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REGULAR ARTICLE

Apple juices processed by high hydrostatic pressure: Interaction between chitosan application and quality drift

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Abstract

Apple juice submitted to high hydrostatic pressure was supplemented with chitosan (0.6 and 0.8 g L-1, added in powder and solution) and ascorbate in powder (0.06% and 0.08%) or solution (1%), to optimize its amounts and forms of application, as well as the implications on juice quality during a storage period of 35 days. Thru these experiments it was found that dissociation of hydrogen ions, protonation and ascorbate oxidation was significantly affected. Accordingly, significant variations were found in the pH, total soluble solids, titrable acidity, Hue, turbidity and phenols, exogenous added ascorbate and sugars contents. It is concluded that addition of 0.8% chitosan as powder has the most effective action to counterbalance total phenols drift, which correlate with the inhibition of enzymatic browning and green/yellow Hue.

Key words: Apple juice, Chitosan, High Hydrostatic Pressure, Quality, Shelf life

Introduction

Industrial processing of unpasteurized fruit juices by high hydrostatic pressure is attached to enzymatic browning (López-Nicolás et al., 2007). In this context, Weemaes and co-workers (1998) found that, to inhibit polyphenol oxidase, preventing enzymatic oxidation of phenols to quinones, apple juice requires 600 MPa. Besides, to inhibit the enzymatic browning, antioxidants might be added during the industrial processing (Özuglu et al., 2002), namely sulfites. Yet, considering public health implications of sulfite in food products, chitosan can replace this chemical entity, as an antioxidant agent (Shahidi et al., 1999), mostly due to its positive ionic charge that can break lipids and acids. As an accumulating effect, this polymer can still change the gelling, stabilizing and thickening properties of food products (Martín-Diana et al., 2009).

Chitosan polymers are non-toxic polycationic compounds, being gastric dehydration the only secondary effect (Fai et al., 2008). Essentially has glucosamine, amino-2-β-2deoxi D-glucose, results from the alkaline deacetylation of chitin (Martín-Diana et al., 2009), being its solubility directly related to the amount of protonated amino groups (Pinto, 2005), whereas the antioxidant properties varies with its molecular weight (Rocha et al., 1999;Wenjun et al., 2002; No et al., 2007).

According to the Council Directive - 2001/112/EC (2002), chitosan addition is not being considered as amended to juices. In this context, this work aims to assess the action of chitosan in unpasteurized apple juices industrially processed by high hydrostatic pressure, to achieve optimization of amounts and forms of application, as well as the implications on quality, so that the inclusion of this product might be consider in the list of additives and treatments Fruit Juice Directive Regulations of the EU.

Materials and Methods

Juice from grinding apples (Granny Smith, Golden Delicious and Fuji), without added water, sugar, preservatives or dyes, were packed and cold-stabilized in a steel compartment containing water and submitted to a high hydrostatic pressure (460

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Mbar for 115 seconds). Following this treatment natural preservation method, chitosan (Sigma-Aldrich, Ref 448 869) and ascorbic acid were added (Table 1), and the apple juice were kept at 4 °C for 35 days.

Total soluble solids in extracted juice, were measured at 20°C with a refractometer Atago ATC-1E.

The pH was measured in samples of 10 mL, at 25°C with a Hanna instruments, Jenway 350 pH meter.

Titratable acidity was determined in 20 mL of fruit juice added with 20 mL of distilled water, with 0.1 N NaOH, to an end point of pH = 8.2, as indicated by a pH meter CD 7000 WPA. The volume of NaOH needed was used to calculate the titratable acidity, applying a multiplication factor of 0.67.

Considering the chromatic coordinates (L* - Lightness; a*, b* - Chroma) measured with a Minolta chromameter (CR-300, Data Processor 301, Japan), the Hue angle (Ho = 180o + Tang-1 [b*/a*]) was determined (Lidon et al., 2012).

Turbidity was measured using a turbidity meter (Merck Turbiquant 1500 IR), after dilution of apple juice controls and treatments in distilled water (1:10, final volume 50 mL).

Sugars were extracted from a mixture of 5 mL of apple juice, with 20 mL of distilled water, after centrifugation (15000xg, 15 min, 4ºC) and filtration (Whatman nº 4 and Millipore 0.45 µm filters). Sugars were identified and quantified by HPLC (Waters binary HPLC), using a Watters 2414 refractive index detector and a reverse Sugar-Pak I (300x6.5mm; Waters) column kept at 90oC (Lidon et al., 2012). A flow rate of 0.5 mL min^{-1} was applied to an aqueous mobile phase of EDTA-Ca (50 µL L^{-1}). A volume of injection of 20 µL was used.

Phenolic compounds and ascorbic acid contents were extracted and measured according to Vieira and co-workers (2009) and Lidon and co-workers (2012), respectively.

Statistical analysis was performed by two-way ANOVA ($P < 0.05$), being each value the mean of triplicates of three independent series. For mean comparison, a Tukey test was applied, considering a 95% confidence level.

Results and Discussion

Hydrogen potential of apple juice in the beginning of the assay (1st day) did not vary significantly among control (C) and treatments (excepting T3), but from the 15th day onwards all samples consistently showed lower values (Table 2), which indicated that until the 35th day of storage, the increased amount of H3O+ was connected to dissociation of hydrogen ions from organic acids. Yet, additions of powder chitosan (T3,4) steadily prompted, relatively to the use of solutions (T1,2), slightly higher hydrogen potential until the end of the experiment, suggesting lower NH2 protonation (Chavasit et al., 1988). Moreover, the levels of added ascorbic acid decreased significantly in the last experimental day through its addition in solution with chitosan, whereas the opposite occurred with added powder (Table 1, 3). As in both cases, significant differences could not be detected in the highest treatment with chitosan (T2 and T4), data suggest that storage time and the diminishing levels of ascorbic acid might be due to sequestration triggered by chitosan addition (Imeri and Knorr, 1988).

Table1. Experimental design for apple juice control (C) and treatments (T_{1-4}).

Treatments	Juice (mL)	Ascorbic Acid (%)		Chitosan (g L^{-1})	
		Powder	Solution[1]	Powder	Solution[2]
C	100				
T_1	100		0.06		0.6
T_2	100		0.08		0.8
T_3	100	0.06		0.6	
T_4	100	0.08		0.8	

[1]Ascorbic acid 1%
[2]Chitosan dissolved in ascorbic acid 1%

The low pH of all juices (Table 2 - between c.a. 3.5 and 3.7) limited the crosslink between chitosan and pectin, which determined that protons recovery after titration with a strong base to specified endpoints (i.e., titrable acidity), among the testing samples and within each experimental day (Table 2), did not vary significantly (excepting T3, at the 28th and 35th day). In each experimental day, the higher values found in most of the chitosan additions as powder (Table 2), further support its acid-binding properties as a polyelectrolyte (No et al., 2007), since electrostatic complexes can be synthesized (Rinaldo, 2006).

Turbidity of the opalescent apple juice resulting of electrostatic complexes from the mixture of cellulose, hemicelluloses, pectins and proteins, in suspension and produced thru cellular wall disruption during maceration (Shahidi et al., 1999), in almost all the testing samples surpassed 100 NTU (Table 3). These quite lower values of turbidity, relatively to the cloudiness apple juices obtained after application of other classical processing technologies (Sorrivas et al., 2006),

results of high hydrostatic pressure specificity. Indeed, proteins denaturation triggered by high hydrostatic pressure, although avoiding changes in the juice taste (Jay et al., 2005), affects juices main structure, decreasing turbidity. Among samples added with chitosan, the lower values of turbidity found with the highest concentrations applied in solution (T2), and at the 35th day with powder (T4), further suggests that with these concentrations the adsorption sites of chitosan became saturated (Shahidi et al., 1999; Rungsardthong et al., 2006; Domingues et al., 2012), limiting the amine functions of chitosan, as coagulant and/or flocculant of anionic components (Rao et al., 2011).

The levels of total soluble solids, which measures and includes carbohydrates, in most cases did not vary significantly until de 35th day (Table 2) but, in contradiction with other reports (Domingues et al., 2012), slightly lower values were found with added chitosan in solution (T3,4). Moreover, the higher amounts of total soluble solids after chitosan addition as powder (Table 2) suggest a direct solvation. Indeed, when chitosan was added as powder (T3,4), sucrose decreased significantly between both experimental periods (Table 3).

In the cases of apple juices treated with chitosan in solution (T1,2) or between powder (T3,4,), significant differences could not be found for glucose (Table 3), but between both experimental periods, significant differences were detected in T1 and T2, although with opposite patterns. Fructose contents also did not vary between both experimental periods (Table 3), but significant differences were found, at the 28th day in T2 and at the 35th day in T1. Between the experimental periods, sorbitol contents, a polyalcohol resulting from glucose degradation, only varied significantly in T1, T2 and T4 (Table 3). Accordingly, a clear pattern of isomerization or hydrolysis could not be found for these sugars in juices added with chitosan (Table 3), which is an indication of a heterogeneous flocculation and removal of some soluble components during samples preparation (Fang et al., 2006), as well as a dissimilar oxidation kinetics during storage.

Table 2. pH, Total soluble solids, titrable acidity and hue parameters of apple juice (controls and treatments) during storage for 35 days. all values are shown as a mean values (N = 3). different letters indicate significant differences ($P \leq 0.05$) among experimental days within each treatment (a,b,c,d) or among treatments within each experimental day (r,s,t).

Treatments	Experimental days				
	1	15	21	28	35
	pH				
C	$3.73^{a,r}$	$3.47^{d,s}$	$3.50^{c,s}$	$3.47^{d,r}$	$3.55^{b,r}$
T_1	$3.70^{a,r,s}$	$3.48^{c,t}$	$3.49^{b,c,s}$	$3.48^{c,r}$	$3.51^{b,s}$
T_2	$3.69^{a,s}$	$3.49^{c,s}$	$3.49^{c,s,t}$	$3.47^{c,r}$	$3.53^{b,r,s}$
T_3	$3.70^{a,r,s}$	$3.53^{b,r}$	$3.50^{c,r,s}$	$3.48^{c,r}$	$3.54^{b,r}$
T_4	$3.71^{a,r,s}$	$3.53^{b,c,r}$	$3.51^{c,d,r,s}$	$3.50^{d,r}$	$3.55^{b,r}$
	Total Soluble Solids (°Brix)				
C	$13.93^{a,r}$	$13.87^{a,r}$	$13.80^{a,r}$	$13.93^{a,r}$	$13.87^{a,r}$
T_1	$13.20^{a,s}$	$13.00^{a,s}$	$13.00^{a,s}$	$13.00^{a,s}$	$13.13^{a,s}$
T_2	$13.00^{a,s}$	$12.93^{a,s}$	$13.00^{a,s}$	$13.00^{a,s}$	$13.00^{a,s}$
T_3	$14.00^{a,r}$	$13.93^{a,r}$	$13.87^{a,r}$	$13.87^{a,r}$	$13.33^{b,s}$
T_4	$14.00^{a,r}$	$14.00^{a,r}$	$14.00^{a,r}$	$14.00^{a,r}$	$14.00^{a,r}$
	Titrable Acidity($g_{malic\ acid}\ L^{-1}$)				
C		$8.68^{a,b,r}$	$9.34^{a,r}$	$8.44^{b,r,s}$	$8.58^{b,s}$
T_1		$8.46^{a,b,r}$	$9.04^{a,r}$	$8.20^{b,r,s}$	$8.61^{a,b,s}$
T_2		$8.42^{a,b,r}$	$8.49^{a,r}$	$7.65^{b,s}$	$8.26^{a,b,s}$
T_3		$8.92^{b,r}$	$9.04^{b,r}$	$9.17^{b,r}$	$11.21^{a,r}$
T_4		$8.65^{a,b,r}$	$8.91^{a,b,r}$	$8.27^{b,r,s}$	$9.08^{a,s}$
	Hue				
C		$83.79^{a,s}$	$79.30^{a,t}$	$79.84^{a,s}$	$82.47^{a,s}$
T_1		$128.52^{a,r}$	$124.54^{a,s}$	$128.59^{a,r}$	$124.26^{a,r}$
T_2		$129.99^{a,b,r}$	$132.28^{a,r}$	$130.91^{a,b,r}$	$127.09^{b,r}$
T_3		$127.50^{a,r,}$	$126.99^{a,r,s}$	$127.24^{a,r}$	$126.49^{a,r}$
T_4		$127.22^{a,b,r}$	$131.75^{a,r}$	$128.46^{a,b,r}$	$126.97^{b,r}$

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Table 3.Turbidity and contents of total phenols, ascorbic acid and sugars of apple juice (controls and treatments) at the 28th and 35th day of storage. all values are shown as mean values (N = 3). different letters indicate significant differences ($P \leq 0.05$) among experimental days within each treatment (a,b) or among treatments within each experimental day (r,s,t,u,v).

Treatments	Experimental days													
	28	35	28	35	28	35	28	35	28	35	28	35	28	35
	Turbidity (NTU)		Total phenols (mg mL^{-1})		Ascorbic acid (mg mL^{-1})		Sugars (g 100 mL^{-1})							
							Sucrose		Glucose		Frutose		Sorbitol	
C	$144.3^{a,r}$	$152.0^{a,r}$	$67.3^{a,s}$	$65.8^{a,r}$	$0.056^{b,r}$	$0.152^{a,r}$	$0.949^{a,r}$	$0.797^{b,r}$	$3.157^{a,r}$	$3.138^{a,r}$	$7.598^{a,r}$	$7.518^{a,r,s}$	$0.495^{a,r}$	$0.484^{a,r}$
T_1	$138.7^{a,r}$	$123.3^{a,r}$	$53.9^{b,r}$	$66.4^{a,r}$	$3.372^{a,t}$	$1.117^{b,s}$	$0.858^{a,r}$	$0.612^{b,r}$	$2.938^{a,r,s}$	$2.630^{b,s}$	$7.079^{a,r,s}$	$6.283^{a,s}$	$0.456^{a,r,s}$	$0.395^{b,s}$
T_2	$108.3^{a,s}$	$96.7^{a,s}$	$74.8^{b,t}$	$101.2^{a,t}$	$10.567^{a,v}$	$8.893^{a,v}$	$0.790^{a,r}$	$0.751^{a,r}$	$2.714^{b,s}$	$3.019^{a,r,s}$	$6.530^{a,s}$	$7.205^{a,r,s}$	$0.418^{b,s}$	$0.462^{a,r}$
T_3	$138.3^{a,r}$	$125.7^{a,r}$	$76.1^{a,t}$	$77.6^{a,s}$	$1.849^{b,s}$	$4.705^{a,t}$	$0.791^{a,r}$	$0.526^{b,s}$	$3.033^{a,r,s}$	$2.843^{a,r,s}$	$7.320^{a,r,s}$	$6.846^{a,r,s}$	$0.477^{a,r}$	$0.438^{a,r}$
T_4	$139.3^{a,r}$	$114.0^{b,s}$	$159.4^{a,u}$	$105.2^{b,t}$	$4.643^{a,u}$	$5.838^{a,u}$	$0.955^{a,r}$	$0.740^{b,r}$	$3.188^{a,r}$	$3.171^{a,r}$	$7.666^{a,r}$	$7.597^{a,r}$	$0.508^{a,r}$	$0.485^{b,r}$

Total phenols, relatively to the control (C), in general showed significantly higher values when chitosan was added as powder to the juice (Table 3), or if was added in solution 0.8% (T2). Outlining the high affinity of chitosan for phenolic compounds (Shahidi et al., 1999), total phenols drift correlate the inhibition of enzymatic browning (Weemaes et al., 1998), countenancing a green/yellow Hue (Table 2).

Between the 15th and the 35th days (Table 3), relatively to the apple juices treated with chitosan (T1-4), Hue of the control samples (C) did not vary significantly, being closely linked to juice browning. Besides, all juice samples incorporating chitosan displayed a Hue (Table 3) ranging between c.a. 124-133 (green/yellow), suggesting the endorsement of electrostatic interactions, as in acid solution charge neutralization can be achieved just with high chitosan contents (Jiang et al., 2011). According with previous reports (No et al., 2007; Martín-Diana et al., 2009), our data further suggest that among the juice samples treated with chitosan (T1-4), the inhibition of the enzymatic browning triggered by polyphenoloxidases (Weemaes et al., 1998; Rocha et al., 1999) implicates two hydroxyl groups and a partially acetylated amino group (Wenjun et al., 2002), through blocking of the binding of the enzyme to prevent the use of oxygen.

Conclusion

Chitosan preserves apple juice in unpasteurized fruit juices stabilized by overpressure, but different concentrations applied in solution or as powder significantly affect intrinsic quality parameters, mostly due to dissociation of hydrogen ions, protonation and ascorbate oxidation. Levels of about 0.8% chitosan, with most effective action as powder, interacted with total phenols drift, which correlate with the inhibition of enzymatic browning and green/yellow Hue.

Author contributions

All authors contributed to the writing of the paper. F. L., C. S. L. and M. G. B. were involved in the overall planing and supervision of the work and paper review. D. B., A. E. L. and I. P. P. were mostly involved in experimental procedures.

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