicate significant differences (SD) among mean values performed by the Hotelling test based on the Bonferroni correction ($P < 0.05$).
Op, opacity.

for 15 min. They observed an increase in L* values for all the concentrations assayed with respect to the control; however, they reported lower a* and b* values.

It is worth noting that the unprocessed juice sample (C7) was the most translucent ($P < 0.0001$) exhibiting opacity of 0.90, while all the treatments reduced translucency (Table 2). Certain treatments turned samples into opaque (Op > 1.0), being the combined treatments pH 5.0, 58°C, 4 min (Op: 1.02) and pH 5.5, 60°C, 4 min (Op: 1.04) the ones which showed the highest opacity ($P < 0.0001$). Consumers generally prefer opaque juices (Okoth *et al.*, 2000); therefore, processing of juices will contribute to enhance appearance and acceptability of carrot juice.

Principal component analysis of functional and colour parameters

A PCA was performed to illustrate how the different evaluated samples were spatially distributed with respect to colour parameters, TAA and α-C and β-C contents. Fig. 3 exhibits a biplot of the principal components 1 and 2 (PC1 and PC2). The cophenetic correlation coefficient was 0.93, indicating that an accurate reduction was achieved with the analysis. Only the first two principal components (PC_1 and PC_2) were retained as they explained 73% of the total variance. The first two PC (Fig. 3) explained 39.6% and 33.3%

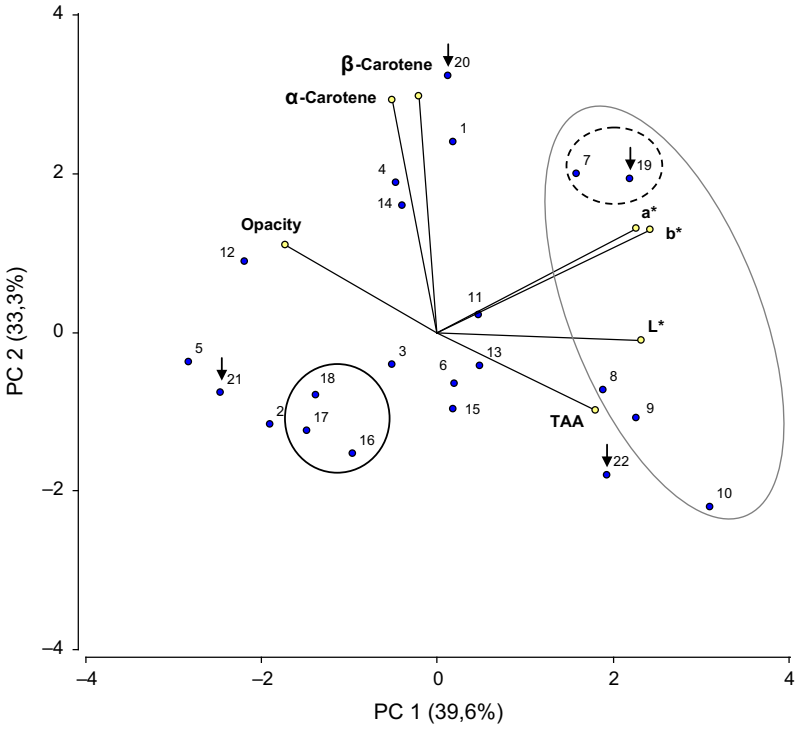

Figure 3 Principal component analysis corresponding to the colour parameters L*, a*, b*, opacity, total antioxidant activity (TAA) and α- and β-carotenes. [Colour figure can be viewed at wileyonlinelibrary.com].

of the variance, respectively. The PC_1 was positively associated with L*, a*, b* parameters and TAA and negatively with opacity, whereas PC_2 showed positive association with α- and β-carotene contents. Overall, treated samples exhibited higher α-C and β-C contents than untreated juice (sample 22) (Fig. 3). The three *T* control samples (samples 16, 17 and 18, corresponding to 56, 58 and 60°C, respectively) exhibited lower TAA than untreated juice, but higher α-C and β-C, exhibiting similar values for all the parameters assayed among them (Fig. 3). Conversely, pH control samples (19, 20 and 21, corresponding to 4.5, 5.0 and 5.5, respectively) were spatially apart from each other; therefore, acidification substantially affected all functional and colour parameters. In that sense, increasing citric acid concentration (pH 4.5) at all *T-t* combinations resulted in the highest L*, a*, b* and TAA values (Fig. 3). On the other hand, systems with lower citric acid concentration exhibited higher opacity (Fig. 3). Samples corresponding to pH control (4.5) and the treatment pH 4.5, 60°C for 4 min (sample 7) exhibited the highest functional quality, as they showed simultaneously high α-C, β-C contents and TAA (dashed circle, Fig. 3). This effect may be explained by the high TAA observed at pH 4.5 and the higher extractability of carotenes at mild temperatures, providing antioxidant and provitamin A activities.

It is worth noting that the combination of mild thermal treatment at 60°C for 4 min at pH 4.5 and refrigerated storage (4°C) of carrot juice ensures a safe product from a microbiological point of view, as suggested by numerous studies conducted on different foodborne pathogens reported in the literature and also by previous studies from our research group. In this sense, mild heating temperatures applied to orange juice (pH 3.5) reduced 5 log-cycle of *Listeria innocua* in 6.2 min at 57°C or 1.7 min at 60°C (Char *et al*., 2009). Moreover, Gayán *et al*. (2012) reported 4.11 and 6.22 log cycles of inactivation of *Salmonella* Typhimurium STCC 878 in McIlvaine buffer (pH 7.0) after 2.23 min of heating at 57.5 and 60°C, respectively. Additionally, Gouma *et al*. (2015) determined the treatment time required to achieve 5 log reductions (5D value) for heat inactivation of *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus* and *L. monocytogenes* in apple juice (pH 3.6) at different temperatures. The most heat-resistant microorganisms were *E. coli* and *L. monocytogenes* requiring a treatment time of 0.89 and 1.03 min, respectively, to reach the desired inactivation at 60°C.

Conclusions

Total antioxidant activity, α- and β-carotene contents and colour parameters of carrot juice were either positively or negatively affected by the different treatment combinations of pH, temperature and exposure time assayed in the present study. In particular, thermal treatments up to 60°C positively influenced the extraction yield of carotenes mainly as a result of the softening and disruption of the remaining cellular tissues, while preserving the biological value as these mild temperatures were not enough to cause isomerisation of carotenes. Furthermore, acidification with citric acid (pH 4.5) enhanced the total antioxidant activity of carrot juice, as well as prevented from browning reactions, turning the samples more luminous, with higher a* and b* values.

The PCA was useful to elucidate the treatments that showed simultaneously high α- and β-carotene contents and antioxidant activity with good colour parameters. According to this analysis, the best treatment condition was pH 4.5 at 60°C for 4 min.

This work showed the impact of processing variables on the increase in the extractability of carotenes and on the preservation of the antioxidant compounds and colour of carrot juice, providing more provitamin A and antioxidant activities than the untreated juice using conventional technologies applicable to small juice producers.

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Conflict of interest

The authors declare no conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Contours of the combined effect of acidification and thermal treatments on total antioxidant activity (TAA) of carrot juice processed for (a) 2; (b) 4; and (c) 6 min.

Table S1. Experimental design: codification of the independent variables.

Table S2. Physical and chemical characterization of raw carrot juice.