Quality of Mango Nectar Processed by High-Pressure Homogenization with Optimized Heat Treatment

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Abstract: This work aimed to evaluate the effect of high-pressure homogenization (HPH) with heat shock on *Aspergillus niger*, vitamin C, and color of mango nectar. The nectar was processed at 200 MPa followed by heat shock, which was optimized by response surface methodology by using mango nectar ratio (45 to 70), heat time (10 to 20), and temperature (60 to 85 °C) as variables. The color of mango nectar and vitamin C retention were evaluated at the optimized treatments, that is, 200 MPa + 61.5 °C/20 min or 73.5 °C/10 min. The mathematical model indicates that heat shock time and temperature showed a positive effect in the mould inactivation, whereas increasing ratio resulted in a protective effect on *A. niger*. The optimized treatments did not increase the retention of vitamin C, but had positive effect for the nectar color, in particular for samples treated at 200 MPa + 61.5 °C/20 min.

Keywords: *Aspergillus niger*, dynamic high pressure, fruit juice, heat shock, non-thermal processing

Practical Application: The results obtained in this study show that the conidia can be inactivated by applying HPH with heat shock, particularly to apply HPH as an option to pasteurize fruit nectar for industries.

Introduction

Heat treatment is the most common process used to preserve perishable food. It is an economic and efficient process to obtain safe and stable products (Ghani and others 1999). However, for some thermo-labile products, heat treatment may cause nutritional and sensory degradation. By contrast, consumers are demanding processed products being as similar as possible to fresh food (Tribst and others 2009a). For such reason, developing nonthermal methods for food processing is being extensively studied—for example, high-hydrostatic pressure, irradiation, UV radiation, pulsed electric field, pulsed light, ultrasound, and high-pressure homogenization (HPH; Diels and others 2003; Tribst and others 2009a). It is expected that products processed by such technologies retain better nutrients and keep sensory characteristics, since such process do not use high temperatures able to speed up food degradation reactions. The application of hurdle technology allows the use of different technologies into more attractive preserved food. It occurs since such use can overcome individual limits of each process and have a potential synergistic effect (Leistner 1994; Chaves-López and others 2009).

HPH process is a forthcoming technology. The treatment fluid is forced under high temperatures to pass through a narrow gap. Thus, it creates a fast acceleration (200 m/s at 340 MPa) (Floury and others 2004) undergoing an extreme drop in pressure as the fluid exits the homogenization valve (Lanciotti and others 1996), which leads to inactivation of microorganisms (Fantin and others 1996) and denaturation of enzymes (Lacroix and others 2005). The mechanism for HPH action is not thoroughly clear, but includes high-speed friction, cavitation collapse, strong impacts, turbulence (Middelberg 1995; Kleinig and Middelberg 1998, Innings and Trägardh 2007), and heating (Diels and others 2004). Such effects result in cell wall rupture and cellular death (Diels and others 2003 2005). The HPH was shown to be an effective process to inactivate vegetative bacteria (Wuytack and others 2002; Campos and Cristianini 2007; Tribst and others 2008), yeasts, and non heat-resistant mould (Fantin and others 1996; Tahiri and others 2006; Campos and Cristianini 2007). However, its low efficacy against bacterial spores turns the HPH into a treatment similar to thermal pasteurization.

Some researchers showed that the process did not present sublethal effect against vegetative cells (Wuytack and others 2002; Diels and others 2005; Briñez and others 2007). However, published studies recently revealed that a heat-resistant *Aspergillus niger* conidia (Tribst and others 2009b) and *Bacillus cereus* and *B. subtilis* spores (Chaves-López and others 2009) were sensitized by HPH treatment followed by moderated thermal treatment. Adversely, no sensitization for such microorganisms was observed when thermal treatment was performed before HPH, indicating that process combination order is important.

Tribst and others (2009b) assessed the effect of thermal treatment and HPH on *A. niger* conidia inoculated in mango nectar. Results indicated a high thermal resistance ($D_{80°C}$ = 5.03 min) and that 300 MPa was able to reduce 5 logs, whereas lower pressures with heat shock showed a synergistic effect. Thus, this study aimed to combine HPH process at 200 MPa with optimized heat shock

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to evaluate the inactivation of the heat-resistant *A. niger* conidia, retention of vitamin C and color changes in mango nectar.

Materials and Methods

Mango nectar preparation

Mango nectar was provided by a Brazilian producer (pH 4.05, 14 brix). For the assays of mathematical modeling and optimization of *A. niger* conidia inactivation, the mango nectar was pretreated at 105 °C/10 min to inactivate natural contaminants.

Mould culture and *A. niger* suspension

The target in the process was *A. niger* (IOC 4573), previously isolated as the most heat-resistant mould of Brazilian mango nectar (Silva and others 2010). Mould growth was obtained in malt extract agar (MEA, pH 6.0) prepared according Pitt and Hocking (1985).

Following the method depicted by Rajashekara and others (1996) with some changes, *A. niger* conidia suspension was prepared. The mould was preincubated in sterile potato dextrose agar (PDA, Difco®) plates for 1 wk at 30 °C. Growth culture was resuspended in sterile distilled water and 0.5 mL was inoculated of each one of the 100 Roux bottles, followed by the incubation for 20 d at 30 °C until 80% of conidia production, which was observed microscopically by using a lacto-fuchsin solution at 0.1%. Conidia were harvested in sterile distilled water with 0.05% of Tween 80 (Rajashekara and others 1996). The suspension was agitated and filtered through sterile glass wool to remove hyphae and mycelium. Afterwards, it was centrifuged 2 times at 11962.6 *g*, then resuspended in sterile water and shaken with glass beads in ultrasonic bath at 0 °C to obtain free conidia (Voldrich and others 2004). The count of working suspension was performed in MEA with incubation for 5 d at 35 °C. Finally, the suspension was stored at 3 °C and used for 6 mo.

High-pressure homogenization

The high-pressure treatments were performed in a Stansted homogenizer, model FPG 7400H:350 (STANSTED Fluid Power Ltd.® Essex, U.K.) at pressure of 200 MPa, with a flow rate of roughly 270 mL/min. T-type thermocouples (needle) were set at the outlet of intensifiers, outlet of homogenizer valve, and at the product cooler. The temperature was recorded at intervals of 10 s by a data logger (model 692–8010 Barnant Co.®, Barrington, Ill., U.S.A.). A heat exchanger was connected to the homogenizer to reduce the temperature of the fluid exiting the homogenizer valve. Water at 20 °C was used as cooling media. The heat exchanger outlet was connected to an aseptic sample collection system. The conidia suspension of *A. niger* was inoculated at a concentration of 10^6 CFU/mL. The nectar was submitted to HPH at 200 MPa. After treatment, the samples were plate-counted in prepared MEA and incubated for 5 d at 35 °C. An initial mould count was determined by direct plating of the inoculated nectar.

Heat shock

The heat shock was performed in thermal death time test tubes (8.0 cm of length, 6.6 mm ID, and 0.7 mm of thickness) submerged in a temperature-controlled water bath, which was monitored continuously by using type T thermocouples connected to an Omega® data logger (model CL526; Conn., U.S.A.). Temperatures ranging from 60 °C to 85 °C were evaluated and lag time was measured with a type T thermocouple inserted in the center of the test tube. After heating, samples were placed in ice–water bath and immediately pleated in MEA and incubated for 5 d at 35 °C.

Kinetic data

Kinetic parameters of *A. niger* inactivation by HPH and heat treatment was previously evaluated by Tribst and others (2009b). The heat resistance was evaluated by determining *D*-value at 80 °C, and the HPH kinetics were determined by mould resistance at 100, 150, 200, and 300 MPa.

Experimental design

A central composite design (CCD) was employed to model and optimize *A. niger* inactivation. *Temperature* (X_1), *time of heat shock* (X_2), and *mango nectar ratio* (X_3) were chosen as independent variables. The range and center point values of 3 independent variables presented in Table 1 were based on the results of previous experiments (Tribst and others 2009b) and in the natural variation of Brazilian mango nectar ratio. The dependent variable was the number of decimal reduction (NDR), calculated considering the survivor's count of each treatment and the initial mould count (Eq. 1).

$$\mathrm{NDR} = \log(\mathrm{initialcount}) - \log(\mathrm{survivor's\ count}). \quad (1)$$

The experimental design consists of 8 factorial points, 6 axial points at a distance of roughly 1.68 from the center, and 3 replicates of the central point. Based on the experimental design results, it was established as a mathematical model following a polynomial equation (Eq. 2) to predict the NDR.

$$\mathrm{NDR} = b_0 + b_{1\times1} + b_{2\times2} + b_{3\times3} + b_{12\times1\times2} + b_{13\times1\times3} + b_{23\times2\times3} + b_{11\times1^2} + b_{22\times2^2} + b_{33\times3^2}. \quad (2)$$

The nectar brix to acid ratio was adjusted maintaining the nectar brix as 14 °B and varying its acidity from 0.2 to 0.31 g citric acid/100 g nectar. The acidity of different samples was adjusted by adding NaOH 0.1 N or 1% of citric acid solution to the nectar. Acidity was performed by titration with NaOH 0.01 N and brix by refractometry.

The conidia suspension of *A. niger* was inoculated in mango nectar at 25 °C, with different ratios. The nectar was submitted to HPH at 200 MPa (the unique sublethal pressure, as described by Tribst and others 2009b) and then submitted to one of the thermal treatment established in the CCD.

Analysis of the experimental design data and calculation of predicted responses were carried out by using Statistica 6.0 (StatSoft, Inc., Tulsa, Okla., U.S.A.). The obtained model was evaluated by its R^2, *P*-value of variables, and analysis of variance (ANOVA) of statistically significant terms.

Color and vitamin C retention

Color and vitamin C analysis was performed in mango nectar submitted to different treatments (able to reach 5 decimal

Table 1–Variables and their levels of experimental design.

	Levels				
Independent variables	$-\alpha$	**−1.00**	**0.00**	**+1.00**	$+\alpha$
Temperature of heat shock (X_1)	60.0	65.0	72.5	80.0	85.0
Time of heat shock (min) (X_2)	10	12	15	18	20
Mango nectar ratio (X_3)	45.0	50.0	57.5	65.0	70.0

reductions), namely, (a) conventional pasteurization for 10 min at 100 °C, (b) HPH at 300 MPa (Tribst and others 2009b), and (c) HPH at 200 MPa followed by heat shock for 10 min at 73.5 °C or for 20 min at 61.5 °C.

Vitamin C was determined using methodology 967.21 described in AOAC (1997). The color analysis was performed by a Hunterlab spectrophotometer, model ColorQuest II, color system CIE $L^*a^*b^*$. Total color difference (TCD) was obtained as described in Eq. (3), being L_0, a_0 and b_0 determined for the samples without treatment. Treatments were evaluated in triplicates.

$$\mathrm{TCD} = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}. \quad (3)$$

Results and Discussion

The *A. niger* presented a high heat resistance, with $D_{80°C}$ of 5.03 min (Tribst and others 2009b). The kinetic data of HPH resistance indicated that at 100 and 150 MPa, it was not observed significant microbial inactivation, with 0.10 and 0.12 decimal reductions, respectively; while 300 MPa was able to reduce more than 6.2 log cycles of the *Aspergillus*. Pressure of 200 MPa promoted 2.03 decimal reductions and was the unique pressure that presented a partial inactivation of the *Aspergillus* (Tribst and others 2009b), which makes impossible the use of pressure as an independent variable in the experimental design. At the pressure of 200 MPa, the temperature of the nectar rose up to 65.4 °C (Tribst and others 2009b), with retention time of 0.7 s (Campos and Cristianini 2007). This heating was not sufficient to promote significant decimal reductions of *A. niger* on nectar, since the $D_{80°C}$ of the microorganism is 5.03 min (Tribst and others 2009b), indicating that the inactivation observed could be attributed only to the effect of HPH.

Table 2 shows the experimental and predicted ($R^2 = 91.4$) results for the tests performed and the deviations between both. The variables with P-value < 0.15 were considered in the mathematical model, which is described in Eq. (4).

$$\mathrm{NDR} = 5.16 + 0.57X_1 + 0.22X_2 - 0.28X_3 - 0.13X_1 \cdot X_2. \quad (4)$$

For the ANOVA, F was calculated to be 31.7, which is almost 10 times higher than the tabulated F (3.26), which ensures a good mathematical model. The deviations for obtained and predicted results were below 0.4 log reduction, revealing that model is reliable to assess microbiological reductions.

Based on the results, we observed that despite using a 2nd-order model, the results generated a 1st order one. Such fact occurred once the quadratic coefficients were not significant until 15%. In addition, the ratio for time and temperature was not significant. It could be observed through the mathematical model that the time and temperature of the heat shock influenced positively the microbial inactivation (positive coefficients) and that, in the range of the study, temperature was the variable that caused the major impact on *A. niger* inactivation. Also, as the ratio increased, the microbial inactivation decreased.

Figure 1 shows the response surface to inactivate the *A. niger* in mango nectar with ratio of 57.5, according to heating temperature and time. It was established that a treatment to reduce 5 log cycles ($\gamma = 5$) for *A. niger* would be appropriate for the mango nectar, especially when the expected contamination level for juice (before processing) is roughly 10^1 of spores per 100 mL of heat-resistant mould (Hocking and Pitt 1984). Further, a probability of nonsterile unit (Pflug 1999) for food industry is of one contaminated container in 10000 (Tribst and others 2009b). Thus, most conditions assessed in this study (time and temperature of heat shock

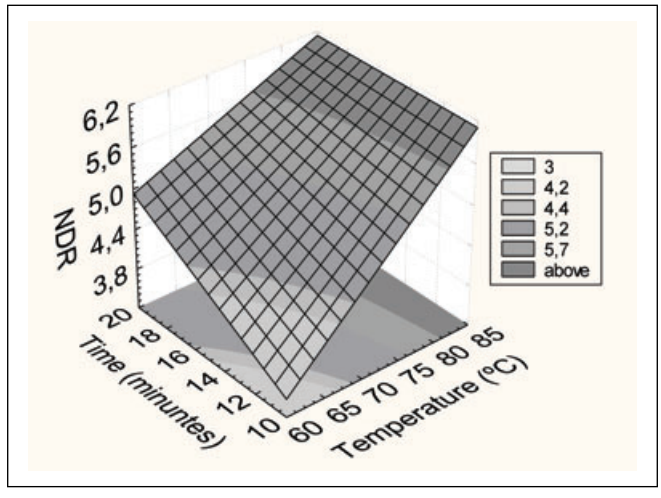

Figure 1–*Aspergillus niger* IOC 4573 inactivation according to heat shock time and temperature (ratio = 57.5).

Table 2–Experimental compared with calculated results by mathematical model.

Test	X_1	X_2	X_3	NDR (experimental)	NDR (mathematical model)	Absolute deviation	Relative deviation
1	−1	−1	−1	4.60	4.52	0.08	−1.6%
2	1	−1	−1	6.08	5.92	0.16	−2.6%
3	−1	1	−1	5.44	5.22	0.22	−4.1%
4	1	1	−1	6.08	6.11	−0.03	0.6%
5	−1	−1	1	3.87	3.96	−0.09	2.2%
6	1	−1	1	5.07	5.36	−0.29	5.7%
7	−1	1	1	4.43	4.65	−0.22	5.0%
8	1	1	1	5.46	5.55	−0.09	1.6%
9	−1.68	0	0	4.01	4.20	−0.19	4.7%
10	1.68	0	0	6.08	6.13	−0.05	0.7%
11	0	−1.68	0	4.77	4.79	−0.02	0.4%
12	0	1.68	0	5.50	5.53	−0.03	0.6%
13	0	0	−1.68	5.34	5.64	−0.30	5.6%
14	0	0	1.68	5.04	4.68	0.36	−7.0%
15	0	0	0	5.32	5.16	0.16	−2.9%
16	0	0	0	5.18	5.16	0.01	−0.3%
17	0	0	0	5.48	5.16	0.32	−5.8%

after HPH, regardless of mango nectar ratio) showed to be able to produce microbiologically stable mango nectar.

Figure 2 shows the response surface on *A. niger* conidia inactivation in mango nectar at 65 °C, according to heating time and nectar ratio. The protective effect of the ratio can be clearly observed as the time needed to promote 5 decimal reductions at higher ratio and was 8 min longer than at low ratio. Such information confirmed the results from Rajashekhara and others (1996) and from Salomão and others (2007). Tournas (1994), who studied mould heat resistance in different media and ratio has also described similar results. The authors pointed out that the lower the ratio or brix, the lower the microorganism heat resistance. Furthermore, results from Bevilacqua and others (2007) indicated that inactivating *Alicyclobacilllus acidoterrestris* spores by HPH is more frequent in lower pH media, which is compatible to lower ratio juice. The results from Briñez and others (2007) also showed a higher vulnerability for vegetable cells in juice than in milk. Considering that similar effects may have occurred to *A. niger* in this study, pretreatment by HPH and heat shock was potentially less efficient in the higher ratio samples, justifying a higher contact time needed at 65 °C to get the same mould inactivation obtained in low-ratio nectar.

From the mathematical model, 2 conditions of heat shock were obtained that would be able to promote 5 decimal reductions of the microorganism when associated with pretreatment to 200 MPa in mango nectar at a ratio of 57.5 (average ratio of samples). The heat shocks were chosen based on the response surface methodology to use extreme conditions (high temperature short time and low temperature high time), obtaining the binomials 61.5 °C/20 min and 73.5 °C/10 min. These treatments were used to assess the effect on color and vitamin C of nectar in comparison to conventional pasteurization and homogenization to 300 MPa (Tribst and others 2009b), conditions under which it is possible to obtain similar inactivation of *A. niger*.

The results of vitamin C retention (Figure 3) were unexpected. All treatment results in similar vitamin losses (roughly 50%). Such results are in disagreement with Welti-Chanes and others (2009), who did not observed a significant vitamin C lost after orange juice homogenization at 250 MPa.

Some processing conditions may have affected negatively the vitamin C retention: the juice was not deaerated before processing, and the presence of oxygen, high shear, and temperature have certainly contributed to oxidize the vitamin. In addition, the pilot equipment used for the homogenization contained seals made of Be–Cu, in which were observed some erosion after processing. According to Ball (2006), the presence of oxygen and trace metals favors the oxidation of the vitamin by creating metal-oxygen-ascorbate with consequent formation of dehydroascorbic acid that is irreversibly oxidized (Gregory 1996).

The nectar color analysis from different process showed that thermal treatment, HPH process, and HPH combined with heat process promoted significant changes in the nectar color, as shown in Table 3. The changes, however, differed according to the treatment. The heating resulted in luminosity loss and increase of yellow-color intensity. The changes caused by 300 MPa treatment or HPH combined with heat processes were

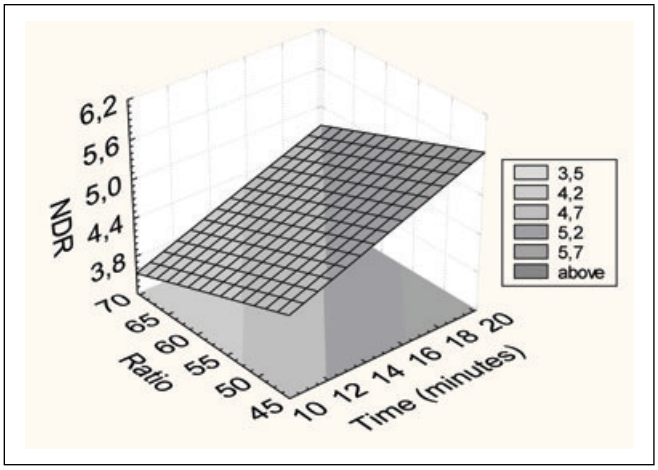

Figure 2–*Aspergillus niger* IOC 4573 inactivation according to heat shock time and mango nectar ratio (temperature = 65 °C).

Table 3– Color changes in mango nectar caused by different treatments (CIE scale $L^*a^*b^*$).

Treatments	L^*	a^*	b^*	TCD
Thermal treatment (100 °C/10 min)	48.42^{**a}	12.23^{a}	50.38^{a}	5.92^{a}
300 MPa	44.15 ^{c,b}	7.48^{b}	45.18^{b}	3.41^{b}
200 MPa + 73.5 °C/10 min	43.71^{b}	8.18^{b}	45.47^{b}	2.73^{b}
200 MPa + 61.5 °C/20 min	43.66^{b}	8.32^{b}	46.51^{b}	2.50^{b,c}
No treatment	44.42^{c}	10.69^{a}	46.29^{b}	

**Subindexes indicate significant difference to 5% of significance level.

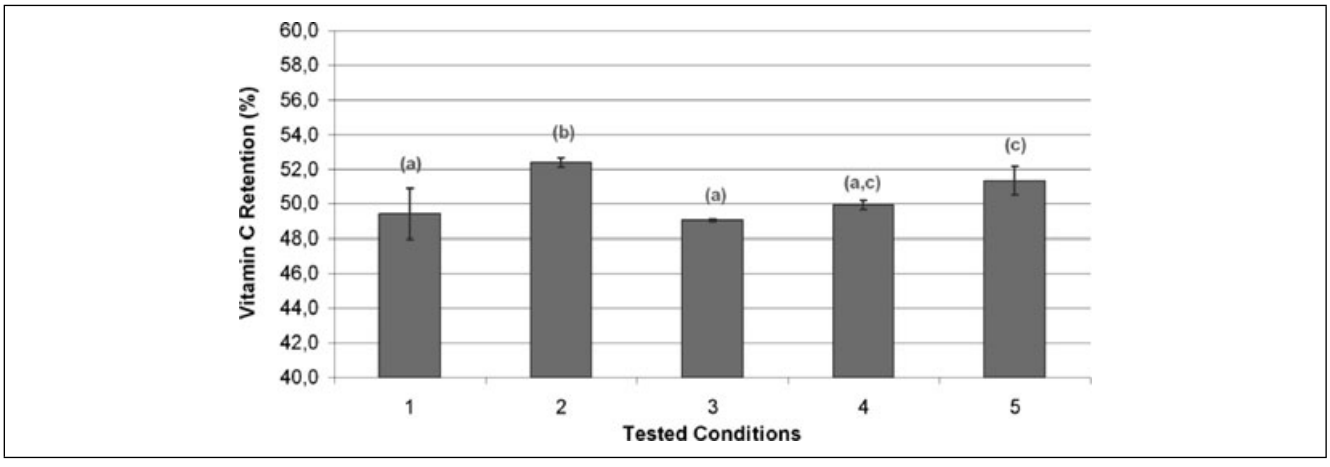

Figure 3–Vitamin C retention in mango nectar treated at 300 MPa (1), 200 MPa (2), 200 MPa + 73.5 °C/10 min (3), 200 MPa + 61.5 °C/20 min (4), and 100 °C/10 min (5). Different letters indicate significant difference to 5% significance level.

lower. They affected primarily the red-nectar intensity, causing its reduction.

By comparing the different treatments by TCD, we observed that the conventional pasteurization was the process that caused greater change of mango nectar color (TCD = 5.92). On the contrary, 200 MPa processing followed by treatment for 20 min at 61.5 °C presented the best nectar color retention. Considering that differences in perceivable color can be analytically classified as very distinct (TCD > 3) (Adekunte and others 2010), the use of combined process, in this case, reduced the changes caused by pressure and temperature processes separately, since it provides the use of milder conditions.

Conclusion

Combining HPH with posterior heat shock was efficient to inactivate heat-resistant mould in mango nectar. Mathematical model indicated that temperature influenced positively the inactivation of *A. niger* whereas juice ratio influenced negatively. The vitamin C retention was similar in all studied cases, indicating a possible vitamin loss by oxidation and evidenced that nectar must be carefully deaerated to ensure the benefits of HPH as a nonthermal process. Furthermore, the combined treatment induces less color change indicating that the HPH combined with heat shock is a potential alternative treatment to reduce sensory damage of fruit products caused by heat treatment.

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