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Effect of High Hydrostatic Pressure on Ascorbic Acid, Phenolic Compounds and Antioxidant Activity of Pera Rio Orange Juice

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Abstract

Orange juice is the most popular juice in the world, representing an important source of bioactive compounds in diet. High hydrostatic pressure (HHP) is an alternative technology that does not use high temperature, being able to preserve flavor and nutritional characteristics of the juice. The influence of HHP treatment conditions, pressure (100-600 MPa), temperature (30-60°C) and time (30-360 s), on ascorbic acid, phenolic compounds and antioxidant activity of orange juice was investigated using response surface methodology. Analysis of variance showed that quadratic polynomial models fitted well with the experimental data for ascorbic acid (R^2=0.92, p<0.01) and antioxidant activity using the ABTS*+ assay (R^2=0.91, p<0.01). The increase in time and temperature of HHP treatment promoted the reduction of ascorbic acid content and antioxidant activity in orange juice. HHP treatment reduced the ascorbic acid content and antioxidant activity of orange juice. The HHP treatment conditions of 100 to 250 MPa, 30 to 40°C and 30 to 125 s were able to produce orange juice with more than 70% of the initial ascorbic acid content and 80% of the antioxidant activity.

Keywords: Antioxidant activity; Ascorbic acid; High hydrostatic pressure (HHP); Orange juice; Response surface methodology (RSM)

Introduction

Orange juice is the most consumed juice in the world, corresponding to 47% worldwide juice consumption [1]. It is an important source of bioactive compounds in diet, like flavonoids and carotenoids, as well as ascorbic acid. Ascorbic acid is considered the major antioxidant compound in orange juice, contributing with more than 90% of the antioxidant activity [2]. Orange juice flavanones, mainly hesperidin and narirutin, also present antioxidant activity [3-5], while carotenoids [6], mostly carotenes and cryptoxanthins, have provitamin A activity, and lutein and zeaxanthin, prevent macular degeneration [7]. Orange juice flavanones have been associated with reduced risk of coronary heart disease [8-10]. Ascorbic acid also contributes to the maintenance of the vascular health and to reduce atherogenesis, regulating the collagen synthesis, prostacyclin production, and nitric oxide [11,12]. Some studies indicated that orange juice consumption may reduce Low Density Lipoprotein Cholesterol (LDL) and improve high density lipoprotein (HDL) cholesterol in hypercholesterolemic subjects, as well as reduce oxidative stress (8-*epi*-$PGF_{2\alpha}$) and uric acid in plasma [13].

The most extensively process used for orange juice is thermal pasteurization, which inactivates vegetative microorganisms and enzymes. But, pasteurization at intense time/temperature conditions induces to ascorbic acid and natural flavor losses, as well as carotenoids and color changes, so affecting the juice's overall quality [14-17].

Due to consumers demand the fruit juice industry has been exploring innovative technologies, which requires minimal heat treatment, able to produce juice with fresh-like and natural-like attributes, to preserve flavor and nutritional aspects [18]. High Hydrostatic Pressure (HHP) reduces orange juice spoilage microflora [19,20] and PME activity [21-23] without using high temperature, therefore reserving sensory and nutritional characteristics. Color, flavor, soluble solids, pH and other compounds of orange juice are not considerably affected. Orange juice spoilage microflora was studied after HHP at 360 MPa, 35°C for 2 min with a 7 log cycle reduction of *Lactobacillus plantarum* and *L. brevis*. HHP treatment of orange juice at 600 MPa during 60 s reduced aerobic microorganisms, and yeasts and molds counts to not detectable levels (<10 CFU/mL) for juice from Valencia and Navel varieties and 400 MPa for 90 s for juice from Hamlin HHP of Navel orange juice at 600 MPa, 20°C for 60 s exhibited a 45% reduction in PME activity, while the same treatment conditions were enough to inactivate 92% of PME orange juice derived from a mixture of three orange varieties. Bisconsin-Junior reported the range of 550 to 600 MPa, 55 to 60°C and 330 to 360 s to produce orange juice with PME residual activity of less than 20% and low microorganism count. PME inactivation depends on the enzyme environment of the particular food system and even on the variety and origin of orange juice.

There are many studies about the influence of some HHP treatments on ascorbic acid and/or antioxidant activity of orange juice [24-28], however the effects of a range of treatment conditions were not determined yet. So, there is no information regarding ascorbic acid and antioxidant activity of orange juice under multiple and simultaneous conditions of HHP.

Bull reported that Valencia orange juice treated at 600 MPa, 20°C for 60 s did not had ascorbic acid significantly affected, however Sanchez-Moreno C found 11% reduction in ascorbic acid of the juice treated at 100 MPa, 60°C during 300 s. Ancos studied the effect of HHP on carotenoids and antioxidant activity of Valencia orange juice. The highest amount of vitamin A and extractable carotenoids in orange juice was obtained at 350 MPa, 30°C for 300 s, although there was a reduction of 22% on antioxidant activity. Sanchéz-Moreno

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reported that after 400 MPa, 40°C for 60 s orange juice showed no significant reduction on vitamin C, but presented higher extractability of carotenoids (54%) and flavonones (34%), however there was no significant difference on antioxidant activity.

The novelty of this work is to evaluate the influence of HHP treatment conditions (pressure, temperature and time) on ascorbic acid, phenolic compounds and antioxidant activity of Pêra Rio orange juice using response surface methodology.

Material and Methods

Chemicals

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, sodium carbonate, and gallic acid were from Sigma-Aldrich (St Louis, MO, USA); ascorbic acid and glucose from Merck (Darmstadt, Alemanha); Folin–Ciocalteu reagent from Imbralab (Ribeirão Preto, SP, Brazil); potassium persulfate from Fluka (Steinheim, Germany); methanol from JT Baker (Philipsburg, PA, USA), sodium hydroxyl, potassium sodium tartrate tetrahydrate, cupric sulfate pentahydrate and potassium phosphate dibasic from Labsynth (Diadema, SP, Brazil); oxalic acid and 2,6-dichloroindophenol sodium salt hydrate from Vetec (Rio de Janeiro, RJ, Brazil).

Orange juice

The fruits were from Pêra Rio variety, supplied by a citrus industry from Araraquara, SP. Fruits were cultivated in Bauru, SP, Brazil (22º 25' 59" S; 49º 10' 31" W), during the 2011/2012 harvest. The extraction of orange juice was performed in a JBT 391B extractor using the premium juice extractor settings and a UFC-35 finisher (0.25 mm sieve) at the JBT FoodTech Citrus System, Araraquara, SP, Brazil. After extraction, the juice was frozen and storage at -18ºC.

Physicochemical evaluation of non-treatment orange juice

Total soluble solids, titratable acidity, pH, ascorbic acid, total e reducing sugars of non-treatment orange juices were determined according to AOAC [29]. Ratio, the total soluble solids/titratable acidity rate, was calculated.

High hydrostatic pressure (HHP) treatment

For the HHP treatment, orange juice (100 mL) was packaged in heat sealed PE bags (Selovac 200B II, Selovac, São Paulo, Brazil), excluding as much air as possible. Orange juice was pressurized, according to the experimental design, in a Stansted Food Lab 9000 (Stansted Fluid Power, Stansted, UK) within a pressure vessel of 500 mL. The maximum nominal operation pressure is 900 MPa and temperature range -20 to 90ºC. The vessel temperature was controlled by water circulation in the outer jacket connected to a heating-cooling system. The pressure transmitting fluid was an ethanol:water solution (70:30, v/v). The compression rate was 7 MPa.s^{-1} and decompression time less than 10 s. Compression and decompression times were not included in the experimental design.

Prior to HHP treatment, the adiabatic heating of orange juice and pressurizing fluid of each experimental condition were evaluated. The adiabatic heating ranged from 2.8 to 3.5ºC per 100 MPa for orange juice and from 3.6 to 6.8ºC per 100 MPa for the pressurizing fluid. Temperature of orange juice bags and vessel was adjusted to a few degrees below the temperature of each experimental condition in order to achieve the temperature according to the central composite rotatable design (CCRD) during pressurization.

Non treated orange juice was used for comparison with the HHP treated orange juice.

Experimental design

The effect of HHP treatment conditions (independent variables), namely pressure, temperature and time, on total phenolic compounds, ascorbic acid and antioxidant activity (response variables) of HHP treated orange juice, were evaluated using the response surface methodology.

A CCRD of three independent variables with five levels, containing a 2^3 factorial design, 6 axials points and 3 repetitions of the central point, totalizing 17 essays was used. The levels of the independent variables were coded as: −1 and +1, representing the levels of 2^3 factorial design; 0 (zero), representing the central point of the design, used to calculate the lack of fit and the pure error of the statistical model; −1.68 and +1.68, representing the axial points, allowing a quadratic statistical model (Table 1). Data from the CCRD were analyzed by multiple regressions to fit the following quadratic polynomial model: (Equation 1)

$$Y=\beta_0 + \beta_1 P + \beta_2 T + \beta_3 t + \beta_{11} P^2 + \beta_{22} T^2 + \beta_{33} t^2 + \beta_{12} PT + \beta_{13} Pt + \beta_{23} Tt$$

where Y=predicted response variable, β_0=constant, β_1, β_2, and β_3=linear coefficients, β_{11}, β_{22}, and β_{33}=quadratic coefficients, β_{12}, β_{13}, and β_{23}=interactive coefficients. The independent variables are P=pressure, T=temperature and t=time. The non significant terms were taken out from the quadratic polynomial model after the ANOVA. A new ANOVA only containing the significant terms was performed to obtain the regression coefficients of the final equation in order to improve accuracy.

Ascorbic acid

Ascorbic acid of the HHP orange juice was based on the reduction of 2,6-dichloroindophenol. Triplicate analyses were performed and results were expressed as mg of ascorbic acid L^{-1} of orange juice [30].

Extraction of antioxidant compounds

The extraction of antioxidant compounds of the HHP orange juice was based on the procedure reported by Stella SP [31]. Orange juice (5 mL) and a methanol: water solution (80:20, v/v) (10 mL) were vortexed for 1 min and then submitted to an ultrasonic bath at room temperature for 15 min. The mixture was centrifuged at 21000 g for 20 min at 20°C and the supernatant collected. The extraction procedure was repeated once using the same conditions.

Total phenolic compounds: The total phenolic compounds of the HHP orange juice were determined using the Folin-Ciocalteu assay as reported by Asami [32] with minor modifications. In a 5 mL volumetric flask, 1 mL of water, 0.2 mL of extract and 0.06 mL of Folin-Ciocalteu reagent were added, mixed and allowed to stand at room temperature for 8 min. After that, 2 mL of a sodium carbonate solution (7%, w/v) was added and then the flask volume was adjusted with distilled water, mixed, and heated at 40ºC for 30 min in a water bath. The absorbance

Independent variables	Values of levels				
	-1.68	-1	0	+1	+1.68
Pressure (MPa)	100	201	350	499	600
Temperature (ºC)	30	36	45	54	60
Time (s)	30	97	195	293	360

Table 1: Levels and corresponding values of the independent variables.

was measured at 740 nm using a spectrophotometer (Evolution 220, Thermo Scientific, USA). Quantification was carried out using a gallic acid calibration curve (50 to 220 µg/mL) and the results were expressed as miligrams of gallic acid.L^{-1} of orange juice. Triplicate analyses were performed.

Antioxidant activity with DPPH· assay: The DPPH· assay was based on the method described by Rufino MSM [33] with some modifications. A methanol solution containing 0,06 mM DPPH· was prepared. A 100 µL aliquot of the whole extract of antioxidant compounds and 1:1 and 4:5 methanol:water (80:20 v/v) solutions of the extracts was mixed with 3 mL DPPH· radical solution. Absorbance readings at 515 nm in a spectrophotometer (Evolution 220, Thermo Scientific, USA) were done after 30 min of reaction. Trolox ethanolic solutions (100–500 µmol L^{-1}) were used for calibration curves. The analyses were performed in triplicate and results expressed as µmol Trolox L^{-1} of orange juice.

Antioxidant activity with $ABTS^{\cdot+}$ assay: The $ABTS^{\cdot+}$ assay was based on the method described by Rufino MSM. The ABTS radical cation ($ABTS^{+}$) was obtained from the reaction between 5.0 mL ABTS (7 mmol L^{-1}) with 88 µL potassium persulfate (140 mmol L^{-1}). The solution was allowed to stand in the dark for 16 h to ensure the complete formation of stable ABTS radical. The ABTS radical solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. A 30 µL aliquot of the whole extract of antioxidant compounds and 1:1 and 4:5 methanol:water (80:20 v/v) solutions of the extracts was mixed with 3 mL ABTS radical solution. Absorbance readings at 734 nm were done after 6 min of reaction in a spectrophotometer (Evolution 220, Thermo Scientific, USA). Trolox ethanolic solutions (100–1200 µmol L^{-1}) were used for calibration curves. The analyses were performed in triplicate and results expressed as µmol Trolox L^{-1} of orange juice.

Data analyses

Results were expressed as mean ± standard deviation of three replicated analyses. ANOVA of the regression equations allowed to verify the adequacy of the model by evaluating the *F* test value, the lack of fit, the coefficient of determination (R^2), and significance of the effects using Statistica software version 10.0 (StatSoft, Tulsa, USA).

Results and Discussion

Physicochemical evaluation of the non-treated orange juice

Total soluble solids was 9.03 ± 0.00 ºBrix, titratable acidity 5.78 ±0.03 g of citric acid.L^{-1}, pH 4.18 ± 0.01, and reducing and total sugar were 33.04 ± 0.26 and 62,62 ± 0.47 g of glucose.L^{-1}, respectively. Ascorbic acid was 859.5 ± 11.4 mg.L^{-1} and ratio (soluble solids/titratable acidity) was 15.57. The physicochemical characteristics were in accordance with the Brazilian law, except for total soluble solids content [34].

Effect of HHP treatment conditions on ascorbic acid

Table 2 shows the ascorbic acid content from the non-treated and HHP treated orange juice under each experimental condition. The non-treated orange juice presented higher ascorbic acid content (859.52 mg L-1) when compared to the Brazilian (82 to 570 mg L^{-1}) and to the Spanish (196 to 634 mg L^{-1}) [35] commercial orange juice. The HHP treated orange juice showed ascorbic acid ranging from 435.9 to 710.2 mg L^{-1}. The experimental condition that most affected ascorbic acid (435.9 mg L^{-1}) was 350 MPa, 45°C and 360 s, which degradation was more than 50%, mainly related to the longest time of process. High ascorbic acid levels are used as quality index of fruits and juices, because ascorbic acid is more sensitive to degradation during process than other bioactive compounds associated to health benefits [36]. The ascorbic acid degradation was higher than that observed in some studies. Orange juice from Valencia and Navel treated at 400 MPa, 40°C during 60 s had 5% and 8% of ascorbic acid degradation, respectively. Sánchez-Moreno C obtained similar results as ours where higher ascorbic acid reduction (11%) resulted from longer time and higher temperature (100 MPa, 60ºC, 300 s), when compared to the orange juice treated at 400 MPa, 40°C for 60 s, which ascorbic acid reduction was 7%.

The ascorbic acid content of orange juice submitted to different conditions of pressure, temperature and time along the 17 experiments according to the CCRD was statistically analyzed. The quadratic model for ascorbic acid was significantly fit to the experimental data, as indicated by the regression model *F* value of 8.80 ($p<0.01$), and presented a satisfactory determination coefficient (R^2=0.92). No

Experiment	Pressure (MPa)	Temperature (°C)	Time (s)	Ascorbic acid (mg L^{-1})	Total phenolic compounds (mg Galic acid.L^{-1})	Total antioxidant activity (µmol Trolox.L^{-1})	
						DPPH	ABTS
Non treated	-	-	-	859.5 ± 11.4	416.7 ± 16.7	2211.7 ± 59.8	3176.7 ± 103.8
1	201	36	97	710.2 ± 4.3	426.4 ± 28.5	2169.4 ± 33.8	2708.6.± 128.3
2	499	36	97	640.1 ± 4.3	447.7 ± 21.1	2182.0 ± 27.2	2737.6 ± 100.3
3	201	54	97	618.7 ± 4.3	421.7 ± 20.4	2021.4 ± 44.3	2563.8 ± 55.7
4	499	54	97	582.2 ± 4.3	434.7 ± 2.3	1991.7 ± 60.8	2550.4 ± 136.3
5	201	36	293	539.5 ± 11.4	427.7 ± 14.1	2048.6 ± 41.7	2621.5 ± 86.4
6	499	36	293	512.1 ± 18.8	419.6 ± 5.5	2039.5 ± 11.0	2353.7 ± 133.2
7	201	54	293	445.0 ± 4.3	402.1 ± 5.1	2015.5 ± 92.8	2266.8 ± 146.4
8	499	54	293	457.2 ± 11.4	401.7 ± 6.8	1823.1 ± 14.5	2062.0 ± 114.2
9	100	45	195	557.8 ± 11.4	386.0 ± 12.2	1876.9 ± 40.4	2416.1 ± 35.8
10	600	45	195	521.2 ± 4.3	404.8 ± 7.8	1864.3 ± 33.9	2304.8 ± 11.2
11	350	30	195	536.4 ± 11.4	406.8 ± 10.2	1761.1 ± 26.7	2351.1 ± 77.6
12	350	60	195	499.9 ± 4.3	418.3 ± 2.7	1811.1 ± 34.9	2120.4 ± 64.9
13	350	45	30	621.8 ± 4.3	425.5 ± 9.2	1759.0 ± 42.8	2935.9 ± 146.6
14	350	45	360	435.9 ± 4.3	404.8 ± 17.5	1751.1 ± 11.8	2112.2 ± 57.6
15	350	45	195	509.0 ± 8.6	421.6± 4.3	1745.3 ± 15.3	2402.8 ± 59.9
16	350	45	195	496.8 ± 7.5	428.8 ± 7.8	1766.0 ± 23.4	2469.6 ± 114.8
17	350	45	195	518.2 ± 4.3	424.6 ± 10.4	1747.8 ± 13.2	2484.0 ± 94.8

Table 2: The central composite rotatable design (CCRD) and experimental response values for HHP treated orange juice.

Source of variation[1]	Ascorbic acid	Total phenolic compounds	Antioxidant activity	
			DPPH	ABTS
Regression model	8.80 [b]	1.87[ns]	1.08[ns]	7.20[b]
P	21.50[c]	18.38 [c]	33.12[c]	16.19[d]
T	83.25[c]	54.18 [c]	147.19[b]	72.98[b]
t	529.04[b]	14.17 [c]	116.60[b]	272.24[c]
P²	33.80[c]	[ns]	763.88[b]	0.25[ns]
T²	12.10[b]	18.08 [c]	359.49[b]	15.30[d]
t²	21.24[c]	23.43[ns]	235.81[b]	15.79[b]
PT	5.84[ns]	[ns]	49.86[c]	0.03[ns]
Pt	9.12[d]	17.30 [c]	36.39[c]	15.87[d]
Tt	[ns]	[ns]	[ns]	6.58[ns]
Lack of fit	12.23[ns]	15.95[ns]	268.74[ns]	8.57[ns]
R²	0.9164	0.51223	0.4462	0.9052

[1]*P*=pressure. *T*=temperature. *t*=time.

[a]p ≤ 0.01. [b]p ≤ 0.05. [c]p ≤ 0.10

[ns]Non-significant.

Table 3: ANOVA (*F* value) of the quadratic model for ascorbic acid content. total phenolic compounds and antioxidant activity of the HHP treated orange juice.

Source of variation[1]	Ascorbic acid content	Antioxidant activity (ABTS)
Mean/Interception	1297.481	2127.168
P	-0.829	0.498
T	-15.442	43.555
t	-1.548	-2.662
P²	(8.371×10^{-4})	-
T²	0.139	-0.608
t²	(1.525×10^{-3})	(5.518×10^{-3})
Pt	(7.829×10^{-4})	$-(4.180 \times 10^{-3})$

[1]*P*=pressure. *T*=temperature. *t*=time.

Table 4: Significant regression coefficients of the quadratic model for ascorbic acid content and antioxidant activity of HHP treated orange juice.

significant lack of fit of the model was found (p>0.05), showing that it fits properly for prediction within the range of the studied HHP treatment conditions. Terms with significant *F* value (p ≤ 0.1) were included in the model. The linear and quadratic terms of pressure (*P*, P^2), temperature (*T*, T^2) and time (*t*, t^2), as well as the interaction term of pressure and time (*Pt*) were significant (Table 3). These statistical parameters confirm the consistency of the model, indicating it is reliable to predict ascorbic acid content in HHP treated orange juice. Using the significant regression coefficients (Table 4) the following model equation for ascorbic acid content was generated: (Equation 2)

$$\text{Ascorbic Acid} = 1297.481 - 0.829P - 15.442T - 1.548t + (8.371 \times 10^{-4})P^2 + 0.139T^2 + (1.525 \times 10^{-3})t^2 + (7.829 \times 10^{-4})Pt$$

where, Ascorbic Acid=ascorbic acid content (mg L^{-1}), P=pressure (MPa), T=temperature (°C) and t=time (s).

The response surface was generated from the regression equation (Eq. 2) to illustrate the effects of the independent variables on the ascorbic acid content (Figure 1). One of the variables was kept at the central point of the design (zero level) while the other two variables were changed within the experimental range. An increase in temperature and time promoted the reduction of the ascorbic acid content in HHP treated orange juice. The independent variable of time was the most important one affecting ascorbic acid reduction in HHP treated orange juice (Figure 1a and 1b). As it can be seen in Figure 1b and 1c, from 100 to 300 MPa ascorbic acid was reduced, but from 500 to 600 MPa a slight increase on ascorbic acid was observed. The increase in ascorbic acid

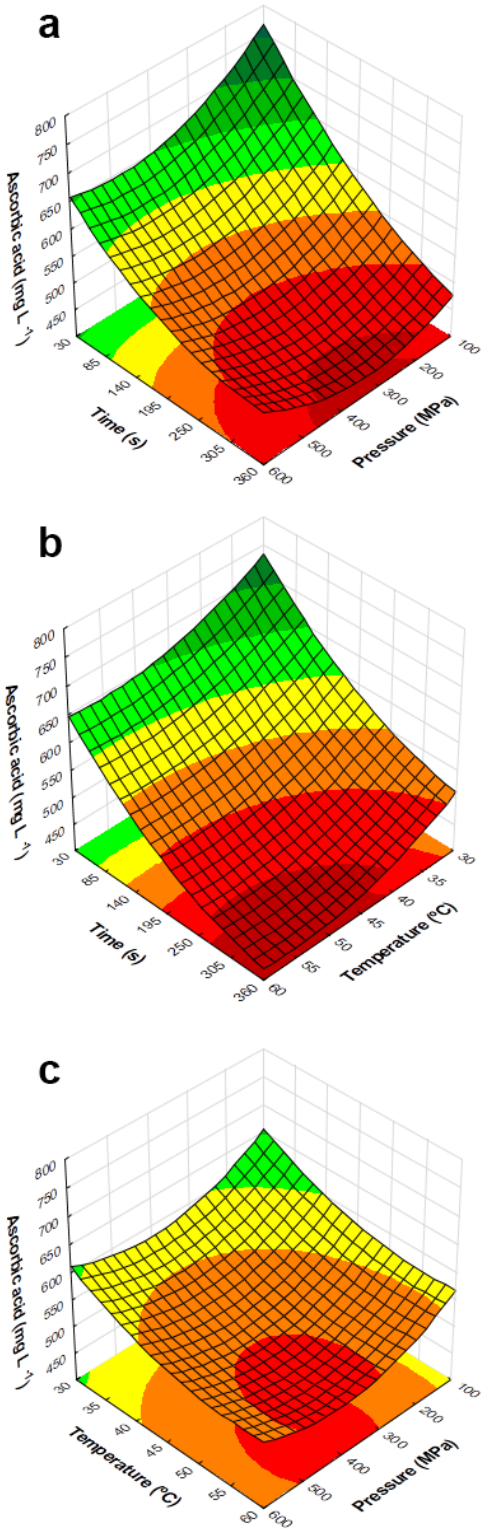

Figure 1: Response surface of the combined effects of time and pressure (a), time and temperature (b), and temperature and pressure (c) on the ascorbic acid content of HHP processed orange juice.

can be resulted from eliminating part of enzyme-catalyzed oxidation (ascorbate oxidase and peroxidase), responsible for vitamin C loss.

According to the model equation (Eq. 2), in order to obtain ascorbic acid content higher than 600 mg L^{-1}, representing 70% of the initial orange juice ascorbic acid content, the range of 100-250 MPa, 30-40°C and 30-125 s HHP treatment conditions should be used.

Effect of HPP treatment conditions on total phenolic compounds

Total phenolic compounds of non-treated orange juice was 416.7 mg acid galic.L^{-1} and for the processed orange juice using HHP, according to the CCRD with 17 experiments, ranged from 386,0 to 447,7 mg acid galic.L^{-1}. The highest content of total phenolic compounds was obtained at 499 MPa, 36°C and 97 s, while at 100 MPa, 45°C and 195 s was the lowest content (Table 2). Sánchez-Moreno C showed that HHP orange juice processed at 400 MPa, 40°C and 60 s had higher content of total flavanones (18.70 mg.100 mL^{-1}), narigenin (4.47 mg.100 mL^{-1}) and hesperitin (14.24 mg.100 mL^{-1}), when compared to the freshly squeezed orange juice, with 13.89 mg.100 mL^{-1} total flavanones, 3.72 mg.100 mL^{-1} narigenin and 10.18 mg.100 mL^{-1} hesperitin. Using different HHP conditions (350 MPa, 30°C and 2.5 min and 400 MPa, 40°C and 1 min) for orange juice, Sánchez-Moreno C reported an increase in hesperitin content but no change was found for narigenin content.

Cao [37] studied the total phenolic content of strawberry pulps submitted to HHP. At 400 MPa there was a decrease in total phenolic content regardless time of HHP treatment. The results were associated with the high residual activity of polyphenol oxidase and peroxidase, which are responsible to catalyze the oxidation of phenols [38]. At 500 and 600 MPa the strawberry pulps had an increase in total phenolic content, related to the higher extractability of some antioxidant compounds.

The statistical analysis indicated that the quadratic model for total phenolic compounds was not significantly fit to the experimental data, as indicated by the regression model *F* value of 1.87 ($p<0.01$), and did not present a satisfactory determination coefficient ($R^2=0.51$) (Table 3). These statistical parameters confirm the model does not have consistency, indicating it is not reliable to predict total phenolic compounds content in HHP treated orange juice.

Effect of HHP treatment conditions on antioxidant activity

Antioxidant activity of orange juice was determined using the DPPH• and $ABTS^{•+}$ assay. The antioxidant activity of non-treated orange juice was 2211.7 µmol Trolox L^{-1} with DPPH• and 3176.7 µmol Trolox L^{-1} with $ABTS^{•+}$, which are slight higher than the values reported by Miller NJ [39] (2469 µmol Trolox L^{-1}) and Stella SP (57.78 to 349.32 µmol Trolox 100 mL^{-1}). The antioxidant activity of the HHP treated orange juice for each experimental condition is in Table 2. HHP treatment reduced antioxidant activity in orange juice, which ranged from 1745.3 to 2182.0 µmol Trolox L^{-1} for DPPH• assay and from 2062.0 to 2935.9 µmol Trolox L^{-1} for $ABTS^{•+}$ assay. The highest antioxidant activity of HHP orange juice using the DPPH• radical reaction was 2182.0 µmol Trolox L^{-1}, at 499 MPa, 36°C, 97 s and for the $ABTS^{•+}$ radical reaction, 2935.9 µmol Trolox L^{-1}, at 350 MPa, 45°C, 30 s. The effect of HHP on antioxidant activity is not the same among food products, as it might influence vitamin stability and extraction yield of some bioactive compounds [40]. Indrawati O reported that HHP treatment increased antioxidant activity of carrot juice, but reduced that for orange juice (*var* Navelinas). According to Table 2 and Figure 2, it is possible to observe that the lower the time and temperature of HHP treatment the higher the antioxidant activity for $ABTS^{•+}$ assay.

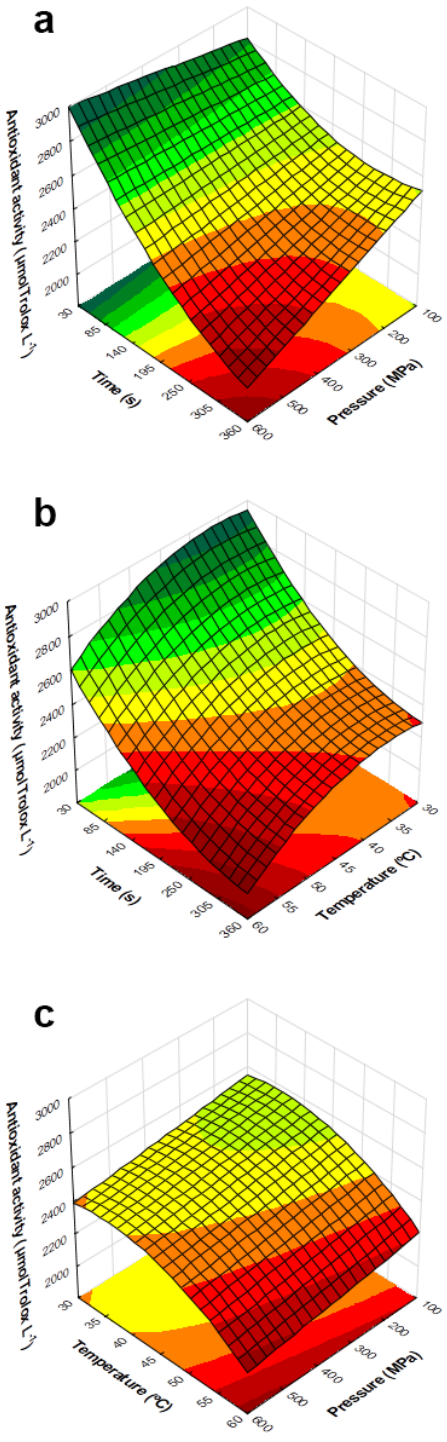

Figure 2: Response surface of the combined effects of time and pressure (a), time and temperature (b), and temperature and pressure (c) on the antioxidant activity of HHP processed orange juice.

The statistical analysis (Table 3) indicated that the quadratic model for antioxidant activity with DPPH• assay was not significantly fit to the experimental data, as indicated by the regression model *F* value of 1.08, and did not present a satisfactory determination coefficient

(R^2=0.45). These statistical parameters confirm the model do not have consistency, indicating it is not reliable to predict antioxidant activity using the DPPH$^{\bullet}$ assay of HHP treated orange juice.

On the other hand, the ANOVA (Table 3) demonstrated that the quadratic model for antioxidant activity using the ABTS$^{\bullet+}$ assay significantly fit the experimental data, as indicated by the regression model *F* value of 7.20 ($p<0.01$), with determination coefficient (R^2) of 0.91. The model presented no significant ($p>0.05$) lack of fit. The linear terms of pressure (*P*), temperature (*T*) and time (*t*), as well as the quadratic terms of temperature (T^2) and time (t^2), and the interaction term of pressure and time (*Pt*) were significant ($p<0.1$). These parameters confirm the reliability of the prediction model for antioxidant activity of HHP orange juice for the ABTS$^{\bullet+}$ assay. Using the significant regression coefficients (Table 4) the following equation for antioxidant activity using ABTS$^{\bullet+}$ assay was developed: (Equation 3)

$$\text{Antioxidant Activity (ABTS}^{\bullet+}\text{)} = 2127.168 + 0.498P + 43.555T - 2.662t - 0.608T^2 + (5.518 \times 10^{-3})t^2 - (4.180 \times 10^{-3})Pt$$

where, Antioxidant Activity (ABTS$^{\bullet+}$)=antioxidant activity (μmol Trolox L^{-1}), P=pressure (MPa), T=temperature (°C) and t=time (s).

Using the regression equation (Eq. 3) the response surface was generated to illustrate the effects of the independent variables on the antioxidant activity using ABTS$^{\bullet+}$ assay (Figure 2). As observed for ascorbic acid content (Figure 1), the increase in temperature and time of HHP orange juice treatment promoted reduction in antioxidant activity (Figure 2). According to Figure 2a, when time was lower than 105 s the increase in pressure enhanced antioxidant activity, but when time was higher than 195 s, pressure was inversely associated to antioxidant activity. Figure 2c shows that the increase in pressure resulted in slight reduction of antioxidant activity. Time was the most important variable affecting the reduction of antioxidant activity of HHP treated orange juice using ABTS$^{\bullet+}$ assay (Figure 2a and 2b). Orange juice antioxidant activity higher than 2550 μmol Trolox L^{-1}, representing ca 80% of the initial orange juice antioxidant activity, can be obtained within the range of 100-320 MPa, 30-42°C and 30-180 s HHP treatment conditions.

Antioxidant activity is related to the bioactive compounds present in food. It is well known that orange juice intake increases vitamin C in plasma, which provided antioxidant related health benefits. The ascorbic acid content and antioxidant activity using ABTS$^{\bullet+}$ assay of HHP treated orange juice showed a positive and strong correlation (R=0.82). Ascorbic acid (Figure 1) and antioxidant activity (Figure 2) showed similar response concerning to the HHP temperature, pressure and time, indicating that the decrease in antioxidant activity could be attributed to the ascorbic acid degradation. These results are in agreement with those reported by Miller NJ, Sánchez-Moreno C and Stella SP, which showed that ascorbic acid is the main antioxidant compound in orange juice.

Conclusion

HHP treatment reduced the ascorbic acid content and antioxidant activity of orange juice. Time, temperature and pressure influenced the response variables. Time of HHP treatment showed the strongest influence on the reduction of ascorbic acid and antioxidant activity. The HHP treatment conditions of 100 to 250 MPa, 30 to 40°C and 30 to 125 s were able to produce orange juice with more than 70% of the initial ascorbic acid content and 80% of the antioxidant activity. The ABTS$^{\bullet+}$ assay fit well the antioxidant activity of orange juice. The effects of HHP treatment conditions on ascorbic acid and antioxidant activity of orange juice allowed establishing the most favorable range of process conditions in order to obtain high nutritional quality of orange juice.

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