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IMPACTO DA TECNOLOGIA DE ALTA PRESSÃO HIDROSTÁTICA SOBRE A QUALIDADE DO SUCO DE LARANJA

Dissertação apresentada ao Programa de Pós-graduação em Alimentos e Nutrição da Faculdade de Ciências Farmacêuticas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, como parte dos requisitos para obtenção do título de Mestre em Alimentos e Nutrição

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SUMÁRIO

LISTA DE FIGURAS

LISTA DE TABELAS

Capítulo 1. OPTIMISATION OF HIGH HYDROSTATIC PRESSURE PROCESSING OF PÊRA RIO ORANGE JUICE

Capítulo 2. EFFECT OF HIGH HYDROSTATIC PRESSURE ON ASCORBIC ACID AND ANTIOXIDANT ACTIVITY OF ORANGE JUICE

Capítulo 3. EFEITO DO PROCESSAMENTO DE ALTA PRESSÃO HIDROSTÁTICA NA QUALIDADE DO SUCO DE LARANJA VAR. PÊRA RIO

RESUMO

O suco de laranja é o suco de fruta mais popular e mais consumido em todo mundo. O Brasil é o maior produtor e exportador mundial de suco de laranja. O consumo de suco de laranja é estimado em mais de 9 bilhões de litros por ano, representando 47% do consumo mundial de suco. A fim de aumentar a vida de prateleira e a estabilidade do suco de laranja, a tecnologia de alta pressão hidrostática (APH) pode ser utilizada como um método alternativo à pasteurização. Este trabalho teve como objetivo otimizar as condições de processamento do suco de laranja da variedade Pêra Rio por APH, pressão (100-600 MPa), temperatura (30-60 °C) e tempo (30-360 s), avaliar o efeito das condições do processamento de APH no ácido ascórbico e na atividade antioxidante do suco de laranja e avaliar a qualidade do suco de laranja processado a 520 MPa, 60 °C por 360 s, comparativamente ao suco pasteurizado e não processado. A metodologia de superfície de resposta foi empregada para estimar a redução da atividade da enzima pectinametilesterase (PME), da contagem de microrganismos, do ácido ascórbico e da atividade antioxidante no suco de laranja processado por APH. Também foram avaliados, as características físico-químicas, a cor instrumental, os compostos fenólicos e a atividade antioxidante do suco de laranja. O aumento da pressão, temperatura e tempo reduziram a atividade da PME e a contagem de microrganismos, exceto pela região entre 170 a 310 MPa, 30 a 45 °C e 30 a 150 s, que aumentou a atividade da PME. A faixa ótima de processamento por APH foi 550 a 600 MPa, 55 a 60 °C e 330 a 360 s, sendo capaz de produzir suco de laranja com população de microrganismos de menos de 2 ciclos logarítmicos de UFC/mL e atividade residual da PME menor que 20%. Ainda, o aumento da temperature e do tempo do processamento por APH reduziu o teor de ácido ascórbico e a atividade antioxidante do suco de laranja. As condições de APH de 100-250 MPa, 30-40 °C e 30-125 s foram capazes de produzir suco de laranja com mais de 70% do teor inicial de ácido ascórbico e 80% da atividade antioxidante. Usando o modelo preditivo para a atividade residual da PME e para a contagem de microrganismos, o suco de laranja Pêra Rio foi processado a 520 MPa, 60 °C por 360 s e comparado ao suco pasteurizado (95 °C por 30 s). O processamento por APH e a pasteurização reduziram a atividade residual da PME para 13% e 4%, respectivamente, e destruíram os microrganismos a níveis não detectáveis.

Ambos os processamentos aumentaram a luminosidade e a cor vermelha e amarela do suco de laranja. O ácido ascórbico foi reduzido pela APH e pasteurização, mas o teor de compostos fenólicos totais e a atividade antioxidante não foram afetados. A tecnologia de APH pode ser utilizada como alternativa à pasteurização do suco de laranja da variedade Pêra Rio.

ABSTRACT

Orange juice is the most popular and consumed fruit juice in the world. Brazil is the main orange juice world producer and exporter. The consumption of orange juice is estimated at more than 9 billion liters per year, which represents 47% of global fruit juice consumption. In order to improve orange juice shelf life and stability, high hydrostatic pressure (HHP) processing can be used as an alternative to pasteurization. The aim of this work was to optimize the HHP processing conditions of Pêra Rio orange juice, namely pressure (100-600 MPa), temperature (30-60 °C) and time (30-360 s), to evaluate the effect of HHP processing conditions on ascorbic acid and antioxidant activity of orange juice and to evaluate the quality of orange juice processed at 520 MPa, 60 °C for 260 s in comparison to pasteurized and non processed orange juice. Response surface methodology was used to estimate the decrease of microflora, pectinmethyl esterase (PME) activity, ascorbic acid and antioxidant activity in HHP processed Pêra Rio orange juice. The physicalchemical characteristics, the instrumental color, the total phenolic compounds and the antioxidant activity of orange juice were also evaluate. The increase in pressure, temperature and time reduced the PME activity and native microflora of orange juice, except at the region between 170 to 310 MPa, 30 to 45 °C and 30 to 150 s, which enhanced PME activity. The range of optimum HHP processing conditions were 550 to 600 MPa, 55 to 60 °C and 330 to 360 s, which was able to produce a stable orange juice with microorganisms population less than 2 log cycles CFU/mL and PME residual activity less than 20%. The increase in time and temperature of HHP exert influence on the reduction of ascorbic acid and antioxidant activity on orange juice. The HHP conditions of 100-250 MPa, 30-40 °C and 30-125 s were able to produce orange juice with more than 70% of the initial ascorbic acid content and 80% of the antioxidant activity. Using the mathematical predictive model for PME residual activity and microorganisms' count, the Pêra Rio orange juice was processed at 520 MPa, 60 °C for 360 s and compared with pasteurized orange juice (95 °C for 30 s). The HHP processing and pasteurization reduced PME residual activity to 13% and 4%, respectively, and inactivated microorganisms to non detectable levels. Both processes increased lightness, red and yellow color of orange juice. Ascorbic acid was reduced by HHP and pasteurization, although total phenolic compounds and antioxidant activity

were not affected. The HHP technology can be considered as an effective alternative to pasteurization of Pêra Rio orange juice.

INTRODUÇÃO

INTRODUÇÃO

O Brasil é o maior produtor e exportador mundial de suco de laranja. Na safra 2012/2013, o país produziu 2,15 milhões de ton de suco de laranja e exportou 2,09 milhões de ton, que representaram 97% do total, dos quais 1,12 milhões de ton foram de suco de laranja não concentrado (Not From Concentrate, NFC), 582 mil ton de suco concentrado e congelado (Frozen Concentrated Orange Juice, FCOJ) e 391 mil ton de suco de laranja destinado a outras bebidas. O volume de suco exportado gerou divisas da ordem de US$ 2,30 bilhões de dólares (CITRUSBR, 2013).

Diversos estudos apontam a preferência do consumidor por suco de laranja espremido na hora do consumo, que mantém o sabor e aroma natural caraterístico de laranja e vem sendo relacionado ao conceito de saudável (FIESP e ITAL, 2010; CAMPOS et al., 2006; MIN, et al., 2003; TORRE, et al., 2003). Por isso, o NFC vem sendo muito valorizado pelo consumidor frente ao FCOJ, que fica exposto à alta temperatura por um período maior que o NCF, o que altera drasticamente o aroma e sabor (JANZANTTI et al., 2011). Contudo, o FCOJ apresenta a vantagem de ter maior estabilidade microbiológica e custo de transporte menor, devido ao processo de concentração (QUEIROZ e MENEZES, 2010).

O tratamento à alta pressão é uma das tecnologias mais inovadoras para processar produtos termossensíveis. O uso de pressões de 100 a 1000 MPa provoca destruição microbiana e retarda significativamente as taxas de reações enzimáticas, minimizando a formação de sabores estranhos e o escurecimento não-enzimático. Desta forma, ocorre pouca perda de nutrientes e vitaminas e as alterações no sabor são praticamente imperceptíveis (TEWARI, 2007).

O processo de alta pressão hidrostática (APH) consiste em submeter o alimento à alta pressão, normalmente de 50 a 1000 MPa, dentro de um tanque pressurizado, contendo um meio liquido, geralmente água potável (HOGAN, KELLY e SUN, 2005). O processo é isostático, ou seja, a pressão é transmitida de maneira uniforme e instantaneamente, e adiabático, o que significa que não importa a forma ou tamanho dos alimentos, diferentemente dos processos térmicos. Além disso, ocorre pouca variação de temperatura com o aumento da pressão. A temperatura aumenta aproximadamente 3 °C para cada acréscimo de

100 MPa no processo, dependendo da composição do alimento (BUZRUL et al., 2008). Essas características impedem que os alimentos sejam deformados ou aquecidos em excesso, o que poderia alterar as suas características nutricionais e sensoriais.

Em termos gerais, a APH aplicada à temperatura ambiente é capaz de destruir células vegetativas e inativar enzimas, com mínima alteração sensorial do alimento (SAN MARTIN, BARBOSA-CANOVAS e SWANSON, 2002). A eficácia do tratamento depende principalmente da pressão aplicada e do tempo de retenção, e a resistência dos microrganismos é variável, dependendo do tipo de organismo e da matriz do alimento (FARKAS e HOOVER, 2000). Atualmente, existem mais de 150 equipamentos industriais de APH em diversos países de todos os continentes, processando tipos variados de alimentos (SHARMA, 2010).

A qualidade do suco de laranja é influenciada pelas características físico-químicas, microbiológicas e enzimáticas, capazes de comprometer, sobretudo, as características sensoriais de aparência, aroma, sabor e turbidez do suco, acarretando rejeição do produto por parte do consumidor (JANZANTTI et al., 2011; QUEIROZ e MENEZES, 2010; FRANCO, 2003).

As características físico-químicas influenciam a qualidade do suco de laranja e têm sido associadas ao tipo de tratamento térmico, às condições de estocagem, ao tipo de embalagem e à presença de luz, entre outros fatores (TEIXEIRA e MONTEIRO, 2006; SHAW, NAGY e ROUSEFF, 1993). O processo de conservação do suco de laranja mais conhecido é a pasteurização, que afeta suas características sensoriais e nutricionais. As condições empregadas na pasteurização podem alterar os compostos voláteis responsáveis pelo sabor característico do suco, que em sua maioria são substâncias termolábeis, sujeitas a rearranjos, ciclização, oxidação, etc., quando submetidas ao aumento de temperatura (FRANCO, 2003).

Existem diversos microorganismos em sucos de fruta. No suco de laranja, devido ao baixo pH, a microflora é limitada às bactérias ácido-tolerantes e aos fungos, sendo as bactérias do gênero *Lactobacillus* e *Leuconostoc* as mais comuns no suco de laranja recém-extraído. Essas bactérias causam deterioração no suco de laranja produzindo dióxido de carbono, acido lático e diacetil, que têm aroma e sabor desagradável (SHAW, NAGY e ROUSEFF, 1993). O tratamento do suco de laranja por APH permite reduzir em até 7 ciclos logarítmicos a população

de *Lactobacillus plantarum* e *Lactobacillus brevis*, e 5 ciclos logarítmicos de *Leuconostoc mesenteroides*, nas condições de 350 MPa a 35 °C por 2 min e 350 MPa a 20 °C por 10 min, respectivamente (KATSAROS et al., 2010; BASAK, RAMASWAMY e PIETTE, 2002). As leveduras resistem a pH ácido e apresentam maior resistência térmica que as bactérias láticas, sendo a espécie *Saccharomyces cerevisiae* a causa mais comum de deterioração nos sucos de fruta. Durante a deterioração são produzidos dióxido de carbono e alcoóis, podendo também haver formação de películas e ocorrer floculação (QUEIROZ e MENEZES, 2010; SHAW, NAGY e ROUSEFF, 1993). Parish (1998) demonstrou que a pressurização do suco de laranja a 400 MPa por cerca de 40 s foi suficiente para diminuir em 4 ciclos logarítmicos a população de *Saccharomyces cerevisiae*.

Um dos principais problemas associados com a qualidade do suco de laranja é a perda da estabilidade, com consequente decantação da matéria sólida e, posteriormente, o suco pode se tornar opaco e gelificar. A estabilidade do suco de laranja é atribuída principalmente à atividade da enzima pectinametilesterase (PME), que também é utilizada para determinar a intensidade do tratamento térmico durante a pasteurização comercial (VERSTEEG et al., 1980). Como a PME apresenta maior resistência ao calor e à pressão comparada àquela dos microrganismos deteriorantes do suco de laranja, sua inativação geralmente é usada como índice de eficiência do tratamento térmico e da APH (GOODNER, BRADDOCK e PARISH, 1998; VERSTEEG et al., 1980).

Geralmente, as características sensoriais relacionadas ao frescor dos sucos e produtos de frutas não são alteradas pelo tratamento de alta pressão, já que os compostos voláteis responsáveis pelo aroma e sabor não são diretamente afetados. Conforme observado em vários estudos, suco de laranja da variedade Navel (BAXTER et al., 2005), suco de goiaba (YEN e LIN, 1999) e polpa de morango (LAMBERT et al., 1999) tratados sob pressão de 200-600 MPa combinados com temperatura ambiente, praticamente não apresentaram alterações no perfil de compostos voláteis.

A atividade da enzima PME, a contagem de microrganismos, as características físico-químicas, o ácido ascórbico, os compostos fenólicos totais e a atividade antioxidante do suco de laranja processado por APH permitirão avaliar o efeito desta tecnologia na qualidade do suco.

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OBJETIVOS

OBJETIVOS

Este trabalho teve como objetivo avaliar o impacto da tecnologia de alta pressão hidrostática (APH) na qualidade do suco de laranja.

Os objetivos específicos foram:

Realizar o estudo de otimização das condições de processamento (pressão, temperatura e tempo) do suco de laranja por APH, utilizando a metodologia de superfície de reposta, a fim de obter a faixa de processamento ótima com relação à atividade enzimática e microbiológica;

Avaliar o efeito das condições de processamento por APH sobre o teor de ácido ascórbico e a atividade antioxidante do suco de laranja utilizando a metodologia de superfície de resposta;

Processar o suco de laranja por APH na condição escolhida;

Avaliar a atividade da enzima pectinametilesterase, a contagem de microrganismos, as características físico-químicas, a atividade antioxidante, os compostos fenólicos totais e a cor instrumental do suco de laranja processado por APH comparativamente ao suco pasteurizado e fresco.

CAPÍTULO 1

OTIMIZAÇÃO DO PROCESSAMENTO POR ALTA PRESSÃO HIDROSTÁTICA DO SUCO DE LARANJA PÊRA RIO

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Optimisation of High Hydrostatic Pressure Processing of Pêra Rio Orange Juice

Short Title: High Pressure Processing of Orange Juice

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Abstract

The influence of high hydrostatic pressure (HHP) on Pêra Rio orange juice was investigated using response surface methodology. A central composite design was used to evaluate the effects of three processing conditions (independent variables), namely pressure (100-600 MPa), temperature (30-60 °C) and time (30-360 s), on the native microflora and pectin methylesterase (PME) activity of orange juice. Analysis of variance showed that second order polynomial models fitted well with the experimental data for PME residual activity (R^2=0.9586, p<0.001) and aerobic microorganisms count (R^2=0.9879, p<0.001). The optimum HHP processing conditions to produce orange juice with PME residual activity less than 20% and low microorganisms count (< 2 log cycles CFU/mL) were 550 to 600 MPa, 55 to 60 °C and 330 to 360 s.

Keywords: High hydrostatic pressure, Orange juice; Pêra Rio variety; Response surface methodology; Pectin methylesterase; Microorganism counts.

Introduction

Orange juice is the most popular fruit juice in the world. Its consumption is estimated at more than 9 billion liters per year, which represents 47% of global fruit juice consumption. Brazil is the main orange juice producer and exporter in the world, having exported 463 thousand tons of frozen concentrated orange juice (FCOJ) and 944 thousand tons of pasteurized juice (NFC, not from concentrate) in the 2011/2012 harvest (CitrusBR 2012). The quality of orange juice is mainly influenced by enzymatic activity and microflora. The activity of several pectin methylesterases (PME) isoenzymes is associated with cloud loss which can further cause gelation of juice (Versteeg et al. 1980). Due to orange juice acidity, the spoilage microflora is limited to yeasts, moulds and lactic acid bacteria that may lead to off flavour, turbidity and gas production (Lawlor et al. 2009).

The most extensively used process for orange juice stabilization is thermal pasteurisation, which inactivates vegetative microorganisms and enzymes improving shelf life. However, pasteurisation at intense time/temperature conditions induces ascorbic acid, carotenoids and flavour losses, as well as colour changes, affecting the juice's overall quality (Naim et al. 1997; Hyoung & Coates 2003; Janzantti et al. 2011).

In order to improve orange juice shelf life and stability high hydrostatic pressure (HHP) processing can be used as alternative method. Vitamin C, carotenoids, colour, flavour, soluble solids, pH and other compounds of orange juice are not considerably affected by HHP (Timmermans et al. 2011; Vervoort et al. 2011; Baxter et al. 2005; Bull et al. 2004). HHP has the potential to reduce orange juice spoilage microflora and PME activity without using high temperatures, therefore preserving sensory and nutritional characteristics.

Some studies reported inactivation of orange juice spoilage microflora by HHP. Lactic acid bacteria are considerably more resistant to HHP than yeasts, while moulds are the most labile spoilage microflora of orange juice (Patterson 2005). Katsaros et al. (2010) developed a mathematical model to predict the destruction of *Lactobacillus plantarum* and *L. brevis* in HHP processed orange juice (100–500 MPa, 20–40 °C) and found that 360 MPa, 35 °C for 2 min was adequate to obtain 7 log cycles reductions of both microorganisms. Basak et al. (2002) reported inactivation of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* in single strength and concentrated orange juice submitted to HHP (100–400 MPa at 20 °C). Kinetics analysis revealed two

different effects in pressure inactivation of microorganisms: an instantaneous pressure kill (dependent on the pressure level) and a first-order inactivation (dependent on holding time).

PME inactivation depends on the enzyme environment of the particular food system and even on the variety and origin of orange juice (Irwe & Olsson 1994). Goodner et al. (1998) studied PME inactivation of Valencia orange juice from Florida (USA) using HHP in the range of 500–900 MPa and found that the labile form of PME was inactivated with almost no effect on the stable form. The use of 50–400 MPa combined at 20–60 ºC showed that only combinations of low pressures and mild temperatures inactivated PME from freshly squeezed orange juice (*Citrus aurantium*, Salustiana variety, Spain), with a maximum reduction (25%) of the initial PME activity after HHP at 200 MPa, 30 °C for 15 min (Cano et al. 1997). Polydera et al. (2004) evaluated the inactivation kinetics of PME in Greek Navel orange juice using 100–800 MPa with 30–60 °C and reported that 600 MPa, 40 °C for 4 min could lead to inactivation of the pressure labile PME. Navel orange juice from Victoria (Australia) processed at 600 MPa, 20 °C for 60 s exhibited a 45% reduction in PME activity (Bull et al. 2004), while the same processing conditions were enough to inactivate 92% of PME orange juice derived from a mixture of Valencia, Pêra and Baladi orange varieties (Vervoort et al. 2011). Basak and Ramaswamy (1996) observed that an increase in soluble solids content (10 to 40 °Brix) decreased PME inactivation rates of HHP processed orange juice. Some studies related lower pH of orange juice with higher inactivation of PME (Basak & Ramaswamy 1996; Bull et al. 2004; Tribess & Tadini 2006).

There is no available literature about Pêra Rio orange juice HHP processing, the most characteristic Brazilian orange variety. The aim of this work was to evaluate the influence of HHP processing conditions (pressure, temperature and time) on PME activity and microbial counts of orange juice from the Pêra Rio variety.

Material and Methods

Material and Chemicals

Orange fruit of the Pêra Rio variety was provided by a citrus industry from Araraquara, SP. The fruit was 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 setting 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 stored at -18 ºC for 2 months.

Citrus pectin was obtained from Sigma-Aldrich (St. Louis, MO, USA); ascorbic acid and glucose were purchased from Merck (Darmstadt, Germany); bromothymol blue and sodium chloride from Qhemis (São Paulo, SP, Brazil); sodium carbonate, 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); Petrifilm^{TM} for aerobic count and yeast and mould count were sourced from 3M^{TM} (St. Paul, MN, USA).

HHP and Thermal Processing of Orange Juice

For the HHP processing, the orange juice was packaged in flexible PE bags (100 mL) and processed in a Stansted Food Lab 9000 (Stansted Fluid Power, S-FL-850-9-W, UK) within a pressure vessel of 500 mL. The equipment has a maximum nominal operation pressure of 900 MPa and a temperature range from -20 °C to 90 °C. The temperature in the vessel was controlled by liquid circulation in the outer jacket connected to a heating-cooling system. The pressure transmitting fluid used was 70 % (v/v) ethanol. The compression rate was 7 MPa.s^{-1} and the decompression time was less than 10 s. Compression and decompression times were not included in the experimental design.

Previous to HHP processing, tests were done in order to determine the adiabatic heating of pressurising fluid and orange juice for each experimental condition. The adiabatic heating ranged from 3.6 to 6.8 ºC/100 MPa for the pressurising fluid and from 2.8 to 3.5 ºC/100 MPa for orange juice. For all experiments, the orange juice PE bags and the vessel had the temperature adjusted to a few degrees below the targeted temperature in order to achieve the desired condition during pressurisation.

For the thermal processing, orange juice (15 mL) was placed in Pyrex glass tubes (outer diameter = 18 mm; inner diameter = 16 mm; height = 180 m), which were immersed in an oil bath at 100 ºC, and heated at 95 ±1 ºC for 30 s. A thermocouple positioned in the juice cold point was used to measure the temperature. The time for the

juice to reach 95 ±1 ºC was less than 2 min. Once the processing conditions were reached, tubes were taken out of the oil bath and immediately cooled in a water/ice mixture.

Non-processed (extracted and filtered) and thermally processed (95 ºC.30s^{-1}) orange juice were used as references for comparison with HHP orange juice.

Experimental design

The response surface methodology was used to evaluate the effect of the independent variables (pressure, temperature and time) on pectin methylesterase (PME) activity and total counts of aerobic microorganisms, and yeasts and moulds (response variables) of HHP orange juice.

A central composite design (CCD) 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 (Rodrigues & Iemma 2009). 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, which made it possible to estimate the lack of fit of the statistical model and the pure error; –1.68 and +1.68, representing the axial points, allowing a quadratic statistical model (Table 1).

PME

The PME activity was evaluated according to Hagerman & Austin (1986). Orange juice and NaCl (8.8% *w/v*) were homogenized (4.5:15, *w/v*) and centrifuged at 18000 g for 20 min at 4 ºC. The supernatant was collected and used as enzymatic extract. The substrate was composed of 2 ml 0.5% citrus pectin (*w/v*), 150 μL 0.01% bromothymol blue (*w/v*) in 0.003 M potassium phosphate buffer and 830 μL distilled water. The substrate and enzymatic extract were adjusted to pH 7.5. Substrate was added of enzymatic extract (20 μL) and absorbance decrease was monitored at 620 nm using a spectrophotometer (Evolution 220, Thermo Scientific, USA). Distilled water was used as the blank. A kinetic curve of the absorbance decrease was obtained and PME activity was calculated from the linear portion of the curve. One unit of PME activity is defined as a decrease of 0.001 in absorbance per min per mL of enzymatic extract. PME activity analyses were performed in triplicate, at 25 ºC.

The PME residual activity (in percent) after each HHP and thermal processing treatment was calculated according to Eq. 1:

(Eq. 1)

$$PME = \frac{PME_p}{PME_0} \times 100$$

where PME = PME residual activity (in percent), PME_p = orange juice PME activity after HHP and thermal process and PME_0 = PME activity of non-processed orange juice.

Physicochemical analyses

The physicochemical characteristics of non-processed orange juice were evaluated in order to verify the accomplishment to the orange juice standards of identity and quality (Brazil 2000). Total soluble solids content, total titratable acidity, pH, ascorbic acid, total and reducing sugars were analysed according to AOAC (1990). All analyses were performed in triplicate.

Microbiological analyses

Orange juice (10 mL) was added to 90 mL sterilised buffered peptone water (BPW). After homogenisation, aliquots were serially diluted in BPW and 1 mL of each dilution was inoculated onto Petrifilm^{TM} 3M^{TM} plates for aerobic, and yeast and mould counts. The aerobic microorganisms count was performed after incubation at 35 ± 1 º C for 48 ± 3 h and yeast and mould count after incubation at 25 ± 1 º C for 120 ± 6 h. The minimum level of detection was 10 CFU/mL (AOAC 2011). The analyses were performed in triplicate.

Data analyses

The results were fitted to a second-order model equation provided by the design. Analyses of variance of the regression equations allowed the adequacy of the model to be determined by evaluating the lack of fit, coefficient of determination (R^2), *F* test value and significance of the effects, using STATISTICA software version 10.0 (StatSoft, Tulsa, USA).

Results and Discussion

Physicochemical characteristics of orange juice

The physicochemical characteristics of non-processed orange juice are shown in Table 2. The orange juice complied with the standard values of the Brazilian legislation, except for total soluble solids (9.03 °Brix) which were lower than the minimum requirement of 10.5 °Brix (Brasil 2000).

Response values and model fitting

In order to optimize the HHP processing of orange juice, the CCD with 17 experiments was employed to evaluate the effect of pressure, temperature and time on PME residual activity and microbial counts. The values of the response variables for HHP processed juice (CCD experiments) and those for non-processed and thermally processed orange juice are listed in Table 3. Non-processed orange juice presented a PME activity of 167 U, considered as 100% of PME residual activity, and counts of $2.6x10^4$ CFU/mL for aerobic microorganisms and $1.7x10^4$ CFU/mL for yeasts and moulds. PME residual activity of the juices from CCD ranged from 15 to 108%, while that of the thermally processed juice was 4%, indicating that the thermal process was more effective to reduce PME activity. The remaining PME activity corresponds to the more heat and pressure resistant isoenzyme (Versteeg et al. 1980; Van Den Broeck et al. 2000). The lowest value of residual PME activity (15%) of orange juice from CCD was obtained when the experimental condition was 600 MPa, 45 °C, 195 s. Nienaber and Shellhammer (2001) reported orange juice residual PME activity of 10% when 600 MPa, 50 °C during 276 s was used. Vervoort et al. (2011) found 8% of residual PME activity in orange juice processed at 600 MPa, 20 °C for 60 s, although at the same processing conditions, Bull et al. (2004) reported only 55% residual PME activity. Concerning to the aerobic microorganisms, and yeasts and moulds, the thermally processed juice (95 ºC/30s) had minimum counts (<10 CFU/mL). Two experiments from CCD (600 MPa, 45 ° C, 195 and 499 MPa, 54 ° C, 293 s) also had minimum counts of aerobic microorganisms. Additionally, CCD experiments employing a pressure of 350 MPa or more resulted in the minimum counts for yeasts and moulds.

Microbial counts below the detection limit of the method (<10 CFU/mL) were expressed as 10 CFU/mL in the analyses of model fitting. The high incidence of

experiments with yeast and mould count <10 CFU/mL meant it was not possible to generate a model for yeasts and moulds.

The analysis of variance (Table 4) showed that the adjusted second order models were significantly fitted to the experimental data, as indicated by the regression model *F* values of 50.95 ($p<0.001$) for PME residual activity and 81.11 ($p<0.001$) for aerobic microorganism count. Terms presenting significant *F* value ($p\leq0.05$) were included in the models. For PME residual activity, the linear effects of pressure (*P*), temperature (*T*) and time (*t*), as well as the quadratic effect of pressure (P^2) and the interaction effect of pressure and temperature (*PT*) were significant. In the same way, for aerobic microorganism count, linear and quadratic effects of pressure (*P*, P^2), temperature (*T*, T^2) and time (*t*, t^2), and interaction effects of pressure and temperature (*PT*) and pressure and time (*Pt*) were significant. Lack of fit of experimental data was not significant ($p>0.05$) for both models. The coefficient of variation (C.V.) for PME residual activity model was 8%, and for aerobic microorganisms count it was 6%. Adequate precision compares the model predicted values to its associated error, in other words a signal to noise ratio. Ratios greater than 4 indicate adequate model discrimination. The models of PME residual activity and aerobic microorganism count showed an adequate precision of 22.39 and 29.87, respectively. The determination coefficient (R^2) for PME residual activity model was 0.96; for aerobic microorganism count it was 0.99, while the adjusted determination coefficient (Adjusted R^2) values were 0.94 and 0.98, respectively. There was a high correlation between the experimental and predicted values. These statistical parameters confirm the consistency of both models, indicating they are reliable to predict PME residual activity and aerobic microorganisms count in Pêra Rio orange juice processed by HHP (Rodrigues & Iemma 2009).

Using the regression coefficients from the adjusted models (Table 4) the following model equations were generated:

(Eq. 2)

$$PME = 47.577 + 0.447P + 0.974T - 0.097t - (5.907 \times 10^{-3})PT - (4.403 \times 10^{-4})P^2$$

where PME = residual activity of PME (in percent), *P* = pressure (in megapascal), *T* = temperature (in degrees Celsius) and *t* = time (in second).

(Eq. 3)

$AMC = 5.457 – (5.306 \times 10^{-3})P + 0{,}038T – (2.833 \times 10^{-3}) + (1.682 \times 10^{-4})PT – (1.228 \times 10^{-5})Pt – (7.373 \times 10^{-6})P^2 –(1.565 \times 10^{-3})T^2 + (6.582 \times 10^{-6})t^2$

where AMC = aerobic microorganism count (in $\log_{10}$ colony forming units per mililitre), P = pressure (in megapascal), T = temperature (in degrees Celsius) and t = time (in second).

Optimisation of the HHP processing

The response surface models were plotted from the regression equations (Eqs. 2 and 3) to illustrate the effects of the independent variables on the PME residual activity and aerobic microorganism count (Fig. 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 pressure, temperature and time promoted the reduction of the PME residual activity and aerobic microorganism count for the orange juice, except for the region between 170 to 310 MPa, 30 to 45 °C and 30 to 150 s, which provided a small increase in PME activity. Cano et al. (1997) also noted PME activation in Salustiana orange juice with HHP processing conditions of 200 to 400 MPa, 20 to 25 °C and 15 min. Furthermore, tomato puree processed from 300 to 700 MPa at ambient temperature resulted in PME activation, with an increase of more than 500% in PME activity (Krebbers et al. 2003). The activation effects could be attributed to reversible configuration and/or conformation changes of the enzyme and/or substrate molecules (Ogawa et al. 1990).

The effects of pressure and temperature at a fixed time (195 s) on the response variables (PME residual activity and aerobic microorganisms count) are in Fig. 1a and 1b. Increasing pressure had a stronger effect in reducing PME activity when temperature levels were high. Pressure higher than 550 MPa with temperature higher than 55 °C promoted more than 90% reduction of PME activity, while at the same pressure with temperature lower than 35 °C only about 40% of PME was inactivated. On the other hand, for aerobic microorganism count, the use of high levels of pressure (higher than 500 MPa) at any temperature led to less than 2 log of CFU/mL.

The effects of pressure and time at 45 °C (Fig. 1c and 1d) showed that pressure had a stronger influence than time in reducing PME activity. Also, an increase in pressure for a longer HPP processing times had a stronger effect in reducing the aerobic

count than shorter periods of time, as expected. Therefore, the use of pressure higher than 500 MPa for time longer than 200 s resulted in aerobic microorganism counts below 1 log CFU/mL. Similar results for aerobic microorganisms count were obtained for orange juice from Valencia and Navel varieties processed by HHP at 600 MPa during 60 s (Bull et al. 2004) and from the Hamlin variety processed at 400 MPa for 90 s (Parish 1998). As shown in Fig. 1e and 1f, increases in temperature and time at 350 MPa (pressure of central point) had a lower influence on the response variables than the other combinations of effects.

Cloud stability is an important quality parameter for orange juice, since it positively affects turbidity, flavour and colour characteristic of the juice. The loss of cloud is attributed to the endogenous PME activity, which demethoxylates soluble pectins causing calcium pectates precipitation and clarification of the juice (Versteeg et al. 1980). However, a low PME residual activity could still preserve cloud stability during the shelf life of the juice. Several studies reported cloud stabilization of HHP processed orange juice with different PME residual activity. Boff et al. (2003) obtained a stable orange juice with 20% of PME residual activity after 120 days stored at 4 and 30 °C. Goodner et al. (1998) reported orange juice presenting PME residual activity of 18% was stable for more than 50 days when stored at 4 °C and Nienaber & Shellhammer (2001) obtained orange juice with 4% of PME, which maintained its cloud stability for longer than 90 days at 4 °C and at 37 °C.

In order to obtain orange juice with PME residual activity 20% or less and low aerobic microorganism count (< 2 log cycle CFU/mL), the optimum levels of the independent variables and their combinations were obtained by analysing the regression equations (Eq. 2 and 3). The processing conditions of 550 to 600 MPa, 55 to 60 °C and 330 to 360 s were suitable to produce a stable orange juice. It is also possible to obtain the same effect at the highest level of pressure (600 MPa) for temperature from 50 to 60 °C and processing time from 300 to 360 s. Furthermore, the same PME residual activity (≤20%) and low microorganism counts can be obtained if temperature of 60 °C and pressure from 520 to 600 MPa is applied during 320 to 360 s. Also, the use of 360 s and pressure from 540 to 600 MPa at 55 to 60 °C confer the same effect.

Conclusions

Response surface methodology was successfully used to optimise the decrease of microflora and PME activity in HHP processed Pêra Rio orange juice. The increase in pressure, temperature and time reduced the PME activity and native microflora of orange juice, except the region between 170 to 310 MPa, 30 to 45 °C and 30 to 150 s, which enhance PME activity. The optimum HHP conditions of 550 to 600 MPa, 55 to 60 °C and 330 to 360 s are able to produce orange juice with less than 2 log cycles CFU/mL and PME residual activity less than 20%.

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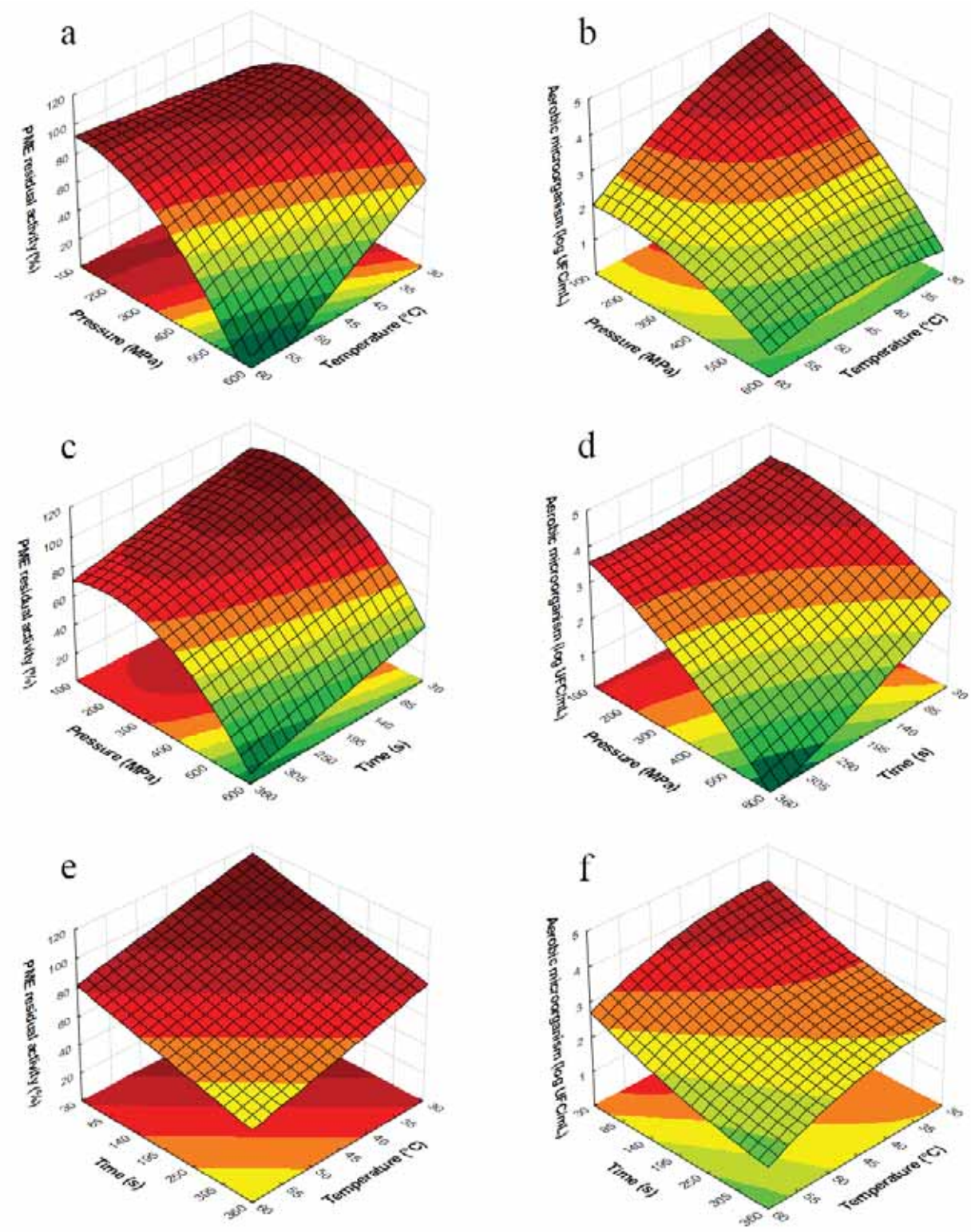

Fig. 1 Response surface of the combined effects of pressure and temperature (a, b), pressure and time (c, d), and temperature and time (e, f) on the PME residual activity and aerobic microorganisms count of HHP- processed orange juice.

Table 1 Levels and corresponding values of the independent variables.

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 2 Physicochemical characteristics of the non-processed orange juice.

Parameter	Value[a]
pH	4.18 ± 0.01
Total soluble solids (°Brix)	9.03 ± 0.00
Titratable acidity (g citric acid/100mL)	0.58 ± 0.01
Ratio (soluble solids/titratable acidity)	15.57 ± 0.08
Ascorbic acid (mg/100mL)	85.95 ± 1.14
Total sugars (g/100mL)	6.26 ± 0.05
Reducing sugars (g/100mL)	3.30 ± 0.03

[a] Mean ± Standard deviation (n=3)

Table 3 The central composite design (CCD) and experimental response values for orange juice.

Experiment	Independent variables			Response variables		
	Pressure (MPa)	Temperature (°C)	Time (s)	PME residual activity (%)	Aerobic microorganisms (CFU/mL)	Yeast and mould (CFU/mL)
Non-processed	-	-	-	100 ± 6	$2.6x10^4 \pm 2x10^3$	$1.71x10^4 \pm 7x10^2$
Thermally processed	-	95	30	4.4 ± 0.7	<10	<10
HHP processed - CCD						
1	201	36	97	108 ± 5	$1.6x10^4 \pm 3x10^3$	$1.30x10^4 \pm 5x10^2$
2	499	36	97	89 ± 6	$3.1x10^2 \pm 4x10^1$	<10
3	201	54	97	92 ± 6	$1x10^3 \pm 2x10^2$	$1.1x10^2 \pm 2x10^1$
4	499	54	97	49 ± 3	$1.5x10^2 \pm 3x10^1$	<10
5	201	36	293	81 ± 4	$7.8x10^3 \pm 8x10^2$	$1.2x10^3 \pm 9x10^2$
6	499	36	293	66 ± 3	33 ± 6	<10
7	201	54	293	79 ± 3	$4.3x10^2 \pm 2x10^1$	$2.0x10^2 \pm 2x10^1$
8	499	54	293	25 ± 2	<10	<10
9	100	45	195	91 ± 5	$3.1x10^3 \pm 5x10^2$	$8x10^3 \pm 2x10^3$
10	600	45	195	15 ± 2	<10	<10
11	350	30	195	90 ± 6	$7.1x10^2 \pm 6x10^1$	<10
12	350	60	195	70 ± 4	$5x10^1 \pm 2x10^1$	<10
13	350	45	30	94 ± 5	$7.9x10^3 \pm 8x10^2$	$1x10^1 \pm 2x10^1$
14	350	45	360	70 ± 3	$9x10^1 \pm 2x10^1$	<10
15	350	45	195	81 ± 7	$6.0x10^2 \pm 5x10^1$	<10
16	350	45	195	84 ± 3	$6.7x10^2 \pm 3x10^1$	<10
17	350	45	195	85 ± 5	$5.5x10^2 \pm 2x10^1$	<10

Table 4 Analysis of variance (*F* value), coefficient of variation, adequate precision and regression coefficients of the second order models for PME residual activity and aerobic microorganism count of orange juice.

Source of variation[1]	PME residual activity		Aerobic microorganism count	
	***F* value**	**Regression coefficients**	***F* value**	**Regression coefficients**
Regression model	50.95 [a]		81.11 [a]	
Mean / Interception		47.577		5.457
Terms				
P	1501.99 [a]	0.447	4334.11 [a]	$-(5.306 \times 10^{-3})$
T	406.25 [b]	0.974	1055.89 [a]	0,038
t	380.09 [b]	–0.097	1398.06 [a]	$-(2.833 \times 10^{-3})$
P^2	382.40 [b]	$-(4.403 \times 10^{-4})$	153.62 [b]	$-(7.373 \times 10^{-6})$
T^2	[ns]	[ns]	90.00 [c]	$-(1.565 \times 10^{-3})$
t^2	[ns]	[ns]	23.18 [c]	(6.582×10^{-6})
PT	155.44 [b]	$-(5.907 \times 10^{-3})$	208.07 [b]	(1.682×10^{-4})
Pt	[ns]	[ns]	131.53 [b]	$-(1.228 \times 10^{-5})$
Tt	[ns]	[ns]	[ns]	[ns]
Lack of fit	13.34 [ns]		14.86 [ns]	
C.V. (%)		8.06		5.77
Adequate Precision		22.39		29.87
R^2		0.9586		0.9879
Adjusted R^2		0.9398		0.9757

[1] *P* = pressure. *T* = temperature. *t* = time.
[a] $p \leq 0.001$. [b] $p \leq 0.01$. [c] $p \leq 0.05$
[ns] Not significant ($p>0.05$).

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Optimisation of High Hydrostatic Pressure Processing of Pêra Rio Orange Juice

--Manuscript Draft--

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Abstract:	The influence of high hydrostatic pressure (HHP) on Pêra Rio orange juice was investigated using response surface methodology. A central composite design was used to evaluate the effects of three processing conditions (independent variables), namely pressure (100-600 MPa), temperature (30-60 °C) and time (30-360 s), on the native microflora and pectin methylesterase (PME) activity of orange juice. Analysis of variance showed that second order polynomial models fitted well with the experimental data for PME residual activity (R^2=0.9586, $p<0.001$) and aerobic microorganisms count (R^2=0.9879, $p<0.001$). The optimum HHP processing conditions to produce orange juice with PME residual activity less than 20% and low microorganisms count (< 2 log cycles CFU/mL) were 550 to 600 MPa, 55 to 60 °C and 330 to 360 s.

CAPÍTULO 2

EFEITO DA ALTA PRESSÃO HIDROSTÁTICA SOBRE O ÁCIDO ASCÓRBICO E A ATIVIDADE ANTIOXIDANTE DO SUCO DE LARANJA

Trabalho enviado para Journal of the Science of Food and Agriculture

EFFECT OF HIGH HYDROSTATIC PRESSURE ON ASCORBIC ACID AND ANTIOXIDANT ACTIVITY OF ORANGE JUICE

Running Title: Ascorbic acid and antioxidant activity of HHP orange juice

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ABSTRACT

BACKGROUND: 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 and antioxidant activity of orange juice was investigated using response surface methodology.

RESULTS: 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 (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.

CONCLUSION: 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: high hydrostatic pressure (HHP); orange juice; ascorbic acid; antioxidant activity; response surface methodology (RSM); ABTS radical.

INTRODUCTION

Orange juice is the most consumed juice in the world, corresponding to 45% worldwide juice consumption (CitrusBR (www.citrusbr.com/en)). Also, orange juice is an important source of bioactive compounds in diet, like flavonoids and carotenoids as well as ascorbic acid. Orange juice flavanones[1], mainly hesperidin and narirutin, present antioxidant activity[2], while carotenoids[3], mostly carotenes and cryptoxanthins, have provitamin A activity, and lutein and zeaxanthin, prevent macular degeneration[4]. Orange juice flavanones have been associated with reduced risk of coronary heart disease[5,6]. Vitamin C is considered the major antioxidant compound in orange juice, contributing with more than 90% of the antioxidant activity[7], while carotenoids and flavanones have minor contribution. Vitamin C also contributes to the maintenance of the vascular health and to reduce atherogenesis, regulating the collagen synthesis, prostacyclin production, and nitric oxide[8,9]. Some studies indicated that orange juice consumption may reduce low density lipoprotein cholesterol (LDL) and improve high density lipoprotein (HDL) cholesterol in hypercholesterolemic subjects[10,11], as well as reduce oxidative stress (8-*epi*-$PGF_{2\alpha}$) and uric acid in plasma[12].

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, affecting the juice's overall quality[13-15].

Due to consumers demand, fruit juice industry has been exploring innovative technologies with minimal heat treatment, able to produce juice with fresh-like and natural-like attributes, to preserve flavor and nutritional aspects[16].

High hydrostatic pressure (HHP) reduces orange juice spoilage microflora[17,18] and PME activity[17,19-22] without using high temperature, therefore preserving sensory and nutritional characteristics. Color, flavor, soluble solids, pH and other compounds of orange juice are not considerably affected[19,20,23] and carotenoids extractability is enhanced, leading to higher bioavailability[24,25]. Orange juice spoilage microflora was studied after HHP at 360 MPa, 35 °C for 2 min; a 7 log cycle reduction of *Lactobacillus plantarum* and *L. brevis* was verified[17]. HHP treatment of orange juice at 600 MPa during 60 s reduced counts of aerobic microorganisms, and yeasts and molds to not detectable levels (<10 CFU/mL) for juice from Valencia and Navel varieties[20] and 400 MPa for 90 s for juice from Hamlin[21]. HHP of Navel orange juice at 600 MPa, 20 °C for 60 s exhibited a 45% reduction in PME activity[20], while the same treatment conditions were enough to inactivate 92% of PME orange juice derived from a mixture of three orange varieties[23]. PME inactivation depends on the enzyme environment of the particular food system and even on the variety and origin of orange juice[22].

There are many studies about the influence of some HHP treatments on ascorbic acid and/or antioxidant activity[20,23-28], however the effects of a range of treatment conditions were not determined yet. Bull *et al.*[20] reported that Valencia orange juice treated at 600 MPa, 20 °C for 60 s did not had ascorbic acid significantly affected, however Sánchez-Moreno *et al.*[26] found 11% reduction in ascorbic acid of the juice treated at 100 MPa, 60 °C during 300 s. Ancos *et al.*[25] studied the effect of HHP on carotenoids and antioxidant activity of Valencia orange juice. It was verified that at 350 MPa, 30 °C for 300 s orange juice with the highest amount of vitamin A and extractable carotenoids was obtained, although there was a reduction of 22% on antioxidant activity. Sánchez-Moreno *et al.*[24]

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 aim of this work was to evaluate the influence of HHP treatment conditions (pressure, temperature and time) on ascorbic acid and antioxidant activity of orange juice using response surface methodology.

MATERIAL AND METHODS

Chemicals

ABTS diammonium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and gallic acid were obtained 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 carbonate, 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

Orange from Pêra Rio variety were 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 stored at -18 ºC for 2 months. Soluble solids, titratable acidity, pH, total and reducing sugars of the orange juice were determined (Table 1) according to AOAC[29]. Ratio 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 90 ºC. 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 pressurising fluid of each experimental condition was evaluated. The adiabatic heating ranged from 2.8 to 3.5 ºC per 100 MPa for orange juice and from 3.6 to 6.8 ºC per 100 MPa for the pressurising fluid. Temperature of orange juice bags and vessel were adjusted to a few degrees below the targeted temperature in each experimental condition in order to achieve the desired condition during pressurisation.

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 central composite rotatable design (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[30].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 2). 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 analysis was based on the reduction of 2,6-dichloroindophenol[29]. Triplicate analyses were performed and results were expressed as mg of ascorbic acid L^{-1} of orange juice.

Extraction of antioxidant compounds

The extraction was based on the procedure reported by Asami *et al.*[31]. Orange juice (5 mL) and methanol:water solution (80:20, v/v) were vortexed for 1 min and then submitted to an ultrasonic bath at room temperature for 15 min. The mixture was centrifuged at 10000 g for 20 min at 20 °C and the supernatant collected. The extraction procedure was repeated once using the same conditions.

Antioxidant activity

Antioxidant activity was evaluated with $ABTS^{+}$, based on the method described by Rufino *et al*.[32]. 5.0 mL ABTS (7 mmol L^{-1}) were added to 88 µL potassium persulfate (140 mmol L^{-1}) to form the ABTS radical solution. 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 753 nm.

Aliquots of orange juice extract, diluted with ethanol (1:1, 4:5 and only orange juice extract), were used to determine antioxidant activity. A 30 µL aliquot

of each of the three orange juice extract was mixed with 3 mL ABTS radical solution. Absorbance readings at 753 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 antioxidant activity analyses were done in triplicate and results were 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

Effect of HPP treatment conditions on ascorbic acid

The non treated orange juice presented higher ascorbic acid content (859.52 mg L^{-1}) when compared to Brazilian (82 to 570 mg L^{-1})[33] and to Spanish (196 to 634 mg L^{-1})[34] commercial orange juice. The orange variety, edaphoclimatic conditions, cultural practices, ripening, harvest, type of process and storage conditions exert strong influence on ascorbic acid content of orange juice[35].

Table 3 shows the ascorbic acid from HHP treated orange juice under each experimental condition, which ranged from 435.9 to 710.2 mg L^{-1}. There was a reduction in ascorbic acid depending on the treatment conditions. The ascorbic acid degradation was higher than that observed in some studies[24,26,27]. Orange

juice from Valencia[27] and Navel[24] treated at 400 MPa, 40 °C during 60 s had 5% and 8% of ascorbic acid degradation, respectively. Difference in ascorbic acid degradation may be related to the pH of orange juice[24,27]. More acidic conditions tend to stabilize ascorbic acid[36,37]. The experimental condition which most affected ascorbic acid (435.9 mg L^{-1}) was 350 MPa, 45 °C and 360 s, with more than 50% of degradation, which may be 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[38].

The statistical analysis (Table 4) indicated that 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 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 ($p\leq0.1$) 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. These statistical parameters confirm the consistency of the model, indicating it is reliable to predict ascorbic acid content in HHP treated orange juice[30]. Using the significant regression coefficients (Table 5) 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. Similar results as ours were obtained by Sánchez-Moreno *et al.*[26]. Higher ascorbic acid reduction (11%) resulted from longer time and higher temperature (100 MPa, 60 ºC, 300 s), when compared to the juice treated at 400 MPa, 40 °C for 60 s, which ascorbic acid reduction was 7%. As 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 independent variable of time was the most important one affecting ascorbic acid reduction in HHP treated orange juice (Figure 1a and 1b). According to the model equation (Eq. 2), ascorbic acid content higher than 600 mg L^{-1}, representing ca 70% of the initial orange juice ascorbic acid content, can be obtained within the range of 100-250 MPa, 30-40 °C and 30-125 s HHP treatment conditions.

Effect of HHP treatment conditions on antioxidant activity

Antioxidant activity of orange juice was determined using the ABTS radical reaction. The antioxidant activity of non treated orange juice was 3176.7 µmol Trolox L^{-1}, which is slight higher than the values reported in the literature[33,39].

Results for antioxidant activity of HHP treated orange juice for each experimental condition are in Table 3. HHP treatment reduced antioxidant activity in orange juice, which ranged from 2062.0 to 2935.9 μmol Trolox L^{-1}. 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]. Idrawati *et al.*[28] reported that HHP treatment increased antioxidant activity of carrot juice, but reduced that for orange juice (*var* Navelinas). The experimental condition with the highest antioxidant activity of orange juice (2935.9 μmol Trolox L^{-1}) was 350 MPa, 45 °C, 30 s. According to Table 3, it is possible to observe that the lower the time of HHP treatment the higher the antioxidant activity.

The ANOVA (Table 4) demonstrated that the quadratic model for antioxidant activity significantly fit the experimental data, as indicated by the regression model *F* value of 7.20 ($p<0.01$), with a 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[30]. Using the significant regression coefficients (Table 5) the following equation for antioxidant activity was developed:

(Equation 3)

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

where, Antioxidant Activity = 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 (Figure 2). As observed for ascorbic acid content (Figure 1), the increase in temperature and time of HHP orange juice treatment caused the reduction of the 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 (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 confers antioxidant related health benefits[12]. The ascorbic acid content and antioxidant activity of HHP treated orange juice showed a positive and strong correlation (R = 0.8248). Ascorbic acid (Figure 1) and antioxidant activity (Figure 2) showed similar response concerning to the HHP pressure, temperature 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

Sánchez-Moreno *et al.*[7], Stella *et al.*[33] and Miller and Rice-Evans[39] which showed that ascorbic acid is the main antioxidant compound in orange juice.

CONCLUSIONS

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 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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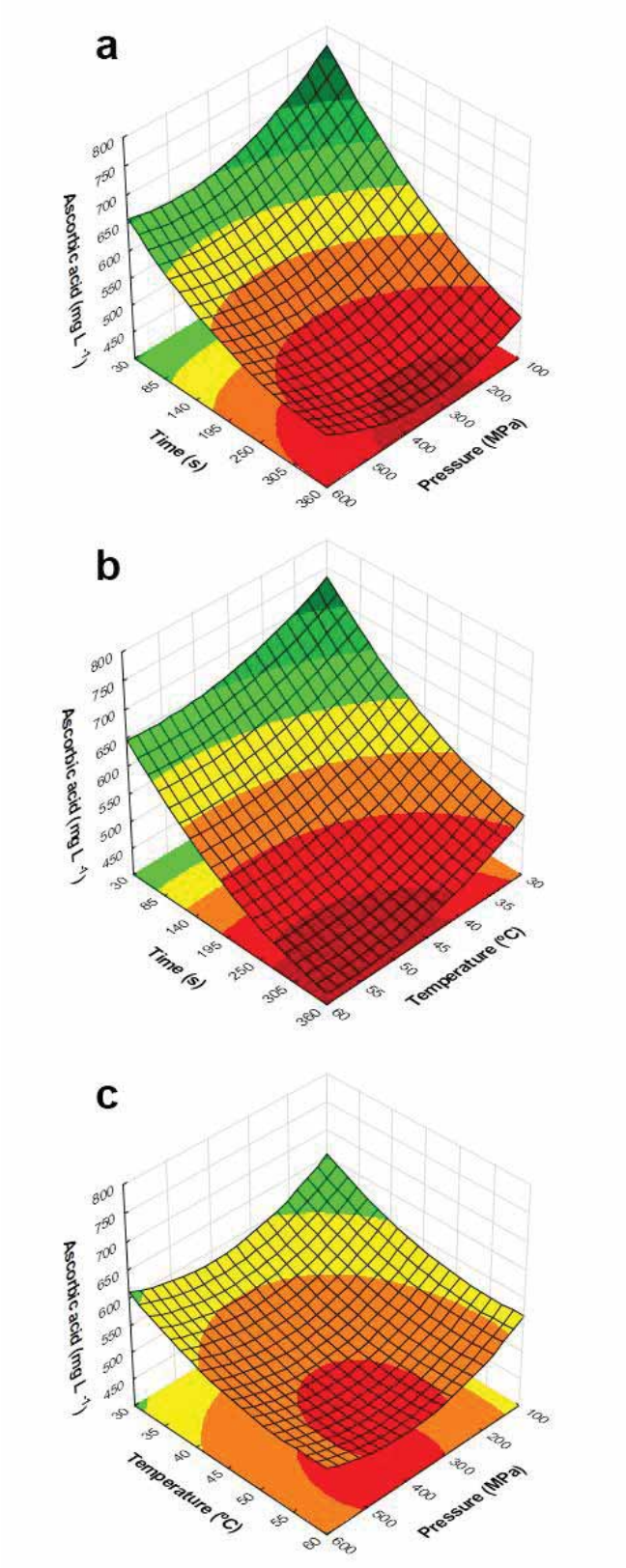

Figure 1. Response surface of the combined effects of time and pressure with temperature at 45 ºC (a), time and temperature with pressure at 350 MPa (b), temperature and pressure with time at 195 s (c) on the ascorbic acid content of HHP treated orange juice.

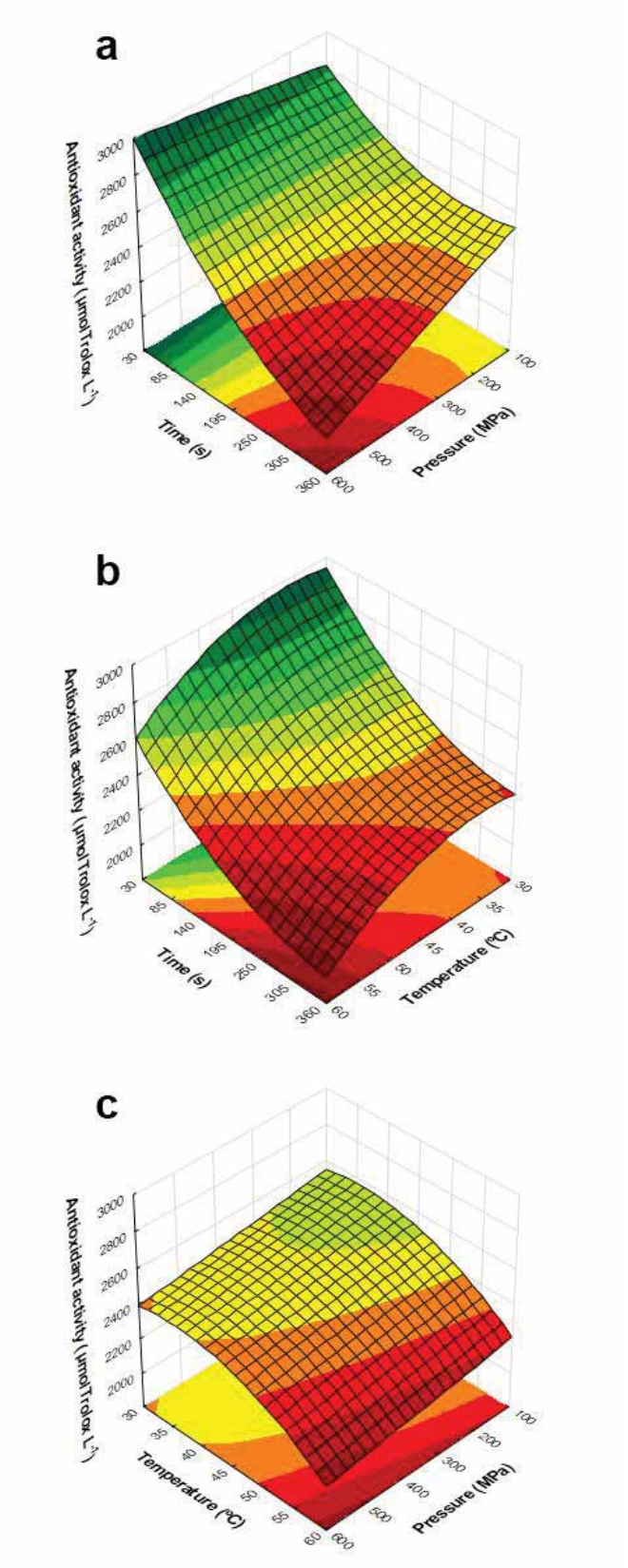

Figure 2. Response surface of the combined effects of time and pressure with temperature at 45 ºC (a), time and temperature with pressure at 350 MPa (b), temperature and pressure with time at 195 s (c) on the antioxidant activity of HHP treated orange juice.

Table 1. Physicochemical characteristics of the non treated orange juice.

Parameter	**Value***
Soluble solids (°Brix)	9.03 ± 0.00
Titratable acidity (g citric acid L^{-1})	5.78 ± 0.03
Ratio (soluble solids/titratable acidity)	15.57 ± 0.08
pH	4.18 ± 0.01
Total sugars (g glucose L^{-1})	62.62 ± 0.47
Reducing sugars (g glucose L^{-1})	33.04 ± 0.26

* Mean ± Standard deviation of three replicate analyses.

Table 2. Levels and corresponding values of the independent variables.

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

Experiment	Independent variables			Response variables	
	Pressure (MPa)	Temperature (°C)	Time (s)	Ascorbic acid (mg L^{-1})	Antioxidant activity (µmol Trolox L^{-1})
1	201	36	97	710.2 ± 4.3	2708.6 ± 128.3
2	499	36	97	640.1 ± 4.3	2737.6 ± 100.3
3	201	54	97	618.7 ± 4.3	2563.8 ± 55.7
4	499	54	97	582.2 ± 4.3	2550.4 ± 136.3
5	201	36	293	539.5 ± 11.4	2621.5 ± 86.4
6	499	36	293	512.1 ± 18.8	2353.7 ± 133.2
7	201	54	293	445.0 ± 4.3	2266.8 ± 146.4
8	499	54	293	457.2 ± 11.4	2062.0 ± 114.2
9	100	45	195	557.8 ± 11.4	2416.1 ± 35.8
10	600	45	195	521.2 ± 4.3	2304.8 ± 11.2
11	350	30	195	536.4 ± 11.4	2351.1 ± 77.6
12	350	60	195	499.9 ± 4.3	2120.4 ± 65.0
13	350	45	30	621.8 ± 4.3	2935.9 ± 146.6
14	350	45	360	435.9 ± 4.3	2112.2 ± 57.6
15	350	45	195	509.0 ± 8.6	2402.8 ± 59.9
16	350	45	195	496.8 ± 7.5	2469.6 ± 114.8
17	350	45	195	518.2 ± 4.3	2484.0 ± 94.8

Table 4. ANOVA (*F* value) of the quadratic model for ascorbic acid content and antioxidant activity of the HHP treated orange juice.

Source of variation[1]	Ascorbic acid content	Antioxidant activity
Regression model	8.80 [a]	7.20 [a]
P	21.50 [b]	16.19 [c]
T	83.25 [b]	72.98 [b]
t	529.04 [a]	272.24 [a]
P²	33.80 [b]	0.25 [ns]
T²	12.10 [c]	15.30 [c]
t²	21.24 [b]	15.79 [c]
PT	5.84 [ns]	0.03 [ns]
Pt	9.12 [c]	15.87 [c]
Tt	0.00 [ns]	6.58 [ns]
Lack of fit	12.23 [ns]	8.57 [ns]
R^2	0.9164	0.9052
Adj. R^2	0.8376	0.8250

[1] *P* = pressure. *T* = temperature. *t* = time.
[a] $p \leq 0.01$. [b] $p \leq 0.05$. [c] $p \leq 0.10$
[ns] Not significant.

Table 5. Significant regression coefficients of the quadratic model for ascorbic acid content and antioxidant activity of HHP treated orange juice.

Source of variation[1]	Ascorbic acid content	Antioxidant activity
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.

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CAPÍTULO 3

EFEITO DO PROCESSAMENTO DE ALTA PRESSÃO HIDROSTÁTICA NA QUALIDADE DO SUCO DE LARANJA VAR. PÊRA RIO

Trabalho a ser enviado para LWT – Food Science and Technology

Efeito do processamento de alta pressão hidrostática na qualidade do suco de laranja *var.* Pêra Rio

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RESUMO

O efeito do processamento de alta pressão hidrostática (APH) (520 MPa, 60 °C por 360 s) na qualidade do suco de laranja da variedade Pêra Rio foi investigado e comparado com a pasteurização (95 °C por 30 s). O processamento de APH e a pasteurização reduziram a atividade residual da PME para 13% e 4 %, respectivamente, e inativaram os microrganismos a níveis não detectáveis (<10 UFC/mL). A cor do suco de laranja foi afetada pela APH e pasteurização, aumentando a claridade, a cor amarela e a cor vermelha. O teor de ácido ascórbico foi menor no suco pressurizado e no suco pasteurizado, porém os compostos fenólicos totais e a atividade antioxidante não apresentaram diferença do suco não processado. A tecnologia de APH pode ser empregada como alternativa à pasteurização, por não afetar sobremaneira a qualidade do suco de laranja e garantir a estabilidade enzimática e microbiológica.

Palavras-chave: suco de laranja; alta pressão hidrostática (APH); variedade Pêra Rio; pectinametilesterase; microrganismos; cor instrumental.

ABSTRACT

The effect of high hydrostatic pressure (HHP) processing (520 MPa, 60 °C for 360 s) on the quality of orange juice *var.* Pêra Rio was investigated and compared with those of pasteurization (95 °C for 30 s). The HHP processing and pasteurization reduced PME residual activity to 13% and 4%, respectively, and inactivated microorganisms to not detectable levels (<10 UFC/mL). Color of orange juice was affected by HHP and pasteurization, enhancing luminosity, red and yellow color. Ascorbic acid was lower in pressurized and pasteurized juice, however total phenolic compounds and antioxidant acitivity did not differ from not processed juice. The HHP technology can be used as alternative for pasteurization, since it does not affect greatly the quality of orange juice ensuring enzymatic and microbiological stability.

1. INTRODUÇÃO

O Brasil é o maior produtor e exportador mundial de suco de laranja. Na safra 2012/2013, o país produziu 2,15 milhões de ton de suco de laranja e exportou 2,09 milhões de ton, que representaram 97% do total, dos quais 1,12 milhões de ton foram de suco de laranja não concentrado (Not From Concentrate, NFC), 582 mil ton de suco concentrado e congelado (Frozen Concentrated Orange Juice, FCOJ) e 391 mil ton de suco de laranja destinado a outras bebidas (CitrusBR, 2013).

A aceitação do suco de laranja está relacionada ao aroma e sabor natural característico da fruta. Por isso, o suco de laranja espremido na hora do consumo e o NFC vêm sendo muito valorizados pelo consumidor, além de serem relacionados ao conceito de saudável (FIESP e ITAL, 2010; Campos et al., 2006; Min, et al., 2003; Torre, et al., 2003). Por outro lado, o FCOJ é exposto à temperatura elevada (95 °C) por um período maior que o NFC, o que altera drasticamente o aroma e sabor (Janzantti et al., 2011), embora apresente a vantagem de ter maior estabilidade microbiológica e custo de transporte menor do que o NFC, devido ao processo de concentração (Queiroz e Menezes, 2010).

O processamento usando alta pressão hidrostática (APH) é uma das tecnologias mais inovadoras para processar produtos termossensíveis. O uso de pressões de 100 a 1000 MPa provoca destruição microbiana e retarda significativamente as taxas de reações enzimáticas, minimizando a formação de sabor estranho e o escurecimento não-enzimático. Desta forma, a perda de nutrientes e as alterações sensoriais são minimizadas (Tewari, 2007).

A tecnologia de APH, considerada alternativa para o processamento do suco de laranja, é capaz de aumentar a vida de prateleira e a estabilidade do produto. Cor, sabor, pH, vitamina C, carotenóides, sólidos solúveis e outros compostos do suco de laranja não são consideravelmente afetados pela APH (Timmermans et al., 2011; Vervoort et al., 2011; Baxter et al., 2005; Bull et al., 2004). A APH tem a capacidade de reduzir microrganismos e a atividade da PME no suco de laranja sem utilizar altas temperaturas, preservando características sensoriais e nutricionais.

Microrganismos deteriorantes do suco de laranja foram avaliados após terem sido submetidos a APH (360 MPa, 35 °C por 2 min), tendo sido obtida redução de 7 ciclos logarítmicos da população de *Lactobacillus plantarum* e de *Lactobacillus brevis* (Katsaros et al., 2010). O processamento do suco de laranja da variedade Valência e Navel por APH usando 600 MPa durante 60 s (Bull et al., 2004) e da variedade Hamlin usando 400 MPa por 90 s (Parish, 1998) reduziu a contagem de microrganismos aeróbios, e de bolores e leveduras a níveis não detectáveis (<10 CFU/mL). O suco de laranja da variedade Navel submetido a 600 MPa, 20 °C por 60 s apresentou redução de 45% na atividade da PME (Bull et al., 2004), enquanto as mesmas condições de processamento foram capazes de inativar 92% da PME do suco de laranja obtido pela mistura das variedades Valência, Pêra e Baladi (Vervoort et al., 2011). A inativação da PME depende das características ambientais, da variedade e da origem do suco de laranja (Irwe e Olsson, 1994).

A pressurização do suco de laranja a 400 MPa, 40 ºC por 60 s não afetou as características de cor, mas reduziu entre 5 e 8% o teor de ácido ascórbico e, praticamente, não alterou os compostos fenólicos totais e a

atividade antioxidante do suco (Plaza et al., 2006; Sánchez-Moreno et al., 2005).

O objetivo deste trabalho foi avaliar o efeito do processamento por APH nas características físico-químicas, atividade da enzima PME, contagem de microrganismos, cor instrumental, compostos fenólicos totais e atividade antioxidante do suco de laranja da variedade Pêra Rio, comparativamente ao suco pressurizado e não processado.

2. MATERIAL E MÉTODOS

2.1. Reagentes e meios de cultura

Pectina cítrica, sal diamônio de ABTS, 2,4,6 - tris(2-pyridyl)-s-triazine, 2,2 - diphenyl-1-picrylhydrazyl, 6 - hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) e ácido gálico foram obtidos da Sigma-Aldrich (St. Louis, MO, EUA); carbonato de sódio, hidróxido de sódio, tartarato de sódio e potássio tetrahidratado, sulfato de cobre pentahidratado e fosfato de potássio dibásico da Labsynth (Diadema, SP, Brasil); ácido ascórbico e glicose foram comprados da Merck (Darmstadt, Alemanha); azul de bromotimol e cloreto de sódio da Qhemis (São Paulo, SP, Brasil); ácido oxálico e sal hidratado de 2,6-dicloroindofenol da Vetec (Rio de Janeiro, RJ, Brasil); persulfato de potássio da Fluka (Steinheim, Alemanha); metanol da JT Baker (Philipsburg, PA, EUA); Reagente de Folin–Ciocalteu da Imbralab (Ribeirão Preto, SP, Brasil); Petrifilm^{TM} para contagem de mesófilos aeróbios, de bolores e leveduras, e de coliformes termotolerantes e totais foram obtidos da 3M^{TM} (St. Paul, MN, EUA).

2.2. Suco de laranja

Laranjas da variedade Pêra Rio foram fornecidas por uma indústria citrícola da região de Araraquara, SP. As frutas foram cultivadas na região de Bauru, SP (22º 25' 59" S; 49º 10' 31" L) durante a safra de 2012/2013. O processamento das frutas para obtenção de suco foi realizado na JBT FoodTech Citrus System, Araraquara, SP. Antes da extração, as frutas foram lavadas por aspersão com água e posteriormente imersas em banho contendo solução de hipoclorito de sódio (100 mg de cloro/L) por 10 min. Todas as peças do extrator receberam o mesmo tratamento de limpeza das frutas. A extração do suco de laranja foi realizada em extrator JBT 391B, utilizando a configuração normalmente empregada para obtenção de suco NFC (*not from contrate*) de alta qualidade (*premium juice extractor*). Posteriormente o suco foi filtrado em *finisher* UFC 35 (malha de 0,25 mm). A seguir, o suco foi congelado e armazenado à -18 C° até a utilização.

2.3. Processamento do suco de laranja usando alta pressão hidrostática (APH)

Para o processamento usando APH, o suco de laranja (100 mL) foi colocado em saco flexível de polietileno, que foi submetido à pressurização no equipamento de APH Stansted Food Lab 9000 (Stansted Fluid Power, S-FL-850-9-W, Reino Unido). As condições usadas no processamento do suco de laranja foram definidas em estudo de otimização previamente desenvolvido (Bisconsin-Junior et al., 2013a). Para o processamento foram empregadas 520 MPa, 60 °C por 360 s, condições capazes de produzir suco de laranja com atividade residual de pectinametilesterase menor que 20% e contagem de

microrganismos aeróbios menor que 2 log UFC/mL. O equipamento de APH utilizado apresenta câmara de compressão de 500 mL, que opera com pressão máxima de 900 MPa, em intervalo de temperatura entre -20 °C e 90 °C. A temperatura na câmara de compressão foi controlada por meio de uma camisa externa ligada a um banho-maria. O fluído pressurizante utilizado foi etanol 70% (v/v). A taxa de compressão foi 3,5 MPa s^{-1} e o tempo de descompressão foi menor que 10 s. O tempo de compressão e descompressão não foi considerado para o processamento. Durante o processamento a temperatura e pressão da câmara de compressão foram monitoradas (Figura 1). Antes da pressurização, a temperatura dos sacos contendo suco de laranja e do fluído pressurizante foi ajustada a 45 °C, com o objetivo de atingir a temperatura de pressurização.

2.4. Pasteurização do suco de laranja

O suco de laranja foi pasteurizado usando trocador de calor tubular Armfield FT25D SSHE (Armfield, Reino Unido) a 95 °C por 30 s (Braddock, 1999), e em seguida resfriado a 20 °C. Após a pasteurização, o suco foi envasado assepticamente em frascos de polietileno de alta densidade (500 mL), previamente higienizados com álcool 70 % (v/v) e secos em estufas a 105 ºC por 30 min.

2.5. Análise físico-química

As características físico-químicas do suco de laranja não processado (extraído e congelado), pressurizado e pasteurizado foram avaliadas. Foram avaliados o teor de sólidos solúveis, acidez total titulável, pH, açúcares

redutores e totais,de acordo com a AOAC (2011), além do *ratio*, que foi calculado (sólidos solúveis/acidez total titulável). Todas as análises foram realizadas em triplicata.

2.6. Atividade da enzima pectinametilesterase (PME)

A atividade da PME foi determinada de acordo com Hagerman e Austin (1986). Suco de laranja (4,5 g) e solução de NaCl (15 ml, 8,8% m/v) foram homogeneizados e centrifugados a 18.000 g por 20 min a 4 ºC (Hitachi, Himac CR 22G II, Japão). O sobrenadante foi coletado e utilizado como extrato enzimático. O substrato foi composto de 2 ml de solução de pectina cítrica 0,5% (m/v), 150 µL da solução do corante azul de bromotimol 0,01% (m/v) em tampão de fosfato de potássio 0,003 M e 830 µL de água destilada. O pH do substrato e do extrato enzimático foi ajustado a 7,5. O extrato enzimático (20 µL) foi adicionado ao substrato e a reação de hidrólise da pectina foi monitorada pelo decréscimo da absorbância a 620 nm em curva cinética usando espectrofotômetro (Evolution 220, Thermo Scientific, EUA). Água destilada foi usada como branco. A atividade da PME foi calculada utilizando a porção linear da curva. Uma unidade da atividade de PME foi expressa pelo decréscimo de 0,001 de absorbância por min por mL de extrato enzimático. As análises da atividade da PME foram realizadas em triplicata a 25 °C.

A atividade residual da PME (%) do suco de laranja foi calculada de acordo com a seguinte equação:

(Eq. 1)

$$PME = \frac{PME_p}{PME_i} \text{ x100}$$

na qual, *PME* = atividade residual da PME (%), PME_p = atividade da PME do suco de laranja (não processado, pressurizado e pasteurizado), e PME_i = atividade da PME do suco de laranja não processado.

2.7. Contagem de microrganismos

Suco de laranja (10 mL) foi adicionado a 90 mL de água peptonada tamponada (APT) estéril. Após homogeneização, alíquotas foram diluídas em série em APT e 1 mL de cada diluição foi inoculado em placa 3M™ Petrifilm™ para contagem de microrganismos mesófilos aeróbios, bolores e leveduras, e coliformes termotolerantes e totais. A contagem de microrganismos mesófilos aeróbios, e coliformes termotolerantes e totais foi realizada após incubação a 35 ± 1 ºC por 48 ± 3 horas, e a contagem de bolores e leveduras, após incubação a 25 ± 1 ºC por 120 ± 6 horas. O nível mínimo para quantificação era 10 UFC/mL (AOAC, 2011). As análises foram feitas em triplicata.

2.8. Cor instrumental

Para a determinação de cor foi utilizado um espectrofotômetro Konica Minolta CM-600D (Konica Minolta Sensing, Osaka, Japão) (Martínez et al., 2005). A configuração do espectrofotômetro utilizou fonte de iluminação D_{65}, ângulo de observação de 10° e abertura de 8 mm. O suco de laranja foi colocado em cubeta de quartzo com caminho ótico de 10 mm (50 x 38 x 10 mm). Foram avaliados os parâmetro de cor L*, a* e b*. O chroma (saturação da

cor), o ângulo *Hue* e a diferença total da cor foram calculados. As análises foram realizadas a 25 °C, em quintuplicata.

2.9. Ácido ascórbico

A determinação de ácido ascórbico foi baseada na redução de 2,6-dicloroindofenol (AOAC, 2011). As análises foram realizadas em triplicata e os resultados expressos como mg de ácido ascórbico/100 mL de suco de laranja.

2.10. Extração dos compostos fenólicos totais (TPC) e antioxidantes

A extração dos TPC e antioxidantes foi baseada no procedimento descrito por Asami et al. (2003). Suco de laranja (5 mL) e solução de metanol:água (80:20, v/v) foram homogeneizados em vortex por 1 min e, em seguida, submetidos a banho de ultrassom à temperatura ambiente por 15 min. A mistura foi centrifugada a 10000 g por 20 min a 20 °C e o sobrenadante foi coletado. O procedimento de extração foi repetido uma vez mais, usando as mesmas condições.

2.11. Compostos fenólicos totais (TPC)

Os TPC foram determinados como descrito por Asami et al. (2003) e Singleton et al. (1999). Uma alíquota de extrato do suco de laranja (0,4 mL) foi adicionada a 0,12 mL de reagente de Folin–Ciocalteu em balão volumétrico de 10 mL. Após 6 min, 4 mL de solução de carbonato de sódio (70 g/L) foram adicionados e o volume foi ajustado até 10 mL com água. A mistura permaneceu à temperatura ambiente por 2 h. Em seguida, a leitura de absorbância foi realizada a 730 nm usando um espectrofotômetro (Evolution

220, Thermo Scientific, EUA). A quantificação foi realizada utilizando curva de calibração preparada com soluções de ácido gálico (72–200 mg/L). As analises de TPC foram realizadas em triplicata e os resultados expressos como mg de ácido gálico/100 mL de suco de laranja.

Foi utilizado um fator de correção para descontar a interferência do ácido ascórbico, que reage com o Folin-Ciocalteu. Soluções padrão de ácido ascórbico (300 a 400 mg/L) foram preparadas na faixa de concentração de ácido ascórbico dos sucos de laranja, e submetidas à reação com Folin-Ciocalteu. Foi obtida uma relação entre ácido ascórbico:ácido gálico de 2,4 x 10^{-2}, que foi deduzida dos valores de TPC dos sucos.

2.12. Atividade antioxidante total (AAT)

A AAT dos sucos de laranja foi avaliada usando a reação de redução do ferro (FRAP) e a reação de captura dos radicais ABTS e DPPH, com base em Rufino et al. (2010). Alíquotas do extrato de suco de laranja diluídas (3:7, 5:5, 7:3, 9:1 e extrato puro) com solução de metanol:água (80:20 v/v) foram utilizadas para determinar a atividade antioxidante.

Para avaliar a reação de redução do ferro (FRAP), a solução do reagente FRAP foi preparada usando mistura de tampão acetato (300 mM), solução de TPTZ (10 mM) e solução de cloreto férrico (20 mM) na proporção de 10:1:1. Uma alíquota de 150µL de cada uma das diluições do extrato de suco de laranja foi homogeneizada com 4 mL de solução de reagente FRAP e o volume do balão volumétrico (5 mL) foi completado com água destilada. A solução foi aquecida a banho-maria por 30 min a 37 °C. A leitura de

absorbância foi realizada a 595 nm em espectrofotômetro (Evolution 220, Thermo Scientific, EUA).

Para a formação do radical ABTS, 88 µL de persulfato de potássio (140 mM) foram adicionados a 5 mL de solução de ABTS (7 mM). A solução permaneceu no escuro por 16 h para garantir a formação completa do radical ABTS. A solução de radical ABTS foi diluída com etanol até que fosse obtida absorbância de 0,70 ± 0,05 a 753 nm. Uma alíquota de 30 µL de cada uma das diluições do extrato do suco foi adicionada a 3 mL de solução de radical ABTS e após 6 min de reação foi realizada a leitura de absorbância em espectrofotômetro a 753 nm.

Para a obtenção do radical de DPPH, uma alíquota da solução metanólica de DPPH (0,06 mM) foi diluída com metanol até obter uma absorbância de 0,75 ± 0,05 a 515 nm. Uma alíquota de 150µL de cada uma das diluições do extrato de suco foi adicionada em um balão volumétrico de 5 mL e o volume foi completado com solução do radical de DPPH. Após 30 min de reação no escuro, foi feita a leitura da absorbância em espectrofotômetro a 515 nm.

Para a curva de calibração foram usadas soluções de Trolox em etanol (100–1400 µmol/L). As análises foram realizadas em triplicata e os resultados expressos como µmol Trolox/100 mL de suco de laranja.

2.13. Análise estatística

Os resultados foram submetidos à análise de variância e teste de Tukey ($p<0,05$) utilizando o software OriginPro 8.5 (Origin Lab, Northampton, Reino Unido).

3. RESULTADOS E DISCUSSÃO

3.1. Avaliação físico-química do suco de laranja

As características físico-químicas do suco de laranja não processado, do suco pressurizado e do suco pasteurizado estão apresentadas na Tabela 1. Não houve diferença no teor de sólidos solúveis entre o suco pressurizado e não processado, enquanto o suco pasteurizado apresentou o maior teor de sólidos solúveis (9,7 ºBrix) ($p \leq 0,05$), indicando que houve perda de água durante o tratamento térmico (Monteiro et al., 2002). O suco de laranja não processado apresentou a maior acidez total titulável e diferiu significativamente ($p \leq 0,05$) do suco pressurizado e do suco pasteurizado, que não diferiram entre si ($p > 0,05$). O *ratio* do suco pressurizado e do suco pasteurizado foi maior ($p \leq 0,05$) do que o do suco não processado. Não houve diferença no pH dos sucos de laranja ($p > 0,05$). O comportamento dos sucos em relação aos açúcares redutores e açúcares totais foi semelhante, sem diferença significativa entre o suco não processado e o suco pasteurizado ($p > 0,05$). O suco pressurizado apresentou o menor teor de açúcares redutores e açúcares totais ($p \leq 0,05$).

A pasteurização também causou aumento no teor de sólidos solúveis do suco de laranja da variedade Navel (Sánchez-Moreno et al., 2005) e Valencia (Bull et al., 2004 e Farnworth et al., 2001), corroborando com os resultados obtidos neste trabalho. Sánchez-Moreno et al. (2005) também reportaram redução da acidez total titulável após pasteurização do suco de laranja Navel.

Todos os sucos de laranja avaliados atenderam aos limites estabelecidos pelo padrão de identidade e qualidade (PIQ) da legislação

brasileira, com exceção dos sólidos solúveis que estavam abaixo de 10,5 °Brix (Brasil, 2000).

3.2. Atividade da PME e contagem de microrganismos do suco de laranja

A atividade residual da PME dos sucos de laranja está apresentada na Tabela 2. O suco de laranja não processado apresentou atividade da PME de 247 U, que foi considerada como 100% da atividade residual. O suco de laranja pasteurizado apresentou 4% de atividade residual da PME, enquanto o suco pressurizado apresentou 13% de atividade residual da PME, próximo à atividade residual prevista (15%) em estudo de otimização anterior, que avaliou o efeito das condições de processamento por APH (pressão, temperatura e tempo) na atividade da PME a na contagem de microrganismos do suco de laranja da variedade Pêra Rio (Bisconsin-Junior et al., 2013a). O processamento térmico empregado (95 °C por 30 s) foi mais efetivo na redução da atividade da PME do que o processamento por APH (520 MPa, 60 °C, 360s). As condições empregadas na pasteurização do suco de laranja estão dentro da faixa de processamento comumente adotada para obtenção de sucos de laranja comerciais, de 90 a 99 °C por 15 a 30 s (Braddock, 1999). Ainda que o suco pressurizado apresente atividade residual da PME, é possível preservar sua estabilidade durante a vida de prateleira. A atividade da enzima PME é responsável em grande parte pela perda de qualidade do suco de laranja, causando redução de viscosidade, perda de turbidez e separação de fases no suco. A PME causa a demetoxilização das pectinas solúveis, resultando na precipitação de pectatos de cálcio e clarificação do suco

(Versteeg et al., 1980). Alguns estudos reportaram a estabilidade do suco de laranja com diferentes atividades residuais da PME após o processamento por APH. Nienaber e Shellhammer (2001) após submeterem suco de laranja a 800 MPa a 25 °C por 60 s, obtiveram suco com 4 % de atividade residual da PME, que permaneceu estável por mais de 90 dias armazenado a 4 °C e a 37 °C. Goodner et al. (1998) reportaram que o suco de laranja processado com 700 MPa por 60s apresentou 18% de PME residual, permanecendo estável por mais de 50 dias a 4 °C.

No suco de laranja não processado foram verificadas contagens de 1,03x10² UFC/mL para microrganismos aeróbios, 3,6x10 UFC/mL para bolores e leveduras e valores abaixo do limite de quantificação (<10 UFC/mL) para coliformes totais, além de não terem sido detectados colônias de coliformes termotolerantes (Tabela 2). A contagem de microrganismos do suco de laranja recém-extraído, geralmente, está entre 10^3 a 10^6 UFC/mL, dependendo do método de extração e da variedade da laranja (Fellers e Higgins, 1988). A sanitização das laranjas e do equipamento de extração com solução de hipoclorito de sódio contribuiu para que a contagem de microrganismos do suco de laranja não processado fosse menor que 10^3 UFC/mL. O suco de laranja pressurizado e o pasteurizado não apresentaram contagem de colônias nas análises microbiológicas, indicando a eficiência da pressurização na destruição dos microrganismos. Resultados semelhantes foram observados no suco de laranja pasteurizado a 95 °C por 30s (Yeom, 2000) e no suco pressurizado a 600 MPa por 60 s (Bull et al., 2004).

3.3. Cor instrumental do suco de laranja

Os sucos de laranja pressurizado e não processado apresentaram luminosidade (L*) (claridade) menor do que o suco pasteurizado ($p \leq 0,05$), indicando que o suco pasteurizado apresentou mais brilho que os demais (Tabela 3). Estes resultados são semelhantes aos reportados por Lee e Coates (2003), que verificaram aumento no L* do suco de laranja (*var.* Valencia) após a pasteurização. Não foi observada alteração de claridade no suco de laranja (*var.* Navel) processado a 400 MPa, 40 °C por 60 s (Sanchéz-Moreno et al., 2005). A concentração e o tipo de carotenoides são responsáveis pela cor do suco de laranja (Lee & Coates, 2003). O parâmetro a*, que expressa a variação entre a cor verde e vermelho, e o parâmetro b*, a variação entre a cor amarelo e azul, tiveram valores mais baixos no suco não processado ($p \leq 0,05$). Houve aumento na cor vermelha e amarela do suco de laranja pasteurizado e pressurizado (Tabela 3). Cortés et al. (2008) também verificaram aumento na cor amarela (parâmetro b*) após a pasteurização do suco de laranja (*var.* Valencia).

O chroma e o ângulo Hue foram mais elevados no suco de laranja pasteurizado, seguido pelo suco pressurizado e pelo suco não processado ($p \leq 0,05$) (Tabela 3). Resultados similares foram obtidos por Cortés et al. (2008), que observaram aumento no chroma após pasteurização do suco de laranja e tratamento com campo elétrico pulsado de alta intensidade (tecnologia não convencional). Lee e Coates (2003) também verificaram aumento do ângulo Hue após a pasteurização do suco de laranja. A diferença total da cor expressa a magnitude da diferença entre a cor do suco não processado com relação ao suco pressurizado e ao suco pasteurizado. O suco

pasteurizado apresentou diferença total da cor maior que a do suco pressurizado e ambos apresentaram valores maiores que 2, indicando que é possível notar visualmente a diferença entre o suco de laranja não processado e os sucos pressurizado e pasteurizado, como relatado por Francis & Clydesdale, 1975.

3.4. Ácido ascórbico, compostos fenólicos totais e atividade antioxidante do suco de laranja

O teor de ácido ascórbico, compostos fenólicos totais e a atividade antioxidante do suco de laranja não processado, do suco pressurizado e do suco pasteurizado estão apresentados na Tabela 4. O teor de ácido ascórbico dos sucos de laranja estava dentro da faixa de ácido ascórbico dos sucos de laranja comerciais brasileiros (Stella et al., 2011) e espanhóis (Meléndez-Martínez et al., 2007). O suco de laranja não processado apresentou o maior teor de ácido ascórbico e diferiu significativamente ($p \leq 0,05$) dos sucos processados por APH e pasteurizado, como esperado. O suco pasteurizado apresentou teor de ácido ascórbico maior que o suco pressurizado ($p \leq 0,05$). A pressurização do suco de laranja promoveu redução de 16% no teor de ácido ascórbico, maior do que aquela descrita para suco de laranja da variedade Valência (5%) (Plaza et al., 2006) e Navel (8%) (Sánchez-Moreno et al., 2005) submetidos a 400 MPa, 40 °C durante 60 s. Nossos resultados mostraram degradação mais acentuada de ácido ascórbico devido ao processamento por APH empregar tempo e temperatura elevados (520 MPa, 60 °C por 360 s). Em estudo anterior (Bisconsin-Junior et al., 2013b), foi verificado que o aumento do tempo e da temperatura durante processamento do suco de laranja por APH

diminuía o teor de ácido ascórbico. A degradação de 13% no ácido ascórbico do suco pasteurizado foi menor que a reportada por Elez-Martínez et al. (2006), de 17%, e maior que aquela descrita por Sánchez-Moreno et al. (2005), de 8%, após pasteurizar suco de laranja a 90 °C por 60 s.

O teor de compostos fenólicos totais (TPC) dos sucos de laranja, da ordem de 53 mg/100mL, não apresentou diferença entre si ($p>0,05$). A atividade antioxidante dos sucos de laranja usando a reação de redução do ferro (FRAP) variou entre 447 e 489 µmol Trolox/100 mL, enquanto que a atividade antioxidante usando as reações de captura dos radicais ABTS e DPPH foram de 294-301 e 220-230 µmol de Trolox/100mL, respectivamente, embora não tenha havido diferença significativa entre os sucos não processado, pressurizado e pasteurizado em nenhuma das reações empregadas ($p>0,05$). Os níveis de TPC e a atividade antioxidante usando a reação com o radical ABTS foram semelhantes aos reportados para sucos de laranja brasileiros (Stella et al., 2011). Gil-Izquierdo et al. (2002) e Sánchez-Moreno et al. (2005) reportaram que o conteúdo de TPC e a atividade antioxidante do suco de laranja não foram afetados significativamente após pasteurização e tratamento por APH usando 400 MPa, 40 °C por 60 s. Contudo, Patras et al. (2009) verificaram que pressões acima de 500 MPa foram capazes de promover aumento nos TPC de polpa de morango e amora.

4. CONCLUSÕES

O processamento do suco de laranja por APH e a pasteurização não causaram alterações expressivas nas características físico-químicas do suco, mas reduziram a atividade da enzima PME e a contagem de microrganismos a

níveis seguros, capazes de garantir a estabilidade do suco de laranja. A cor do suco de laranja foi afetada pela pressurização e pasteurização, aumentando a luminosidade, a cor amarela e a cor vermelha. O teor de ácido ascórbico foi menor no suco pressurizado e no suco pasteurizado, porém o TPC e a atividade antioxidante não apresentaram diferença do suco não processado. A tecnologia de APH pode ser empregada como alternativa à pasteurização, por não afetar sobremaneira a qualidade do suco de laranja e garantir a estabilidade enzimática e microbiológica.

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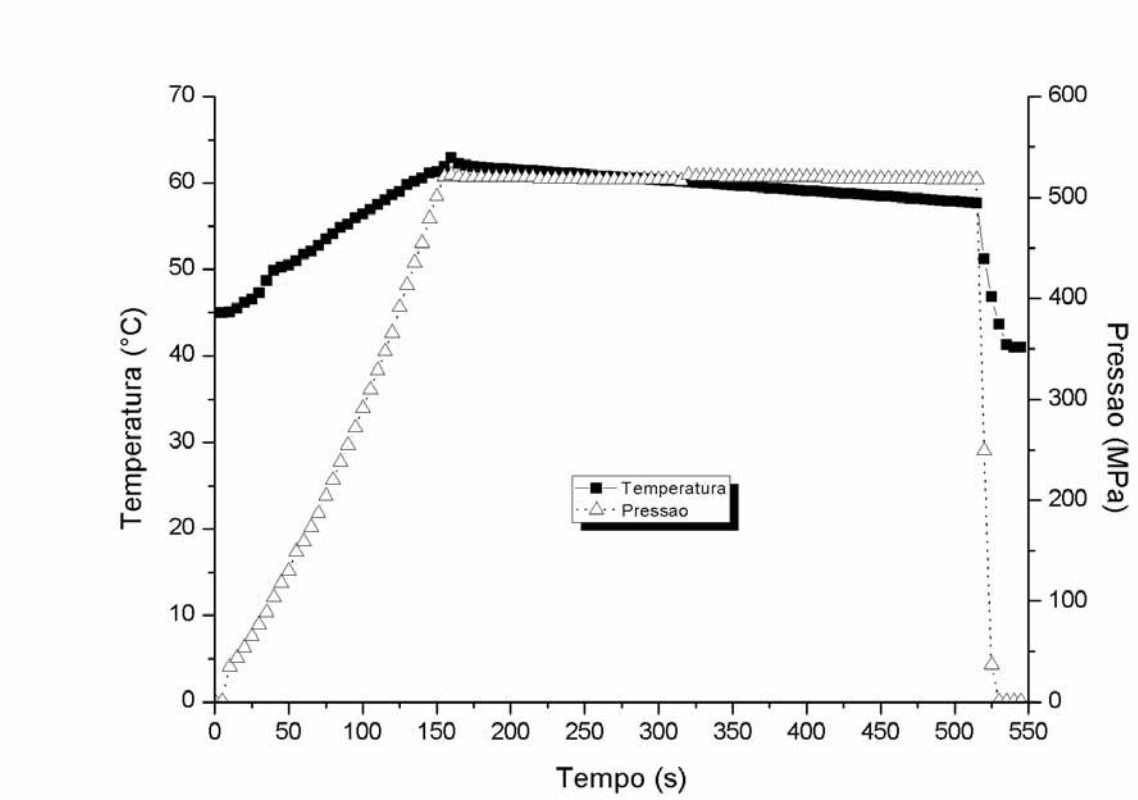

Figura 1. Temperatura e pressão da câmara de compressão durante o processamento por APH do suco de laranja.

Tabela 1. Características físico-químicas do suco de laranja não processado, do suco processado por APH e do suco pasteurizado.

Parâmetro	Suco de laranja		
	Não processado	**APH**	**Pasteurizado**
Sólidos solúveis (°Brix)	$9,46^{b} \pm 0,07$	$9,55^{b} \pm 0,08$	$9,71^{a} \pm 0,07$
Acidez total titulável (g ácido cítrico/100mL)	$0,67^{a} \pm 0,01$	$0,60^{b} \pm 0,01$	$0,61^{b} \pm 0,01$
Ratio	$14,2^{b} \pm 0,2$	$16,0^{a} \pm 0,2$	$15,6^{a} \pm 0,3$
pH	$4,06^{a} \pm 0,01$	$4,07^{a} \pm 0,04$	$4,05^{a} \pm 0,04$
Açúcares redutores (g glicose/100mL)	$3,74^{a} \pm 0,04$	$3,52^{b} \pm 0,04$	$3,73^{a} \pm 0,05$
Açúcares totais (g glicose/100mL)	$7,11^{a} \pm 0,05$	$6,64^{b} \pm 0,02$	$7,10^{a} \pm 0,07$

Médias com letras iguais na mesma linha não diferem entre si no teste de Tukey ($p \leq 0,05$).

Tabela 2. Atividade residual da enzima PME e contagem de microrganismos mesófilos aeróbios, bolores e leveduras, coliformes termotolerantes e totais dos sucos de laranja não processado, processado por APH e pasteurizado.

Parâmetro	**Não processado**	**APH**	**Pasteurizado**
Atividade residual PME (%)	100,00 ± 6,85	13,19 ± 2,15	4,24 ± 0,29
Mesófilos aeróbios (UFC/mL)	$1,03x10^2 \pm 2,1x10$	ND	ND
Bolores e leveduras (UFC/mL)	3,6x10 ± 1,5x10	ND	ND
Coliformes totais (UFC/mL)	<10	ND	ND
Coliformes termotolerantes (UFC/mL)	ND	ND	ND

ND Não detectado
UFC - Unidade formadora de colônia

Tabela 3. Cor instrumental dos sucos de laranja não processado, processado por APH e pasteurizado.

Parâmetro	Não processado	APH	Pasteurizado
L*	41,38^{c} ± 0,16	42,47^{b} ± 0,27	45,68^{a} ± 0,07
a*	-1,78^{b} ± 0,03	-1,32^{a} ± 0,11	-1,21^{a} ± 0,05
b*	16,13^{c} ± 0,24	18,35^{b} ± 0,42	22,21^{a} ± 0,12
Chroma[1]	16,22^{c} ± 0,23	18,40^{b} ± 0,41	22,24^{a} ± 0,12
Ângulo Hue[2]	83,70^{c} ± 0,17	85,87^{b} ± 0,43	86,87^{a} ± 0,13
Diferença total da cor[3]	0	2,52 ± 0,77	7,52 ± 0,13

Médias com letras iguais na mesma linha não diferiram entre si no teste de Tukey (p≤0,05).

[1] Chroma = $\sqrt{a^{*2}+b^{*2}}$

[2] Ângulo Hue = arcotangente (b*/a*)

[3] Diferença total da cor = $\sqrt{(L^{*'}-L^{*}0)^{2} + (a^{*'}-a^{*}0)^{2} + (b^{*'}-b^{*}0)^{2}}$

Tabela 4. Ácido ascórbico, compostos fenólicos totais (TPC) e atividade antioxidante usando a reação de redução do ferro (FRAP) e a captura dos radicais ABTS e DPPH dos sucos de laranja não processado, processado por APH e pasteurizado.

Parâmetro	Não processado	APH	Pasteurizado
Ácido ascórbico (mg/100mL)	38,34^{a} ± 0,35	32,22^{b} ± 0,22	33,27^{c} ± 0,26
TPC (mg ácido gálico/100 mL)	53,24^{a} ± 2,77	53,14^{a} ± 3,89	53,01^{a} ± 2,18
FRAP (µmol Trolox/100 mL)	447,61^{a} ± 41,27	480,29^{a} ± 8,55	489,25^{a} ± 3,72
ABTS (µmol Trolox/100 mL)	294,88^{a} ± 6,84	300,59^{a} ± 16,36	294,26^{a} ± 6,16
DPPH (µmol Trolox/100 mL)	220,35^{a} ± 27,21	228,89^{a} ± 9,22	229,72^{a} ± 21,11

Médias com letras iguais na mesma linha não diferiram entre si no teste de Tukey (p≤0,05).

CONCLUSÕES

Os resultados obtidos nos permite concluir que os objetivos deste trabalho foram atingidos, sendo apresentadas a seguir as principais conclusões de cada capítulo:

1) No processamento do suco de laranja por alta pressão hidrostática (APH), o aumento da pressão, temperatura e tempo diminuiu a contagem de microrganismos nativos e a atividade da enzima pectinametilesterase. As condições ótimas de processamento foram de 550 a 600 MPa, 55 a 60 °C e 330 a 360 s, capazes de fornecer suco de laranja estável com contagem de microrganismos abaixo de 2 ciclos logarítmicos UFC/mL e atividade residual da enzima pectinametilesterase menor que 20%. A metodologia de superfície de resposta foi considerada uma ferramenta eficaz para otimizar o processamento do suco de laranja por APH.

2) O processamento do suco de laranja por APH reduziu o teor de ácido ascórbico e a atividade antioxidante do suco de laranja, sob as condições avaliadas. Todas as variáveis de processamento (tempo, temperatura e pressão) foram significativas, contudo o tempo foi a variável que exerceu maior influência na diminuição do ácido ascórbico e da atividade antioxidante. As condições de processamento entre 100 a 250 MPa, 30 a 40 °C e 30 a 125 s foram capazes de produzir suco de laranja com mais de 70% do teor inicial de ácido ascórbico e 80% da atividade antioxidante inicial. A metodologia de superfície de resposta também foi considerada efetiva para descrever o efeito das condições de processamento por APH no ácido ascórbico e na atividade antioxidante do suco de laranja.

3) Usando o modelo preditivo para a atividade residual da PME e para a contagem de microrganismos, o suco de laranja Pêra Rio foi processado a 520 MPa, 60 °C por 360 s e pasteurizado (95 ºC por 30 s). O processamento por APH e a pasteurização não causaram alterações expressivas nas características físico-químicas do suco de laranja. No entanto, a pasteurização e a APH reduziram a atividade da enzima PME e a contagem de microrganismos a níveis seguros, capazes de garantir a estabilidade do suco de laranja. A cor do suco de laranja foi afetada pela

APH e pasteurização, com aumento da luminosidade, da cor amarela e da cor vermelha do suco. O teor de ácido ascórbico foi reduzido pela pressurização e pasteurização do suco, porém o teor de compostos fenólicos totais e a atividade antioxidante não foram afetados.

Estes resultados indicam que a tecnologia de APH pode ser utilizada como alternativa à pasteurização do suco de laranja da variedade Pêra Rio.

ANEXO

ARTIGO PUBLICADO

OPTIMISATION OF HIGH HYDROSTATIC PRESSURE PROCESSING OF PÊRA RIO ORANGE JUICE

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ORIGINAL PAPER

Optimisation of High Hydrostatic Pressure Processing of Pêra Rio Orange Juice

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Abstract The influence of high hydrostatic pressure (HHP) on Pêra Rio orange juice was investigated using response surface methodology. A central composite design was used to evaluate the effects of three processing conditions (independent variables), namely pressure (100–600 MPa), temperature (30–60 °C) and time (30–360 s), on the native microflora and pectin methylesterase (PME) activity of orange juice. Analysis of variance showed that second-order polynomial models fitted well with the experimental data for PME residual activity (R^2=0.9586, p<0.001) and aerobic microorganism count (R^2=0.9879, p<0.001). The optimum HHP processing conditions to produce orange juice with PME residual activity of less than 20 % and low microorganism count (<2 log cycles CFU/mL) were 550 to 600 MPa, 55 to 60 °C and 330 to 360 s.

Keywords High hydrostatic pressure · Orange juice · Pêra Rio variety · Response surface methodology · Pectin methylesterase · Microorganism counts

Introduction

Orange juice is the most popular fruit juice in the world. Its consumption is estimated at more than 9 billion litres per year, which represents 47 % of global fruit juice consumption. Brazil is the main orange juice producer and exporter in the world, having exported 463 thousand tons of frozen concentrated orange juice and 944 thousand tons of pasteurised juice (not from concentrate) in the 2011/2012 harvest (CitrusBR 2012). The quality of orange juice is mainly influenced by enzymatic activity and microflora. The activity of several pectin methylesterase (PME) isoenzymes is associated with cloud loss which can further cause gelation of juice (Versteeg et al. 1980). Due to orange juice acidity, the spoilage microflora is limited to yeasts, moulds and lactic acid bacteria that may lead to off flavour, turbidity and gas production (Lawlor et al. 2009).

The most extensively used process for orange juice stabilisation is thermal pasteurisation, which inactivates vegetative microorganisms and enzymes improving shelf life. However, pasteurisation at intense time/temperature conditions induces ascorbic acid, carotenoids and flavour losses, as well as colour changes, affecting the juice's overall quality (Naim et al. 1997; Hyoung and Coates 2003; Janzantti et al. 2011).

In order to improve orange juice shelf life and stability, high hydrostatic pressure (HHP) processing can be used as an alternative method. Vitamin C, carotenoids, colour, flavour, soluble solids, pH and other compounds of orange juice are not considerably affected by HHP (Timmermans et al. 2011; Vervoort et al. 2011; Baxter et al. 2005; Bull et al. 2004). HHP has the potential to reduce orange juice spoilage microflora and PME activity without using high temperatures, therefore preserving sensory and nutritional characteristics.

Some studies reported inactivation of orange juice spoilage microflora by HHP. Lactic acid bacteria are considerably more resistant to HHP than yeasts, while moulds are the most labile spoilage microflora of orange juice (Patterson 2005). Katsaros et al. (2010) developed a mathematical model to predict the destruction of *Lactobacillus plantarum* and *Lactobacillus brevis* in HHP-processed orange juice (100–500 MPa, 20–40 °C) and found that 360 MPa at 35 °C for 2 min was adequate to obtain 7 log cycle reductions of both microorganisms. Basak et al. (2002) reported inactivation of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* in single strength and

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concentrated orange juice submitted to HHP (100–400 MPa at 20 °C). Kinetics analysis revealed two different effects in pressure inactivation of microorganisms: an instantaneous pressure kill (dependent on the pressure level) and a first-order inactivation (dependent on holding time).

PME inactivation depends on the enzyme environment of the particular food system and even on the variety and origin of orange juice (Irwe and Olsson 1994). Goodner et al. (1998) studied PME inactivation of Valencia orange juice from Florida (USA) using HHP in the range of 500–900 MPa and found that the labile form of PME was inactivated with almost no effect on the stable form. The use of 50–400 MPa combined at 20–60 °C showed that only combinations of low pressures and mild temperatures inactivated PME from freshly squeezed orange juice (*Citrus aurantium*, Salustiana variety, Spain), with a maximum reduction (25 %) of the initial PME activity after HHP at 200 MPa, 30 °C for 15 min (Cano et al. 1997). Polydera et al. (2004) evaluated the inactivation kinetics of PME in Greek Navel orange juice using 100–800 MPa with 30–60 °C and reported that 600 MPa, 40 °C for 4 min, could lead to inactivation of the pressure-labile PME. Navel orange juice from Victoria (Australia) processed at 600 MPa, 20 °C for 60 s exhibited a 45 % reduction in PME activity (Bull et al. 2004), while the same processing conditions were enough to inactivate 92 % of PME orange juice derived from a mixture of Valencia, Pêra and Baladi orange varieties (Vervoort et al. 2011). Basak and Ramaswamy (1996) observed that an increase in total soluble solids content (10 to 40 °Brix) decreased PME inactivation rates of HHP-processed orange juice. Some studies related lower pH of orange juice with higher inactivation of PME (Basak and Ramaswamy 1996; Bull et al. 2004; Tribess and Tadini 2006).

There is no available literature about Pêra Rio orange juice HHP processing, the most characteristic Brazilian orange variety. The aim of this work was to evaluate the influence of HHP processing conditions (pressure, temperature and time) on PME activity and microbial counts of orange juice from the Pêra Rio variety.

Materials and Methods

Materials and Chemicals

Orange fruit of the Pêra Rio variety was provided by a citrus industry from Araraquara, SP, Brazil. The fruit was cultivated in Bauru, SP, Brazil (22°25′59″ S, 49°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 setting and a UFC-35 finisher (sieve 0.25 mm) at the JBT FoodTech Citrus System, Araraquara, SP, Brazil. After extraction, the juice was frozen and stored at −18 °C for 2 months.

Citrus pectin was obtained from Sigma-Aldrich (St. Louis, MO, USA); ascorbic acid and glucose were purchased from Merck (Darmstadt, Germany); bromothymol blue and sodium chloride were from Qhemis (São Paulo, SP, Brazil); sodium carbonate, sodium hydroxyl, potassium sodium tartrate tetrahydrate, cupric sulfate pentahydrate and potassium phosphate dibasic were from Labsynth (Diadema, SP, Brazil); oxalic acid and 2.6-dichloroindophenol sodium salt hydrate were from Vetec (Rio de Janeiro, RJ, Brazil); and Petrifilm™ for aerobic count and yeast and mould count were sourced from 3M™ (St. Paul, MN, USA).

HHP and Thermal Processing of Orange Juice

For the HHP processing, the orange juice was packaged in flexible PE bags (100 mL) and processed in Stansted Food Lab 9000 (Stansted Fluid Power, S-FL-850-9-W, UK) within a pressure vessel of 500 mL. The equipment has a maximum nominal operation pressure of 900 MPa and a temperature which ranged from −20 to 90 °C. The temperature in the vessel was controlled by liquid circulation in the outer jacket connected to a heating–cooling system. The pressure transmitting fluid used was 70 % (*v*/*v*) ethanol. The compression rate was 7 MPa s^{-1} and the decompression time was less than 10 s. Compression and decompression times were not included in the experimental design.

Previous to HHP processing, tests were done in order to determine the adiabatic heating of pressurising fluid and orange juice for each experimental condition. The adiabatic heating ranged from 3.6 to 6.8 °C/100 MPa for the pressurising fluid and from 2.8 to 3.5 °C/100 MPa for orange juice. For all experiments, the orange juice PE bags and the vessel had the temperature adjusted to a few degrees below the targeted temperature in order to achieve the desired condition during pressurisation.

For the thermal processing, orange juice (15 mL) was placed in Pyrex glass tubes (outer diameter=18 mm, inner diameter=16 mm, height=180 m), which were immersed in an oil bath at 100 °C, and heated at 95±1 °C for 30 s. A thermocouple positioned in the juice cold point was used to measure the temperature. The time for the juice to reach 95± 1 °C was less than 2 min. Once the processing conditions were reached, the tubes were taken out of the oil bath and immediately cooled in a water/ice mixture.

Non-processed (extracted and filtered) and thermally processed (95 °C, $30s^{-1}$) orange juices were used as references for comparison with HHP orange juice.

Experimental Design

The response surface methodology was used to evaluate the effect of the independent variables (pressure, temperature and time) on PME activity and total counts of aerobic

microorganisms and yeasts and moulds (response variables) of HHP orange juice.

A central composite design (CCD) of three independent variables with five levels, containing a 2^3 factorial design, 6 axial points and 3 repetitions of the central point, totalizing 17 essays was used (Rodrigues and Iemma 2009). 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, which made it possible to estimate the lack of fit of the statistical model and the pure error; and –1.68 and +1.68, representing the axial points, allowing a quadratic statistical model (Table 1).

PME

The PME activity was evaluated according to Hagerman and Austin (1986). Orange juice and NaCl (8.8 % *w*/*v*) were homogenised (4.5:15, *w*/*v*) and centrifuged at 18,000×*g* for 20 min at 4 °C. The supernatant was collected and used as enzymatic extract. The substrate was composed of 2 mL 0.5 % citrus pectin (*w*/*v*), 150 μL of 0.01 % bromothymol blue (*w*/*v*) in 0.003 M potassium phosphate buffer and 830 μL distilled water. The substrate and enzymatic extract were adjusted to pH 7.5. Substrate was added to enzymatic extract (20 μL) and absorbance decrease was monitored at 620 nm using a spectrophotometer (Evolution 220, Thermo Scientific, USA). Distilled water was used as the blank. A kinetic curve of the absorbance decrease was obtained and PME activity was calculated from the linear portion of the curve. One unit of PME activity is defined as a decrease of 0.001 in absorbance per minute per millilitre of enzymatic extract. PME activity analyses were performed in triplicate, at 25 °C.

The PME residual activity (in percent) after each HHP and thermal processing treatment was calculated according to Eq. 1:

$$PME = \frac{PME_p}{PME_0} \times 100 \quad (1)$$

where PME = PME residual activity (in percent), PME_p = orange juice PME activity after HHP and thermal process and PME_0 = PME activity of non-processed orange juice.

Table 1 Levels and corresponding values of the independent variables

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

Physicochemical Analyses

The physicochemical characteristics of non-processed orange juice were evaluated in order to verify the accomplishment to the orange juice standards of identity and quality (Brasil 2000). Total soluble solids content, total titratable acidity, pH, ascorbic acid, total and reducing sugars were analysed according to AOAC (1990). All analyses were performed in triplicate.

Microbiological Analyses

Orange juice (10 mL) was added to 90 mL sterilised buffered peptone water (BPW). After homogenisation, aliquots were serially diluted in BPW and 1 mL of each dilution was inoculated onto Petrifilm™ 3M™ plates for aerobic and yeast and mould counts. The aerobic microorganism count was performed after incubation at 35±1 °C for 48±3 h and yeast and mould count after incubation at 25±1 °C for 120±6 h. The minimum level of detection was 10 CFU/mL (AOAC 2011). The analyses were performed in triplicate.

Data Analyses

The results were fitted to a second-order model equation provided by the design. Analyses of variance of the regression equations allowed the adequacy of the model to be determined by evaluating the lack of fit, coefficient of determination (R^2), F test value and significance of the effects, using STATISTICA software version 10.0 (StatSoft, Tulsa, USA).

Results and Discussion

Physicochemical Characteristics of Orange Juice

The physicochemical characteristics of non-processed orange juice are shown in Table 2. The orange juice complied with the

Table 2 Physicochemical characteristics of the non-processed orange juice

Parameter	Value[a]
pH	4.18±0.01
Total soluble solids (°Brix)	9.03±0.00
Titratable acidity (g citric acid/100 mL)	0.58±0.01
Ratio (soluble solids/titratable acidity)	15.57±0.08
Ascorbic acid (mg/100 mL)	85.95±1.14
Total sugars (g/100 mL)	6.26±0.05
Reducing sugars (g/100 mL)	3.30±0.03

[a] Mean ± standard deviation

standard values of the Brazilian legislation, except for total soluble solids (9.03 °Brix) which were lower than the minimum requirement of 10.5 °Brix (Brasil 2000).

Response Values and Model Fitting

In order to optimise the HHP processing of orange juice, the CCD with 17 experiments was employed to evaluate the effect of pressure, temperature and time on PME residual activity and microbial counts. The values of the response variables for HHP-processed juice (CCD experiments) and those for non-processed and thermally processed orange juice are listed in Table 3. Non-processed orange juice presented a PME activity of 167 U, considered as 100 % of PME residual activity, and counts of 2.6×10^4 CFU/mL for aerobic microorganisms and 1.7×10^4 CFU/mL for yeasts and moulds. PME residual activity of the juices from CCD ranged from 15 to 108 %, while that of the thermally processed juice was 4 %, indicating that the thermal process was more effective to reduce PME activity. The remaining PME activity corresponds to the more heat- and pressure-resistant isoenzyme (Versteeg et al. 1980; Van Den Broeck et al. 2000). The lowest value of PME residual activity (15 %) of orange juice from CCD was obtained when the experimental condition was 600 MPa, 45 °C and 195 s. Nienaber and Shellhammer (2001) reported orange juice PME residual activity of 10 % when 600 MPa, 50 °C during 276 s, was used. Vervoort et al. (2011) found 8 % of PME residual activity in orange juice processed at 600 MPa, 20 °C for 60 s, although at the same processing conditions, Bull et al. (2004) reported only 55 % PME residual activity. Concerning to the aerobic microorganisms and yeasts and moulds, the thermally processed juice (95 °C/30 s) had minimum counts (<10 CFU/mL). Two experiments from CCD (600 MPa, 45 °C; 195 and 499 MPa, 54 °C, 293 s) also had minimum counts of aerobic microorganisms. Additionally, CCD experiments employing a pressure of 350 MPa or more resulted in the minimum counts for yeasts and moulds.

Microbial counts below the detection limit of the method (<10 CFU/mL) were expressed as 10 CFU/mL in the analyses of model fitting. The high incidence of experiments with yeast and mould count <10 CFU/mL meant it was not possible to generate a model for yeasts and moulds.

The analysis of variance (Table 4) showed that the adjusted second-order models were significantly fitted to the experimental data, as indicated by the regression model F values of 50.95 ($p<0.001$) for PME residual activity and 81.11 ($p<0.001$) for aerobic microorganism count. Terms presenting a significant F value ($p\leq0.05$) were included in the models. For PME residual activity, the linear effects of pressure (P), temperature (T) and time (t), as well as the quadratic effect of pressure (P^2) and the

Table 3 The central composite design (CCD) and experimental response values for orange juice

Experiment	Independent variables			Response variables		
	Pressure (MPa)	Temperature (°C)	Time (s)	PME residual activity (%)	Aerobic microorganisms (CFU/mL)	Yeast and mould (CFU/mL)
Non-processed	–	–	–	100±6	$2.6\times10^4\pm2\times10^3$	$1.71\times10^4\pm7\times10^2$
Thermally processed	–	95	30	4.4±0.7	<10	<10
HHP-processed—CCD						
1	201	36	97	108±5	$1.6\times10^4\pm3\times10^3$	$1.30\times10^4\pm5\times10^2$
2	499	36	97	89±6	$3.1\times10^2\pm4\times10^1$	<10
3	201	54	97	92±6	$1\times10^3\pm2\times10^2$	$1.1\times10^2\pm2\times10^1$
4	499	54	97	49±3	$1.5\times10^2\pm3\times10^1$	<10
5	201	36	293	81±4	$7.8\times10^3\pm8\times10^2$	$1.2\times10^3\pm9\times10^2$
6	499	36	293	66±3	33±6	<10
7	201	54	293	79±3	$4.3\times10^2\pm2\times10^1$	$2.0\times10^2\pm2\times10^1$
8	499	54	293	25±2	<10	<10
9	100	45	195	91±5	$3.1\times10^3\pm5\times10^2$	$8\times10^3\pm2\times10^3$
10	600	45	195	15±2	<10	<10
11	350	30	195	90±6	$7.1\times10^2\pm6\times10^1$	<10
12	350	60	195	70±4	$5\times10^1\pm2\times10^1$	<10
13	350	45	30	94±5	$7.9\times10^3\pm8\times10^2$	$1\times10^1\pm2\times10^1$
14	350	45	360	70±3	$9\times10^1\pm2\times10^1$	<10
15	350	45	195	81±7	$6.0\times10^2\pm5\times10^1$	<10
16	350	45	195	84±3	$6.7\times10^2\pm3\times10^1$	<10
17	350	45	195	85±5	$5.5\times10^2\pm2\times10^1$	<10

Table 4 Analysis of variance (F value), coefficient of variation, adequate precision and regression coefficients of the second-order models for PME residual activity and aerobic microorganism count of orange juice

	PME residual activity		Aerobic microorganism count	
	F value	Regression coefficients	F value	Regression coefficients
Source of variation				
Regression model	50.95*		81.11*	
Mean/interception		47.577		5.457
Terms				
P	1,501.99*	0.447	4,334.11*	$-(5.306\times10^{-3})$
T	406.25**	0.974	1,055.89*	0.038
t	380.09**	−0.097	1,398.06*	$-(2.833\times10^{-3})$
P^2	382.40**	$-(4.403\times10^{-4})$	153.62**	$-(7.373\times10^{-6})$
T^2	ns	ns	90.00***	$-(1.565\times10^{-3})$
t^2	ns	ns	23.18***	(6.582×10^{-6})
PT	155.44**	$-(5.907\times10^{-3})$	208.07**	(1.682×10^{-4})
Pt	ns	ns	131.53**	$-(1.228\times10^{-5})$
Tt	ns	ns	ns	ns
Lack of fit	13.34, ns		14.86, ns	
C.V. (%)	8.06		5.77	
Adequate precision	22.39		29.87	
R^2	0.9586		0.9879	
Adjusted R^2	0.9398		0.9757	

P pressure, T temperature, t time, *ns* not significant ($p>0.05$)

*$p\leq0.001$; **$p\leq0.01$; ***$p\leq0.05$

interaction effect of pressure and temperature (PT), were significant. In the same way, for aerobic microorganism count, linear and quadratic effects of pressure (P, P^2), temperature (T, T^2) and time (t, t^2) and interaction effects of pressure and temperature (PT) and pressure and time (Pt) were significant. Lack of fit of experimental data was not significant ($p>0.05$) for both models. The coefficient of variation (C.V.) for PME residual activity model was 8 %, and for aerobic microorganism count, it was 6 %. Adequate precision compares the model predicted values to its associated error, in other words a signal-to-noise ratio. Ratios greater than 4 indicate adequate model discrimination. The models of PME residual activity and aerobic microorganism count showed an adequate precision of 22.39 and 29.87, respectively. The determination coefficient (R^2) for PME residual activity model and aerobic microorganism count was 0.96 and 0.99, respectively, while the adjusted determination coefficient (adjusted R^2) values were 0.94 and 0.98, respectively. There was a high correlation between the experimental and predicted values. These statistical parameters confirm the consistency of both models, indicating they are reliable to predict PME residual activity and aerobic microorganism count in Pêra Rio orange juice processed by HHP (Rodrigues and Iemma 2009).

Using the regression coefficients from the adjusted models (Table 4), the following model equations were generated:

$$PME = 47.577 + 0.447P + 0.974T - 0.097t - \left(5.907\times10^{-3}\right)PT - \left(4.403\times10^{-4}\right)P^2 \quad (2)$$

where PME = residual activity of PME (in percent), P = pressure (in megapascal), T = temperature (in degrees Celsius) and t = time (in second).

$$AMC = 5.457 - \left(5.306\times10^{-3}\right)P + 0.038T - \left(2.833\times10^{-3}\right) + \left(1.682\times10^{-4}\right)PT - \left(1.228\times10^{-5}\right)Pt - \left(7.373\times10^{-6}\right)P^2 - \left(1.565\times10^{-3}\right)T^2 + \left(6.582\times10^{-6}\right)t^2 \quad (3)$$

where AMC = aerobic microorganism count (in $\log_{10}$ colony-forming units per millilitre), P = pressure (in megapascal), T = temperature (in degrees Celsius) and t = time (in second).

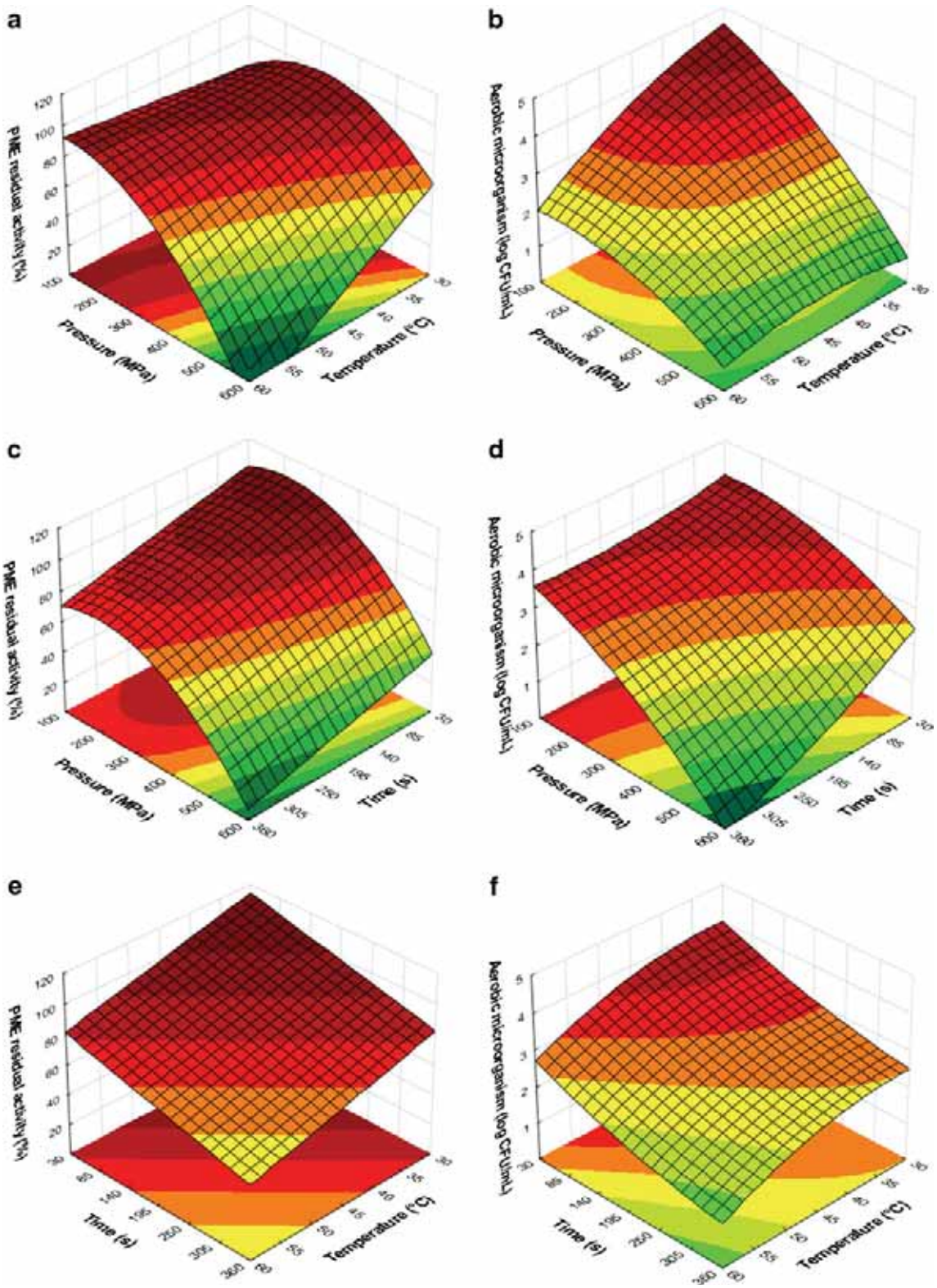

Fig. 1 Response surface of the combined effects of pressure and temperature (**a**, **b**), pressure and time (**c**, **d**) and temperature and time (**e**, **f**) on the PME residual activity and aerobic microorganism count of HHP-processed orange juice

Optimisation of the HHP Processing

The response surface models were plotted from the regression equations (Eqs. 2 and 3) to illustrate the effects of the independent variables on the PME residual activity and aerobic microorganism count (Fig. 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 pressure, temperature and time promoted the reduction of the PME residual activity and aerobic microorganism count for the orange juice, except for the region between 170 and 310 MPa, between 30 and 45 °C and between 30 and 150 s, which provided a small increase in PME activity. Cano et al. (1997) also noted PME activation in Salustiana orange juice with HHP processing conditions of 200 to 400 MPa, 20 to 25 °C and 15 min. Furthermore, tomato puree processed from 300 to 700 MPa at ambient temperature resulted in PME activation, with an increase of more than 500 % in PME activity (Krebbrers et al. 2003). The activation effects could be attributed to reversible configuration and/or conformation changes of the enzyme and/or substrate molecules (Ogawa et al. 1990).

The effects of pressure and temperature at a fixed time (195 s) on the response variables (PME residual activity and aerobic microorganism count) are presented in Fig. 1a, b. Increasing pressure had a stronger effect in reducing PME activity when temperature levels were high. Pressure higher than 550 MPa with temperature higher than 55 °C promoted more than 90 % reduction of PME activity, while at the same pressure with temperature lower than 35 °C, only about 40 % of PME activity was inactivated. On the other hand, for aerobic microorganism count, the use of high levels of pressure (higher than 500 MPa) at any temperature led to less than 2 log CFU/mL.

The effects of pressure and time at 45 °C (Fig. 1c, d) showed that pressure had a stronger influence than time in reducing PME activity. Also, an increase in pressure for a longer HPP processing time had a stronger effect in reducing the aerobic count than shorter periods of time, as expected. Therefore, the use of pressure higher than 500 MPa for time longer than 200 s resulted in aerobic microorganism counts below 1 log CFU/mL. Similar results for aerobic microorganism count were obtained for orange juice from the Valencia and Navel varieties processed by HHP at 600 MPa during 60 s (Bull et al. 2004) and from the Hamlin variety processed at 400 MPa for 90 s (Parish 1998). As shown in Fig. 1e, f, increases in temperature and time at 350 MPa (pressure of central point) had a lower influence on the response variables than the other combinations of effects.

Cloud stability is an important quality parameter for orange juice, since it positively affects turbidity, flavour and colour characteristic of the juice. The loss of cloud is attributed to the endogenous PME activity, which demethoxylates soluble pectins causing calcium pectate precipitation and clarification of the juice (Versteeg et al. 1980). However, a low PME residual activity could still preserve cloud stability during the shelf life of the juice. Several studies reported cloud stabilisation of HHP-processed orange juice with different PME residual activities. Boff et al. (2003) obtained a stable orange juice with 20 % of PME residual activity after it was stored for 120 days at 4 and 30 °C. Goodner et al. (1998) reported that orange juice presenting PME residual activity of 18 % was stable for more than 50 days when stored at 4 °C, and Nienaber and Shellhammer (2001) obtained orange juice with 4 % of PME, which maintained its cloud stability for longer than 90 days at 4 and at 37 °C.

In order to obtain orange juice with PME residual activity of 20 % or less and low aerobic microorganism count (<2 log cycle CFU/mL), the optimum levels of the independent variables and their combinations were obtained by analysing the regression equations (Eqs. 2 and 3). The processing conditions of 550 to 600 MPa, 55 to 60 °C and 330 to 360 s were suitable to produce a stable orange juice. It is also possible to obtain the same effect at the highest level of pressure (600 MPa) for temperature from 50 to 60 °C and processing time from 300 to 360 s. Furthermore, the same PME residual activity (≤20 %) and low microorganism counts can be obtained if temperature of 60 °C and pressure from 520 to 600 MPa are applied during 320 to 360 s. Also, the use of 360 s and pressure from 540 to 600 MPa at 55 to 60 °C confers the same effect.

Conclusions

Response surface methodology was successfully used to optimise the decrease of microflora and PME activity in HHP-processed Pêra Rio orange juice. The increase in pressure, temperature and time reduced the PME activity and native microflora of orange juice, except the region between 170 and 310 MPa, between 30 and 45 °C and between 30 and 150 s, which enhance PME activity. The optimum HHP conditions of 550 to 600 MPa, 55 to 60 °C and 330 to 360 s are able to produce orange juice with less than 2 log cycles CFU/mL and PME residual activity less than 20 %.

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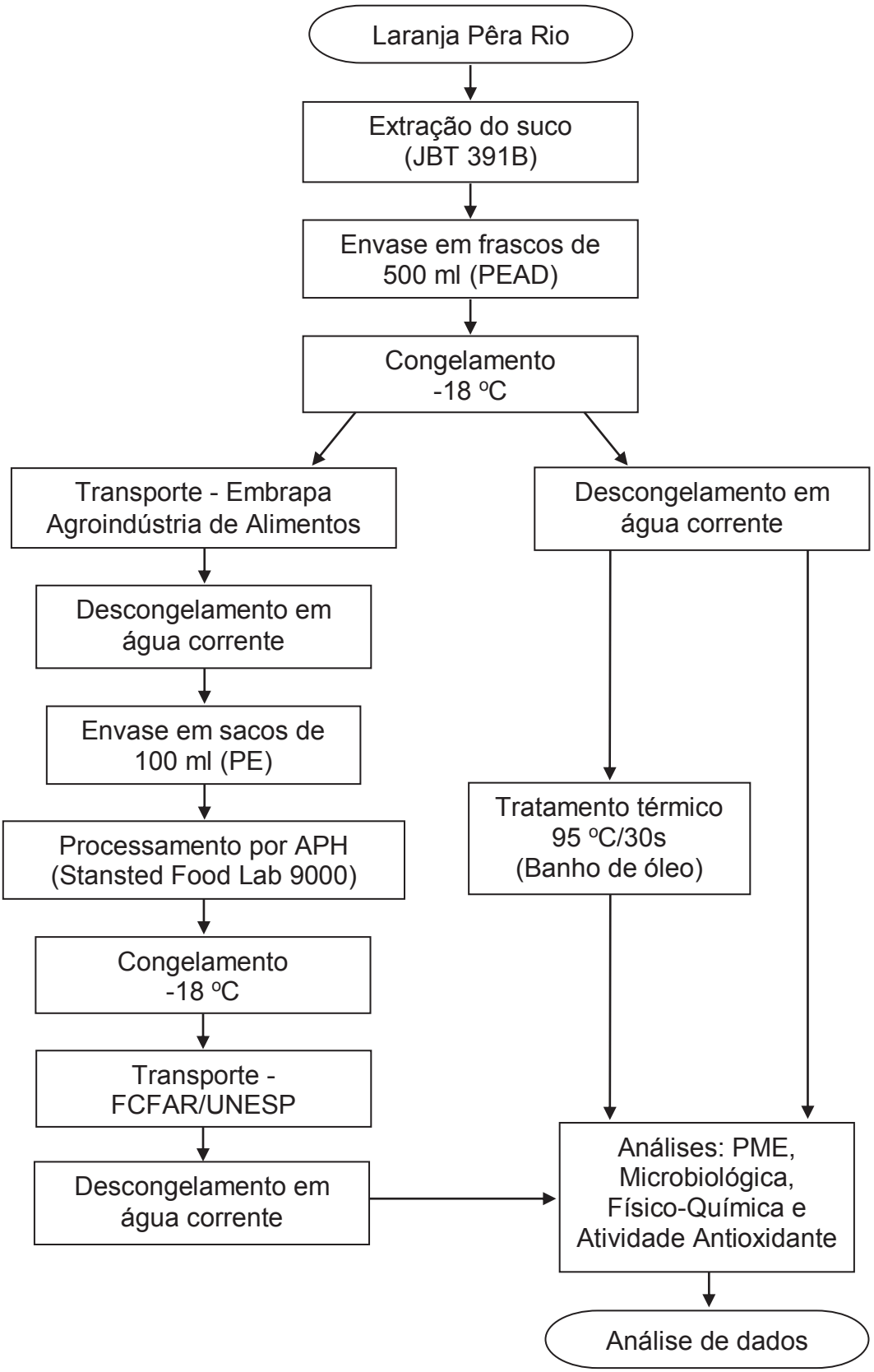
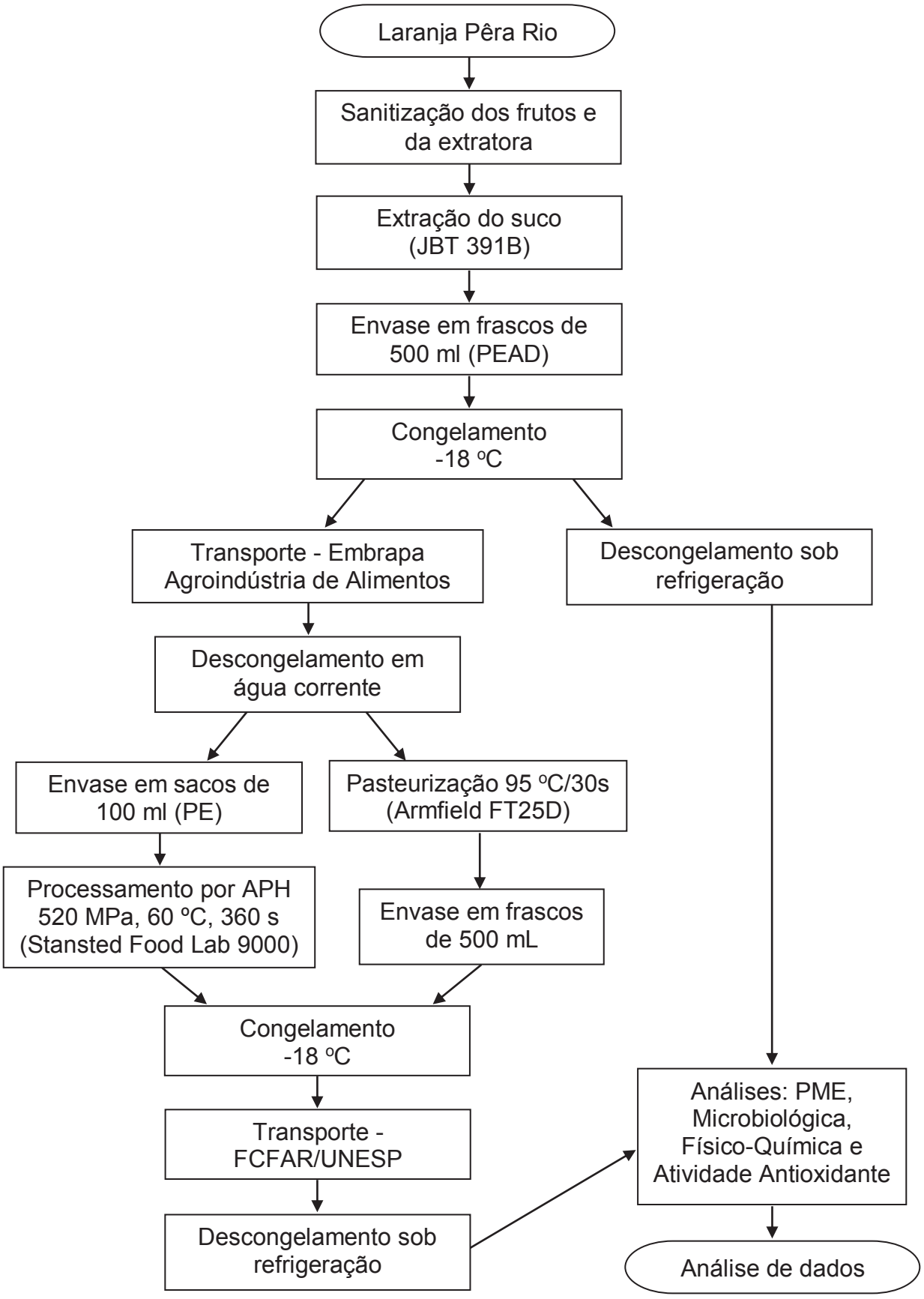
APÊNDICES

FLUXOGRAMAS

SUCO DE LARANJA DA SAFRA 2011/2012 (Capítulo 1 e 2)



SUCO DE LARANJA DA SAFRA 2012/2013 (Capítulo 3)



FOTOS

FOTOS DOS EQUIPAMENTOS UTILIZADOS NOS PROCESSAMENTOS

Figura 1. Extratora JBT FoodTech 391B.

Figura 2. Equipamento de alta pressão hidrostática Stansted Food Lab 9000.

Figura 3. Pasteurizador Armfield FT 25D.

Figura 4. Unidade de enchimento ultra limpo.