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Effect of high hydrostatic pressure treatment on post processing antioxidant activity of fresh Navel orange juice

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Abstract

Total antioxidant activity of high pressure processed (600 MPa, 40 °C, 4 min) compared to thermally pasteurised (80 °C, 60 s) fresh Navel orange juice was studied as a function of storage at different isothermal conditions (0–30 °C). The contribution of ascorbic acid – among the other antioxidant compounds of orange juice – to the total antioxidant activity was also evaluated. The reaction rate constant of *n*th order kinetics of the decolourisation of ABTS radical cation solution, after addition of orange juice, was used as a measure of the total antioxidant activity. A mathematical description of the above reaction rate constant as a function of storage temperature and time was established. Total antioxidant activity of both juices decreased during storage mainly due to ascorbic acid loss. High pressure treatment led to a better retention of the antioxidant activity of orange juice compared to conventional pasteurisation.

Keywords: Antioxidant activity; High pressure; Pasteurisation; Storage; Orange juice

1. Introduction

High hydrostatic pressure (HHP) processing is a new non-thermal technology that has been extensively proposed for application on acid fruit juices like orange juice. HHP has been found to inactivate microorganisms and denature several enzymes (Basak, Ramaswamy, & Simpson, 2001; Goodner, Braddock, & Parish, 1998; Nienaber & Shellhammer, 2001a; Parish, 1998a; Teo, Ravishankar, & Sizer, 2001; Zook, Parish, Braddock, & Balaban, 1999) without greatly affecting low molecular weight food compounds, such as vitamins, pigments, flavouring agents and other compounds related to sensory, nutritional and health related qualities of the product (Fernández-García, Butz, & Tauscher, 2000; Fernández-García, Butz, Bognar, & Tauscher, 2001a; Fernández-García, Butz, & Tauscher, 2001b; Nienaber & Shellhammer, 2001b; Parish, 1998b; Sancho et al., 1999). The above mentioned effect of HHP on the quality characteristics of food has been mainly attributed to the stability of covalent bonds to high pressure (Knorr, 1993).

A great number of antioxidants are naturally present in orange juice, being responsible for the potential protective action of orange juice against certain degenerative diseases. According to recent epidemiological studies, high consumption of orange juice is associated with a reduced risk for free radical related oxidative damage and diseases such as different types of cancer, cardiovascular or neurological diseases (Diplock, 1994;

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Hollman, Hertog, & Katan, 1996; Vinson et al., 2002). L-Ascorbic acid, carotenoids, flavonoids and other polyphenolic compounds are the most important substances of orange juice contributing to its total antioxidant activity (Gardner, White, McPhail, & Duthie, 2000; Miller & Rice-Evans, 1997; Rapisarda et al., 1999). The effect of processing or storage on the antioxidant activity of orange juice is an important issue for study. A number of different events (loss of naturally occurring antioxidants, improvement of their antioxidant activity, formation of various compounds having antioxidant or pro-oxidant properties) can occur during process or post processing storage of orange juice affecting its overall antioxidant activity (Arena, Fallico, & Maccarone, 2001; Goyle & Ojha, 1998; Lee, 1992; Lee & Chen, 1998; Lindley, 1998; Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997; Nicoli, Anese, & Parpinel, 1999).

Few works have been published on the effect of HHP on post processing total antioxidant activity of orange juice or other food systems. Fernández-Garcıa et al. (2001a) found that HHP treatment and storage up to 21 days at 4 °C caused no significant differences in antioxidant capacity, vitamin C, or carotene content of orange juice and an orange–lemon–carrot juice product. Similar or better retention of antioxidant capacity of high pressure treated products after 1 month storage at 4 °C, compared to untreated samples, was reported in apple juice and tomato puree (Fernández-Garcıa et al., 2000, 2001b). Butz et al. (2002) found that high pressure did not induce loss of health promoting substances (e.g., vitamins, antioxidants, antimutagens) of vegetables. De Ancos, Gonzalez, and Pilar Cano (2000) reported stability or even improvement of radical scavenging activity of persimmon fruit purees after HHP treatment due to the stability of carotenoids. Polydera, Stoforos, and Taoukis (2004a), working with reconstituted from frozen concentrate juice, found higher antioxidant activities for high pressurised compared to thermally pasteurised orange juice during their storage.

The objective of the present work was to comparatively study the effect of HHP and thermal pasteurisation on post processing antioxidant activity of a Greek Navel variety of fresh orange juice. Based on a new kinetic approach, the work focused on mathematically describing total antioxidant activity as a function of storage temperature and time.

2. Materials and methods

2.1. Juice samples

Orange juice of Greek Navel variety (*Citrus sinensis*), produced in an FMC (Food Manufacturing Coalition) line of a commercial juice plant in Southern Greece was obtained. No thermal treatment was applied. The juice was immediately frozen in a forced circulation freezer (MDF-U442, SANYO Electric Co., Ltd., Japan) and kept at −40 °C until use.

2.2. Selection of processing conditions

Thermal pasteurisation was conducted at 80 °C for 60 s and HHP processing at 600 MPa and 40 °C for 4 min. The selection of conditions was mainly based on pectinmethylesterase (PME) inactivation kinetics obtained in previous experiments (Polydera, Galanou, Stoforos, & Taoukis, 2004). Since PME, an enzyme responsible for cloud loss and quality deterioration of orange juice during storage, is known to be more heat and pressure resistant than the common spoilage microorganisms of orange juice (Goodner et al., 1998; Snir, Koehler, Sims, & Wicker, 1996), inactivation of PME is generally used to determine the intensity of processing conditions during thermal or HHP pasteurisation (Goodner et al., 1998; Versteeg, Rombouts, Spaansen, & Pilnik, 1980). From the conditions providing adequate PME inactivation, the ones resulting in optimum sensory quality were selected. These conditions also exceeded process requirements for microbial stability of orange juice (Polydera, Stoforos, & Taoukis, 2003).

2.3. High pressure processing

High pressure treatments were achieved using a laboratory pilot scale HHP equipment with a maximum operating pressure of 1000 MPa (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Holland) consisting of an operation high pressure unit with a pressure intensifier, a high pressure vessel of 1.5 l in volume and a multivessel system consisting of six vessels of 45 ml capacity each. All high pressure vessels were surrounded by a liquid circulating jacket connected to a heating-cooling system. The pressure transmitting fluid used was polyglycol ISO viscosity class VG 15 (Resato International BV, Roden, Holland).

For the HHP experiments, polypropylene bottles of 150 ml capacity with screw-cup closures were used. Fifty bottles were filled with orange juice and placed into the large vessel for processing. The desired value of pressure (600 MPa) was set and after pressure build-up (about 1 min), the pressure vessel was isolated. This point defined the time zero of the process. Pressure was released after a preset time interval (4 min) by opening the pressure valve. The initial temperature increase during pressure build-up (about 3 °C/100 MPa) was taken into consideration in order to achieve an average operating temperature of 40 °C during pressurisation. In order to achieve this, the samples and the pressure transmitting fluid were initially kept at a temperature of 27 °C. After the pressure increased, the temperature also increased because of adiabatic heating, reaching approximately 45 °C (at

time zero of the process). The temperature decreased to 40 °C in a relatively short time (about 60 s), while in the remaining 180 s it changed from 40 to 36 °C, giving an average operating temperature of ≈40 °C. Pressure and temperature were constantly monitored and recorded (in 1 s intervals) during the process.

2.4. Thermal pasteurisation

Orange juice was pasteurised in a pilot scale pasteuriser with a tubular heat exchanger (Armfield FT74, HTST/UHT Processing Unit, Hampshire, England) at 80 °C for a holding time of 60 s and then cooled to 20 °C. The pasteurised juice was aseptically transferred into packages identical to the ones used for high pressure pasteurisation.

2.5. Storage study

Samples of thermally and high pressure pasteurised orange juice were stored immediately after processing (time 0) at five different isothermal conditions (0, 5, 10, 15 and 30 °C) in temperature programmable control cabinets (Sanyo MIR 153, Sanyo Electric Co, Ova-Gun, Gunma, Japan). Ten samples per process and storage condition were used. Storage temperature was constantly recorded by type T thermocouples and a multichanell datalogger (CR10X, Campbell Scientific, Shepshed, Lougborough, UK). Samples were evaluated at time 0 and at regular time intervals for a total of 1–3 months depending on storage temperature. Total antioxidant activity and L-ascorbic acid were measured.

2.6. Antioxidant activity determination

An improved ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt) radical cation decolorization assay was used, which involves the direct production of the blue/green $ABTS^{\cdot+}$ chromophore through the reaction between ABTS (Sigma, Steinheim, Germany) and potassium persulfate (Merck, Darmstadt, Germany). Addition of antioxidants to the preformed radical cation reduces it to ABTS, to an extent and on a time-scale depending on the antioxidant activity, the concentration of the antioxidant and the duration of the reaction (Re et al., 1999). ABTS was dissolved in water to a 7 mM concentration. $ABTS^{\cdot+}$ was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.7 (±0.02) at 752 nm (30 °C).

In order to measure the antioxidant activity of orange juice samples stored at a particular temperature for a specific time period, 20 μl of orange juice were added to 4 ml of diluted $ABTS^{\cdot+}$ solution and the absorbance was recorded every 30 s for a total of 30 min. Each orange juice sample was analysed in triplicate. Appropriate solvent blanks were run in each assay.

In most cases, Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) is used as a standard for comparison for the determination of the antioxidant activity of a compound. The results are reported as the Trolox equivalent antioxidant capacity (TEAC), which is the concentration of the antioxidant giving the same percentage decrease of absorbance of the ABTS radical cation as 1 mM Trolox, at a specific time point (Re et al., 1999; Van den Berg, Haenen, Van den Berg, Van den Vijgh, & Bast, 2000). Such an approach may be suitable for the description of the antioxidant activity of compounds that react instantaneously with free radicals (e.g., ascorbic acid) without being affected by the reaction time. On the contrary, when scavenging of free radicals by an antioxidant compound (e.g., flavonoids) is a function of time, the antioxidant activity depends not only on the amount of the reduced free radicals at a specific time period, but also on the reaction rate. The time dependence mentioned above is usually observed for the antioxidant activity of a mixture of compounds or a real food, such as orange juice. In the present work, the decrease of absorbance of the $ABTS^{\cdot+}$ solution after the addition of orange juice was monitored as a function of time and was mathematically described. The calculated parameters were used as a measure of antioxidant activity.

The contribution of different antioxidant compounds of orange juice to its total antioxidant activity was determined by the methodology described by Polydera et al. (2004a). The $ABTS^{\cdot+}$ decolourisation curve after the addition of orange juice was divided in two different stages: an initial abrupt decrease of absorbance, from 0.7 to a value depending on ascorbic acid concentration, and a further time dependent decrease of absorbance due to other antioxidant compounds, such as flavonoids. In order to estimate the fraction of the total antioxidant activity that could be attributed to ascorbic acid content of the juice samples, the antioxidant activity of samples of ascorbic acid solutions of known concentration, was determined. L-Ascorbic acid (Merck KGaA, Darmstadt, Germany) was prepared as stock solution in water to a concentration of 5 mM and diluted to concentrations in the range of 6 to 20 μM. 20 μl of each L-ascorbic acid solution was added to 4 ml of diluted $ABTS^{\cdot+}$ solution. The absorbance decrease, which was instant, was linearly plotted as a function of L-ascorbic acid concentration. The relationship determined, describing the residual absorbance (A_r) as a function of ascorbic acid concentration (C, mg/100 ml orange juice), is given in the following equation:

$$A_r = 0.7 - 0.00949 \cdot C. \quad (1)$$

2.7. Determination of L-ascorbic acid

L-Ascorbic acid concentration was determined using an HPLC method. Samples of 1 ml of orange juice were extracted with equal volumes of 4.5% (w/v) metaphosphoric acid solution and filtered through a 0.45 μm GHP Acrodisc filter. An aliquot then was injected into the chromatographic column. The chromatographic system (HP 1100 Series, Waldbronn, Germany) consisted of a quaternary pump, a vacuum degasser, a Rheodyne 20 μl injection loop, a Diode-Array Detector, and it was controlled through HP ChemStation software. A Hypersil ODS column (250 × 4.6 mm, particle size 5 μm) fitted with a Hypersil ODS guard column was utilised with a mobile phase of HPLC grade water with metaphosphoric acid to pH 2.2 at a flow rate of 0.5 ml/min. The detection was at 245 nm (Oruña-Concha, González-Castro, López-Hernández, & Simal-Lozano, 1998). Results were calculated as mg of L-ascorbic acid per 100 ml of orange juice. Each sample was prepared and analysed in duplicate.

2.8. Statistical analysis

Parameters of Eqs. (2)–(4) were determined from the experimental data by non-linear regression (SYSTAT® 8.0 Statistics, 1998, SPSS Inc., Chicago, IL, USA). Parameters of Eq. (5) were estimated through the non-linear regression routine of Sigma Plot, Version 7, 2001, SPSS Inc.

3. Results and discussion

3.1. Total antioxidant activity of orange juice during storage

The change of absorbance as a function of time during $ABTS^{\bullet+}$ decolourisation, after the addition of orange juice as a source of antioxidants, was found to follow

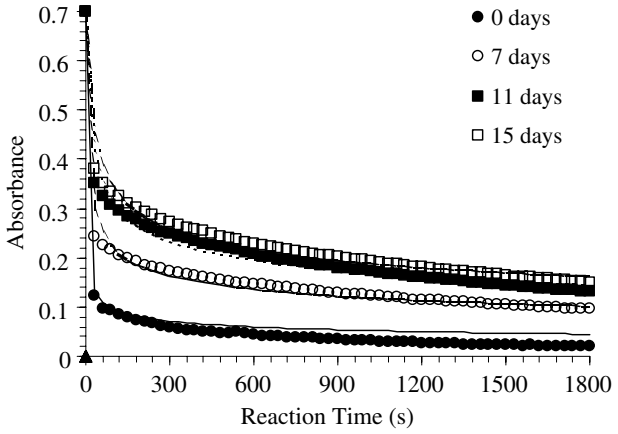

Fig. 1. $ABTS^{\bullet+}$ decolourisation curve indicating total antioxidant activity of high pressurised orange juice as a function of storage time at 30 °C. Symbols refer to experimental data, while lines represent predicted values determined through Eq. (1).

*n*th order kinetics (Eq. (2)), as shown in Fig. 1 for high pressurised orange juice stored at 30 °C

$$A = [(n-1)\cdot k\cdot t + A_O^{1-n}]^{1/1-n}, \quad (2)$$

where A is the absorbance of the solution at time t, $A_O = 0.7$ the initial absorbance of $ABTS^{\bullet+}$ solution before addition of orange juice, n the apparent reaction order and k a constant related with the reaction rate.

For the determination of total antioxidant activity of each orange juice sample, parameters n and k of Eq. (2) were estimated through non-linear regression for all different combinations of storage temperature and time for both treatments. Very small variation in the value of parameter n was obtained for all conditions tested close to an average value equal to 4.81, while k values depended on process and storage conditions. For the established n value, the reaction rate constant, k, which is a measure of the total antioxidant activity of each orange juice sample, was determined for each process and each storage temperature and time combination (Tables 1 and 2).

Table 1
Rate constant, k (s^{-1}), of the decolourisation reaction of $ABTS^{\bullet+}$ solution after the addition of high pressure processed orange juice as a function of storage temperature and time

Storage time (days)	Storage temperature (°C)				
	0	5	10	15	30
0	20.0 (0.98[a])	20.0 (0.98)	20.0 (0.98)	20.0 (0.98)	20.0 (0.98)
7	–	–	10.74 (0.99)	1.91 (0.99)	0.837 (0.99)
15	15.8 (0.99)	1.47 (0.98)	2.20 (0.99)	0.882 (0.98)	0.146 (0.97)
27	1.07 (0.98)	0.357 (0.94)	0.406 (0.95)	0.152 (0.95)	0.218 (0.98)
46	–	–	0.225 (0.96)	0.108 (0.94)	–
64	4.18 (0.98)	–	0.662 (0.96)	0.085 (0.94)	–
90	0.411 (0.97)	0.498 (0.96)	–	–	–

[a] R^2: regression correlation coefficient for fitting of Eq. (2) on experimental data ($n = 4.81$).

Table 2
Rate constant, k (s^{-1}), of the decolourisation reaction of $ABTS^{\bullet+}$ solution after the addition of thermally pasteurised orange juice as a function of storage temperature and time

Storage time (days)	Storage temperature (°C)				
	0	5	10	15	30
0	10.4 (0.99[a])	10.4 (0.99)	10.4 (0.99)	10.4 (0.99)	10.4 (0.99)
5	–	–	–	–	0.812 (0.98)
7	–	–	0.852 (0.98)	0.210 (0.96)	–
10	3.53 (0.99)	7.92 (0.99)	–	–	0.294 (0.98)
17	–	–	–	–	0.140 (0.95)
20	–	–	0.939 (0.99)	0.151 (0.97)	–
27	1.53 (0.99)	0.297 (0.97)	1.09 (0.99)	0.172 (0.96)	–
41	3.770 (0.99)	0.344 (0.97)	0.053 (0.94)	0.042 (0.95)	–
60	0.564 (0.98)	0.124 (0.96)	0.032 (0.95)	0.009 (0.98)	–
90	0.223 (0.95)	0.289 (0.92)	–	–	–

[a] R^2 : regression correlation coefficient for fitting of Eq. (2) on experimental data ($n = 4.81$).

A decrease of k values and therefore a decrease of total antioxidant activity of both high pressure and thermally pasteurised orange juice was observed as a function of storage time at each temperature studied, as it is shown in Tables 1 and 2, respectively. The decrease of total antioxidant activity of high pressure treated orange juice during storage at 30 °C is indicatively illustrated in Fig. 1. The loss of antioxidant activity during storage is clearly shown by the progressive reduction in the rate of change in absorbance between day 0 and day 15. Increase of storage temperature resulted in a small decrease of total antioxidant activity of both treated orange juices (Tables 1 and 2).

Compared to conventional pasteurization, high pressure treatment led to higher total antioxidant activity of orange juice immediately after processing (time 0 of storage), as well as during storage at 0–30 °C (Tables 1 and 2). This is in agreement with results in orange juice reconstituted from frozen concentate (Polydera et al., 2004a). The $ABTS^{\bullet+}$ decolourisation curves after the addition of high pressure or thermally pasteurised orange juice used for the determination of total antioxidant activity just after processing (time zero of storage), are comparatively presented in Fig. 2. The greater absorbance decrease observed for high pressure treatment compared to thermal pasteurisation corresponds to higher total antioxidant activity.

In order to investigate more systematically the effect of storage time and temperature on the total antioxidant activity of orange juice, the rate constant, k, of the $ABTS^{\bullet+}$ decolourisation was expressed mathematically as a function of the above parameters for both high pressure and thermally treated juice. An exponential decay of the reaction rate, k, was observed with storage time of orange juice at constant temperature for both treatments, fitting experimental data better compared to other models tested. Considering the previous mentioned exponential relationship, Eq. (2) was transformed into the following equation:

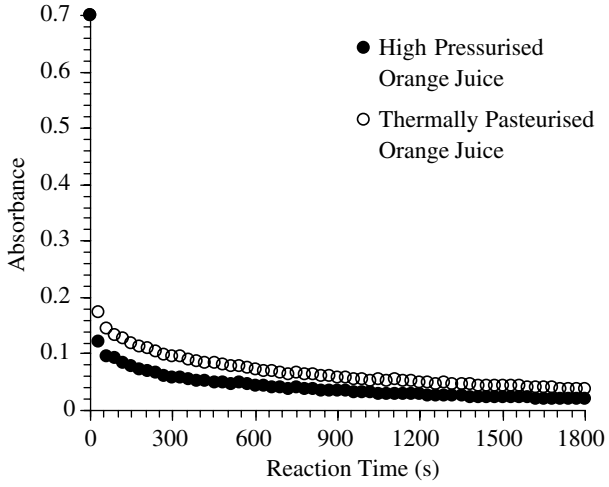

Fig. 2. $ABTS^{\bullet+}$ decolourisation curve indicating total antioxidant activity of both high pressurised and thermally pasteurised orange juice immediately after processing (zero storage time).

$$A = [(n-1)\cdot k_O \cdot \exp(-b\cdot t_{st})\cdot t + A_O^{1-n}]^{1/1-n} \quad (3)$$

where A is the absorbance of the solution at time t, $A_O = 0.7$ the initial absorbance of $ABTS^{\bullet+}$ solution before addition of orange juice, n the apparent reaction order, k_O (s^{-1}) the reaction rate after addition of orange juice at zero storage time, and b ($days^{-1}$) a constant describing the effect of storage time (t_{st}, days) on the reaction rate.

The values of parameter b for each storage temperature and process were estimated through a non-linear regression from Eq. (3) for $n = 4.81$ and k_O equal to 20.0 and 10.4 s^{-1} for high pressure and thermally treated orange juice, respectively, and presented in Table 3. A similar average value of n was determined through Eq. (3) for all experimental data when it was not defined to be equal to the particular value of 4.81. An increase of storage temperature led to an increase of parameter b, meaning a more rapid decrease of reaction rate k

Table 3
Parameter b (days^{-1}) of Eq. (3) for each storage temperature and each type of processing

Storage temperature (°C)	High pressure processed orange juice		Thermally pasteurised orange juice	
	b (days^{-1})	R^2	b (days^{-1})	R^2
0	0.041	0.90	0.044	0.96
5	0.045	0.75	0.051	0.76
10	0.070	0.81	0.103	0.90
15	0.096	0.80	0.125	0.89
30	0.217	0.94	0.273	0.91

(and therefore total antioxidant activity of orange juice) as a function of storage time at constant temperature. Lower b values were calculated for high pressure processed compared to thermally pasteurised orange juice for each storage temperature, indicating a smaller decrease of total antioxidant activity of high pressurised juice as a function of storage time.

Taking into consideration the exponential dependence of the reaction rate k on storage time, t_{st}, as well as a linear correlation of parameter b with storage temperature, T, the rate constant, k, of the ABTS$^{\cdot+}$ decolourisation (Eq. (2)), which is used for the quantification of total antioxidant activity of orange juice, was expressed as:

$$k = k_O \cdot \exp[-(a \cdot T + c) \cdot t_{st}], \quad (4)$$

where k_O (s^{-1}) is the reaction rate after addition of orange juice at zero storage time, a [(days °C)$^{-1}$] a constant describing the effect of storage temperature (T, °C) on parameter b, and c (days^{-1}) the parameter b value at 0 °C.

The linear correlation of parameter b and storage temperature for both treatments was preferred among other equations tested, since it was found to lead to a better correlation coefficient, R^2, for the description of the reaction rate k as a function of storage temperature and time. The parameters of Eq. (4) were determined through a non-linear regression for the total of experimental data of reaction rate k at all combinations of storage temperature and time. The estimated parameter values are presented in Tables 4 and 5 for high pressure and thermally pasteurised orange juice, respectively. Similar temperature dependence of parameter b was found for both processes (0.012 and 0.013 (days °C)$^{-1}$ for high pressure and thermally pasteurised orange juice, respectively). Slightly lower c value (b value for storage at 0 °C) was determined for high pressurised compared to thermally pasteurised orange juice, indicating a relatively smaller effect of storage time on total antioxidant activity of high pressurised juice.

Table 4
Estimation of parameters of Eq. (4) for high pressure processed orange juice ($R^2 = 0.94$)

Parameter	Value	95% Confidence interval	
		Lower limit	Upper limit
K_O (s^{-1})	20.23	18.3	22.2
a [(days °C)$^{-1}$]	0.012	0.007	0.018
c (days^{-1})	0.042	0.025	0.059

Table 5
Estimation of parameters of Eq. (4) for thermally pasteurised orange juice ($R^2 = 0.90$)

Parameter	Value	95% Confidence interval	
		Lower limit	Upper limit
K_O (s^{-1})	10.2	9.01	11.5
a [(days °C)$^{-1}$]	0.013	0.005	0.020
c (days^{-1})	0.052	0.026	0.078

3.2. Contribution of different antioxidant compounds of orange juice to its total antioxidant activity

Among compounds exhibiting antioxidant activity in orange juice, vitamin C is the most important antioxidant compound accounting for 65–90% of the total antioxidant activity of orange juice (Gardner et al., 2000; Miller & Rice-Evans, 1997). The reaction between L-ascorbic acid and ABTS radical cation practically occurs instantaneously (Re et al., 1999). On the contrary, the reaction between most flavonoids and ABTS radical cation is time dependent resulting in continuously decreasing values of absorbance as a function of time (Madsen, Andersen, Jørgensen, & Skibsted, 2000).

In order to study the contribution of L-ascorbic acid to the total antioxidant activity of orange juice according to the methodology described previously, the absorbance (A_r) at the end of the first stage of the ABTS$^{\cdot+}$ decolourisation curve, after addition of each orange juice sample to the ABTS$^{\cdot+}$ solution, was calculated through Eq. (1). The % change (decrease) of absorbance during the first stage of each curve, which is a measure of the antioxidant activity of orange juice due to its ascorbic acid concentration, was calculated as a function of storage time at all different isothermal conditions for both high pressure processed and thermally pasteurised orange juice. This absorbance change is depicted indicatively for different storage temperature and time conditions for both differently processed juices in Fig. 3. The greater the % change of absorb-

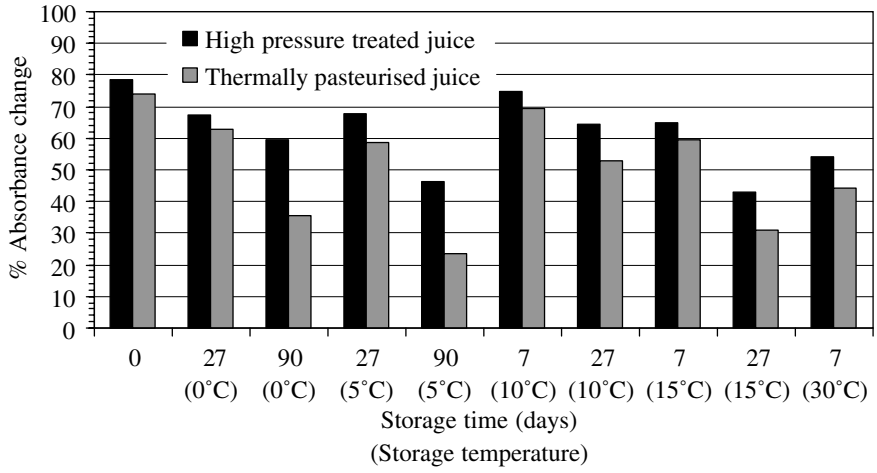

Fig. 3. The % decrease of absorbance caused in the ABTS$^{\cdot+}$ solution by the addition of high pressurised and thermally pasteurised orange juice, due to ascorbic acid antioxidant activity (first stage), at different storage temperature and time conditions.

ance (taller columns in Fig. 3), the higher the antioxidant activity of the respective sample. Therefore, a decrease of the antioxidant activity – due to ascorbic acid loss – was observed as a function of storage time for both high pressurised and conventionally pasteurised orange juice at 0–30 °C. Furthermore, better retention of the antioxidant activity of orange juice due to ascorbic acid contribution was found in case of high pressurised juice, in agreement to previous findings (Polydera et al., 2004a).

The change of absorbance during the second stage of the ABTS$^{\cdot+}$ decolourisation curve, which is the result of the antioxidant activity of other orange juice constituents besides L-ascorbic acid (such as flavonoids or other phenolic compounds), was described by a four-parameter exponential decay model (Polydera et al., 2004a):

$$A = a' \cdot \exp(-b' \cdot t) + c' \cdot \exp(-d' \cdot t), \quad (5)$$

where A the absorbance of ABTS$^{\cdot+}$ solution at time t, a' and c', parameters, the sum of which is equal to the final absorbance value of the previous stage (that is, A_r), b' and d', parameters relative to reaction rate and, therefore, to antioxidant activity, and t the time of decolourisation assay.

Setting the final absorbance of the first stage of ABTS$^{\cdot+}$ decolourisation curve (A_r) as the initial absorbance of the second stage, parameters a', b', c', and d' of Eq. (5) were determined through non-linear regression for the orange juice samples of both treatments during storage at 0–30 °C. Values for parameter b' were significantly greater than respective values of parameter d'. Thus, the rate of the reaction of ABTS$^{\cdot+}$ radicals with the compounds of higher antioxidant activity except for ascorbic acid, as described by parameter b', was used as a measure of antioxidant activity of each sample during the second stage of the decolourisation curve. Parameter b' values for storage at 15 °C and different storage times are presented in Table 6 for both high pressure and thermally treated juice.

Table 6
Parameter b' describing the second stage of ABTS$^{\cdot+}$ decolourisation curve during storage at 15 °C of high pressurised (b'_{HP}) and thermally pasteurised (b'_{TP}) orange juice

Storage time (days)	b'_{HP}	R^2	b'_{TP}	R^2
0	0.0119 ± 0.0006*	0.99	0.0069 ± 0.0004	0.99
7	0.0075 ± 0.0003	0.99	0.0035 ± 0.0009	0.98
20	–		0.0112 ± 0.0010	0.99
27	0.0103 ± 0.0007	0.99	0.0217 ± 0.0021	0.99
41	–		0.0193 ± 0.0018	0.99
46	0.0143 ± 0.0011	0.99	–	
60	–		0.0117 ± 0.0011	0.99
64	0.0240 ± 0.0018	0.99	–	

* standard error, $P < 0.0001$ for all storage times.

Storage time did not seem to affect to a great extent the antioxidant activity of orange juice during the second stage of the decolourisation curve for both processes. A general tendency of b' values (and therefore antioxidant activity during the second stage) to slightly increase as a function of storage time was observed for both high pressurised and pasteurised orange juice after prolonged storage at higher temperatures (e.g., 15 °C, Table 6). The above described change of antioxidant activity was possibly the result of different actions that can take place during storage of orange juice, among them the formation of new antioxidant compounds, e.g., later Maillard reaction products (Arena et al., 2001; Lindley, 1998; Nicoli et al., 1997, 1999).

The type of processing used, high pressure or thermal treatment, did not seem to substantially affect the antioxidant activity of orange juice during the second stage of the decolourisation curve. Similar antioxidant activity change as a function of storage time was observed for the two processes (Table 6).

The total antioxidant activity of orange juice was the sum of the activities during the two stages of the decolourisation curve, with ascorbic acid constituting the most important antioxidant contributor of orange juice (40–80% contribution to total antioxidant activity). The decrease of the total antioxidant activity of orange juice that was found during storage (Fig. 1 for 30 °C, Tables 1 and 2) can be therefore mainly attributed to ascorbic acid loss. Furthermore, the higher overall antioxidant activity of high pressure compared to thermally treated orange juice during storage at 0–30 °C (Fig. 2, Tables 1 and 2) was the result of a better retention of ascorbic acid which was observed for high pressurised juice in a parallel study (Polydera & Taoukis, 2004b).

4. Conclusions

Total antioxidant activity of orange juice, which was expressed through the rate constant of *n*th order kinetics of the $ABTS^{\bullet+}$ decolourisation curve after addition of orange juice, was described mathematically as a function of storage time and temperature conditions. Different constituents contributed to the total antioxidant activity of orange juice. L-Ascorbic acid constituted the most important antioxidant compound reacting instantaneously with free radicals, while the reaction of other antioxidants of orange juice (mainly flavonoids and other polyphenolic compounds) was time dependent, being described by the sum of two exponential decay functions of different rate. A high pressure treatment of 600 MPa at 40 °C for 4 min led to a better retention of antioxidant activity during post processing storage of orange juice at 0–30 °C compared to conventional thermal pasteurisation (80 °C, 60 s), mainly due to lower ascorbic acid degradation rates.

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