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Effects of supercritical CO_2 and N_2O pasteurisation on the quality of fresh apple juice

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ABSTRACT

Supercritical pasteurisation is receiving increasing attention as an alternative technology for foodstuff pasteurisation, but often the possible effects on the perceptible quality are not sufficiently considered.

To address this latter issue, besides standard microbial analysis, we here investigate the impact of CO_2/N_2O supercritical pasteurisation (100 bar, 36 °C and 10 min treatment time) on the quality traits of fresh apple juice, linked to consumer perception. Discriminative sensory analysis (triangle test) and basic chemical characterization (total solids, sugars, organic acids, polyphenols) could not clearly demonstrate any induced modification of the treated juice, while head space analysis of volatile compounds (both by GC–MS and PTR–MS) indicated a general depletion of the volatile compounds that must be considered in the development of a stabilization method based on supercritical gases.

1. Introduction

Commercial pasteurisation processes are based mostly on thermal energy to eliminate potential food-borne illness. Recently, technologies, such as high pressure and pulsed electric fields, have been investigated to reduce microbial populations in food without introducing the negative effects on quality caused by heating. In particular, as far as high pressure processes are concerned, previous studies demonstrate the feasibility of both hydrostatic pressure and dense gas treatments as alternative techniques for pasteurisation of different substrates and elimination of different kinds of bacteria commonly present in foodstuffs (Spilimbergo & Bertucco, 2001; Yuste, Capellas, Pla, Fung, & Mor-Mur, 2001).

In recent years, a review on dense phase CO_2 (Damar & Balaban, 2006), a few patents (Van Ginneken, Weyten, Willems, & Lodewijckx, 2004; Wildasin, 2004) and several new articles, focusing on microbial, enzyme inactivation and the effects on food quality, have been published (Gunes, Blum, & Hotchkiss, 2006; Liu, Zhang, & Li, 2005): they confirm the feasibility and the effectiveness of this innovative technique. The main advantage of CO_2 treatment, in comparison with heat treatments, consists in the low temperature applied which induces a much lower impact on nutritional and chemicophysical properties of food (Connery, Shah, Coleman, & Hunek, 2005). In addition, if compared to high-hydrostatic pressure treatments, the relatively mild pressure conditions applied lead to an easier controlled, more feasible and less expensive process.

Little information, however, is available about the effects on perceivable quality and nutritional properties of different liquid foods immediately after CO_2 treatment and during storage.

Observations reported in the literature are scarce and conflicting and seem to depend on the food system investigated: mainly, orange juice has been tested (Arreola et al., 1991; Balaban et al., 1991; Boff, Truoung, Min, & Shellhammer, 2003; Wei, Balaban, Fernando, & Peplow, 1991), while few observations concern other juices, in particular carrot (Park, Lee, & Park, 2002), grape (Gunes et al., 2005), coconut (Damar & Balaban, 2005), mandarin (Lim, Vagiz, & Balaban, 2006) and watermelon (Lecky, 2005).

Physical and chemical properties, e.g. pH, Brix values and titratable acidity, orange juice do not appear to be influenced by CO_2 treatment. Yellowness and lightness seem to increase while redness seems to decrease (Arreola et al., 1991; Park et al., 2002; Wei et al., 1991).

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Two recent papers report physical changes of apple products: no visual changes, or modifications in total soluble solids content of apple cider have been found (Van Ginneken et al., 2004) while Gui et al. (2006) reported a significant reduction of the browning degree in CO_2-treated apple juice after storage at 4 °C.

Nutritional attributes have been scarcely examined so far: ascorbic acid retention was higher in the treated orange juice compared to the untreated sample (Arreola et al., 1991), while no significant differences between vitamin C and folic acid in treated and untreated orange juice (Ho, 2003) have been observed. In the case of grape juice, no changes in anthocyanins, total phenolics and antioxidant capacity after CO_2 treatment have been reported (Del Pozo-Insfran, Balaban, & Talcott, 2006).

A limited number of published studies focus on the sensory modifications induced by CO_2 treatment in food (Wildasin, 2004). The effects of stabilization treatments on perceivable quality are, however, of outmost importance because they are the key factors for consumer acceptance. The very few data so far collected indicate that high pressure-treated orange juice was almost indistinguishable from the untreated one (Damar & Balaban, 2006).

The literature on N_2O appears even scarcer and less clear: Enomoto, Nakamura, Nagai, Hashimoto, and Hakoda (1997) reported a considerable reduction of *S. cerevisiae* using N_2O at 40 bar and 40 °C for 240 min, while Fraser (1951) observed a poorer reduction of *E. coli* (compared to CO_2), after a treatment at 38 °C and 52 bar for 3 min. Recently, Spilimbergo and Mantoan (2006) demonstrated total inactivation of fresh apple juice after a treatment of 5 min, at 100 bar and 36 °C in a mixed multi-batch device. To the best of our knowledge, no paper reports on the effect of N_2O on chemicophysical, nutritional or sensory features of foodstuffs.

On the basis of these considerations, the objective of the present paper is three-fold:

(a) to confirm the efficiency of CO_2 pasteurisation and to investigate the effect of N_2O in the microbial inactivation of fresh apple juice;
(b) to verify whether the treatments induce sensory modifications, potentially perceptible by consumers, by means of discriminative analysis performed with a trained panel;
(c) to investigate the effect of high pressure on quality traits, linked to consumer perception and likeability, by means of chemical analysis of (i) soluble compounds, important for tastes related to sugar, organic acid and polyphenols content (ii) volatile organic compounds responsible of odour and flavour, performed by two head space techniques: solid-phase micro extraction coupled with gas chromatography–mass spectrometry (SPME–GC–MS) and proton transfer reaction–mass spectrometry (PTR–MS).

2. Materials and methods

2.1. Apple juice

Fifty litres of freshly squeezed apple juice were produced at Macè Srl (Italy), using a blend of Golden Delicious and Granny Smith apples. The juices produced were sealed in plastic bags (1000 ml) and stored at −20 °C. The day before any trial or further treatment, the juice bags were thawed at 4 °C (overnight). Before each experimental run, a certain quantity of the thawed juice was maintained at 4 °C and not treated (Reference juice). After the treatment, reference and treated juices were stored again at −20 °C prior to analysis.

To assess the microbiological inactivation as a function of the treatment time, the thawed juice, before use, was incubated for 1 day at 25 °C in order to increase the total microbial count up to about 5×10^2 cfu/ml.

2.2. High pressure equipment

The trials were performed with the multi-batch pilot plant described in Spilimbergo and Mantoan (2006). The liquid CO_2 (RIVOIRA, purity 99.990%) or N_2O (RIVOIRA, purity 99.95%) was cooled down to 4 °C and then pumped into high pressure vessels by a volumetric pump (LEWA, mod. LCD1/M910 s) with a maximum flow rate of 13 l/h. The vessels consist of two 310 ml cylinders (for the investigation of final product quality by sensory and chemical analysis/trial A) and of ten 15 ml cylinders (used only for the investigation of the effect of treatment time on microbial inactivation and headspace total concentration by PTR–MS analysis/trial B) provided with a magnetic system for stirring (VETROTECNICA, micro-stirrer, Velp, about 300 rpm).

All the vessels, immersed in a water bath, were thermally controlled by a temperature probe (Pt100) inside the reactor. Further details of the equipment and the procedure are described in Spilimbergo, Mantoan, and Cavazza (2007).

2.3. Supercritical pasteurisation

All the experimental runs were carried out at a constant temperature of 36 °C and pressure of 100 bar. Previous studies (Spilimbergo et al., 2006, Spilimbergo et al., 2007) demonstrated that these conditions represent the best ones for increasing the efficiency of the process without probably affecting the quality of the product.

Trial A: A 75 ml of juice were introduced to each vessel (V_{max} = 310 ml) and exposed to supercritical gas at 100 bar and 36 °C for 10 min with a stirring rate of 300 rpm. Twelve consecutive experimental runs were performed to produce the total volume of 900 ml needed for sensory and chemical analyses. For each treatment (with CO_2 and N_2O), six replicates were carried out.

Trial B: A 5 ml of juice were introduced to each vessel (V_{max} = 15 ml) and exposed to supercritical gas at 100 bar and 36 °C for different treatment time (0, 5, 10 and 20 min) with a stirring rate of 300 rpm. For each process condition, three replicates were performed.

2.4. Microbiological analysis

Before and after each treatment total microbial survivals were determined by standard plating techniques (Speck, 1976).

Every sample was diluted (1:10) in peptonated water, then plated in WL medium (composition: 4 g yeast extract, 5 g tryptone, 50 g glucose, 0.55 g H_2PO_4, 0.425 g KCl, 0.125 g $CaCl_2$, 0.125 g $MgSO_4$, 0.0025 g $FeCl_3$, 0.0025 g $MnSO_4$, 20 g Agar, 0.022 g bromocresol green and water up to 1000 ml). Plates were incubated for 2 days at 25 ± 1 °C and then the colonies were counted.

The results are expressed as survival%, N/N_0 %, where N represents the number of colonies in the treated sample and N_0 is the number in the untreated sample, calculated as the mean value of the three replicates.

2.5. Sensory analysis

We decided to investigate possible unspecific sensory differences in treated juice using the triangle test, an overall difference test that provides a sensitive measure of any sensory changes (Meilgaard, Civille, & Carr, 1999). In this context, the selection and the training of the judges are necessary steps to guarantee reproducible assessments and good discriminatory ability.

The panel consisted of 22 trained judges (13 male and 9 female), selected among the 33 candidates of a training course on the recognition of basic sensory stimuli (odours and tastes) and the triangle test procedure (26 training tests in all). In particular, the judges were trained in order to increase their ability to recognize slight differences induced in fresh apple juices.

In each of the following six sessions, judges were individually checked using two blind triangle tests: a control-odour triangle test (reference juice versus flavoured juice obtained by the addition of 1 mg/kg of hexyl acetate, responsible for apple "peel/green apple" odour) and a control-taste triangle test (reference apple juice versus acidified apple juice obtained by the addition of 1 g/kg of citric acid in order to increase the "acid" taste). On the basis of results of the latter tests, we further selected judges, excluding those (3 judges) that did not score a minimum of 50% of correct answers.

The products were compared according to the standard triangle test procedure (ISO, 2004) and statistical analysis of data was based on binomial distribution with $p = 1/3$ (Schlich, 1993). Four consecutive triangle tests were performed per session: two tests to measure the effect of treatments (Trial-CO_2 and Trial-N_2O) and 2 tests to control panel performances (control-odour and control-taste). Six sessions were undertaken in consecutive weeks. Test order was balanced over judges and sessions. Samples (20 ml) were presented in 50 ml disposable transparent cups coded with 3 digit random numbers.

The hypothesis of constant panel performances in the different sessions has been checked by χ^2 test. A significance level of 95% is assumed if not otherwise stated.

2.6. Chemical analysis of soluble compounds

Total soluble solids were measured using a refractometer RFM 81 (Bellingham & Stanley Ltd., Tunbridge Wells) and expressed as °Brix (Reg.CEE N.2676/90 All. 2).

Fructose, glucose and sucrose were measured with a HPLC Waters LC Module I plus, equipped with a Waters 410 Differential Refractometer detector (Waters Inc., Milford, USA). Separation was achieved with a polymer-based ion-exchange column (Varian Res-Elut-CHO-Ca 8% 7.5 μm, 300 mm × 7.75 mm), using water as eluent under isocratic conditions and at the column temperature of 85 °C.

Titratable acidity was measured as g/kg of citric acid with an automated titrator (Crison Compact III, Crison Instrument, Barcelona), following the procedure of Reg.CEE N.2676/90 All. 13.

HPLC analysis of organic acids (citric, malic and ascorbic acid) was performed following the procedure reported by Vrhovsek, Giongo, Mattivi, and Viola (2007).

The total amount of polyphenols was measured as mg/kg of catechin according to the optimized Folin–Ciocalteu method (Rigo et al., 2000).

2.7. Head space analysis of volatile compounds

2.7.1. General

Volatile compounds are strictly related to odour and flavour and are usually profoundly modified by pasteurisation processes. Both SPME/GC–MS and PTR–MS allow a direct monitoring of the volatile compounds present in the headspace: the genuine mixture directly interacting with our senses.

The head space composition of the treated and reference juices evaluated by sensory and basic chemical analyses has been measured by both SPME GC–MS and PTR–MS. The latter methodology has also been used to evaluate the effect of different treatment times (0, 5, 10, 20, 40 min) on the headspace of the juices produced in the small reactors (trial B).

2.7.2. SPME GC–MS

Solid-phase microextraction is a convenient and rapid means of isolating volatile compounds prior to gas chromatographic analysis, widely used in many applications in food analysis and particularly to detect characteristic aromas, off-flavour and pesticides (Kataoka, Lord, & Pawliszyn, 2000).

For the extraction and concentration of volatile compounds from juice headspace, a manual SPME holder (Supelco Bellafonte, PA), coated with 2 cm 50/30 μm DVB/Car/PDMS (Supelco Bellefonte, PA 57348), was used. Every day, before the analyses, the fibre was preconditioned for 5 min at 250 °C.

A 20 ml aliquot of the juice was transferred to a 30 ml vial, adding 6 g of NaCl to enhance the release of the volatiles into the sample headspace. After the addition of 40 μl of internal standard (2-octanol, 25 mg/l), the vial was crimp-closed with a Teflon-lined silica cap (Supelco) and equilibrated at 30 °C for 10 min under constant stirring. After that, the SPME fibre was exposed to the sample headspace for 30 min.

Thermal desorption of the compounds from the fibre coating took place in the GC injector at 250 °C in splitless mode. A Perkin Elmer AutoSystem XL gas chromatograph, coupled with a Turbo-Mass Gold (PerkinElmer, Norwalk CT) mass spectrometer, was used. Separation was achieved on a DBWax fused-silica capillary column (60 m, 0.32 mm ID, 0.5 μm film thickness, J&W Scientific Agilent Technologies Palo Alto, CA). The oven temperature was programmed as follows: after 2 min at 50 °C the temperature was raised to 200 °C (3 °C/min, held 10 min) and then to 230 °C (10 °C/min, held 10 min). Helium was used as carrier gas with a flow rate of 1.2 ml/min. Transfer line temperature was 220 °C. The mass spectrometer was operated in electron ionization mode (70 eV) with a scan range from m/z 30–300. According to their peak resolution, the areas were either calculated from the total ion current (TIC) or estimated from the integrations performed on selected ions. Quantification of the aroma compounds was achieved by the internal standard method (2-octanol). Peak identification was based on mass spectral interpretation and on the standard library NIST-98/Wiley and, when available, also on authentic standards.

The repeatability of the method, including SPME extraction, was tested by analysing five samples of the reference apple juice.

2.7.3. PTR–MS

Proton transfer reaction mass spectrometry (PTR–MS) is a relatively new mass spectrometric technique founded on a particular implementation of chemical ionization, based on the proton transfer from hydronium ions (H_3O^+) to the volatile compounds to be detected (Lindinger, Hirber, & Paretzke, 1993). It is characterised by its high sensitivity and it is a rapid, analytical procedure that allows direct injection of the gaseous sample. This technique has been described in several papers (Lindinger, Hansel, & Jordan, 1998) and successfully exploited to carry out food analysis. Examples of food science application are the characterisation of products and processes (Biasioli et al.,2003a, 2003b; Yeretzian, Jordan, & Lindinger, 2003; Aprea et al.,2007a, 2007b) or food spoilage detection (Aprea et al., 2006a; Aprea, Biasioli, Carlin, Märk, & Gasperi, 2008) and the study of the correlation with sensory evaluation (Aprea, Biasioli, Gasperi, Märk, & van Ruth, 2006b; Biasioli et al, 2006; Gasperi et al., 2001).

A commercial high sensitivity PTR–MS (Ionicon Analytik GmbH, Innsbruck, Austria) was used for a fast characterisation of juice head space composition (Biasioli et al., 2003a). For each sample, 5 ml of juice were placed in a 120 ml silicon-septum closed glass bottle (Supelco, Bellefonte, USA) for 45 min in a water bath (36 °C). The VOCs released into the headspace were then transferred through a heated (70 °C) capillary line (uncoated deactivated fused silica tubing with an inner diameter of 0.25 mm;

Table 1
Microbial inactivation data of apple juice by high pressure CO_2 and N_2O at 100 bar and 36 °C for different treatment times.

CO_2		N_2O	
Time (min)	N/N_0 %	Time (min)	N/N_0 %
1	8.01	1	7.42
5	0.35	5	0.45
10	0	10	0
15	0	15	0

Supelco, Bellefonte, USA) directly into the drift tube of the PTR–MS at a rate of about 10 cm^3/s. The headspace was replaced by a flow of pure nitrogen gas (SOL s.p.a., Italy; purity: 99.999%). Details of this sampling method can be found in Biasioli et al. (2003a). To avoid memory effects, the samples were measured in random order and nitrogen was flushed, for 5 min, between two consecutive samples.

The mass spectrometric data were collected over a mass range of m/z from 20 up to 260, using a dwell time of 0.2 s per mass under controlled drift tube condition (570 V, 50 °C, 2.04 mbar). After blank subtraction, data were converted into ppb (Aprea et al., 2007b). Each sample was measured for 5 cycles; the first two cycles were disregarded and then the data of the next 3 were averaged and used for further data analysis.

2.8. Statistical analysis

In order to find differences among the juices in terms of chemical parameters, one-way ANOVA, followed by Tukey's HSD test was performed by means of the software STATISTICA 5.1 (StatSoft, Inc., Tulsa, OK, USA). Principal component analysis (PCA) of volatile compounds data was computed by the software, The Unscrambler 8.5 (CAMO PROCESS AS, OSLO, Norway).

3. Results and discussion

3.1. Microbial inactivation

Kinetic analysis was performed at 36 °C (this value was considered low enough to maintain the sensory properties of the product, and sufficiently high to reach a satisfactory rate of inhibition in a short treatment time), 100 bar, 300 rpm mixing rate and with a sample volume of 75 ml. These are the optimal values for the operative parameters of the high-pressure apparatus used as demonstrated in previous experiments reported by Spilimbergo and Mantoan (2006).

A total inactivation of the microorganisms initially present in the fresh juice was obtained (Table 1) both with CO_2 and N_2O, at 100 bar and 36 °C, after 10 min of treatment.

As a consequence, the treatment time for all the experimental runs, carried out for the chemical and sensory analyses (Trial A), was set at 10 min, under the same operative conditions (100 bar, 36 °C, 300 rpm).

The data confirm the effectiveness of both dense CO_2 and dense N_2O against microorganisms at mild values of temperature (Spilimbergo et al., 2006; Spilimbergo et al., 2007).

3.2. Sensory analysis

Table 2 lists the results of all triangle tests performed by the panel, comparing the reference juice with the modified juices (control-odour and control-taste) and with the treated juices (CO_2 and N_2O).

In the case of the control-odour test, the results achieved during the 6 sessions indicate a clear difference (>99.9) with a panel resembling a population with a very large "percentage above chance" (Schlich, 1993). The χ^2 test indicates that the results are compatible (99% confidence) with a constant probability in all sessions.

For the control-taste test, only in the last two sessions there was a significant difference between reference and acidified juice (>99.9 significance). On average, the panel corresponds to a small difference above chance and again the χ^2 test indicates that the data are compatible with constant panel performance in the case of little difference between the products.

Regarding CO_2 treatment, the 1st, 3rd and 5th comparisons indicate a clear difference (>99.9% significance) while others show differences with minor levels of significance. In this case, the χ^2 value is not compatible with constant panel performances and product characteristics.

In the case of the N_2O treatment, the 4th, 5th and 6th replicates indicate a significant difference (>97% significance), while the 1st, 2nd and 3rt replicates show differences with minor levels of significance. These data are not compatible with constant panel performances and products characteristics.

The results of the control tests demonstrate the good repeatability of the panel through all the sessions in the case of both a large difference (control-odour) and a small difference (control-taste) and therefore the differences found by the panel in the treated juices can be considered reliable results. For this reason, we believe that the data indicate a high variability of the processed juice and that, with proper process control, it is possible to reduce the impact of supercritical pasteurisation below the sensitivity of our panel.

3.3. Chemical analysis of soluble compounds

The results shown in Table 3 indicate no significant differences (ANOVA, $p<0.05$) induced by supercritical pasteurisation (both N_2O

Table 2
Results of the triangle tests in the 6 replicates for the two control tests on modified juices, in the 1st column for odour and in the 2nd column for taste, and for the two tests on supercritical CO_2- and N_2O-treated juices (treatment conditions: 75 ml juice, 100 bar, 36 °C, 300 rpm, 10 min) in the last two columns: number of correct responses of each comparison and the related p value.

Session	Total responses	Control-odour		Control-taste		CO_2		N_2O	
		Correct responses	p	Correct responses	p	Correct responses	p	Correct responses	p
1	18	16	<0.001	9	0.108	14	<0.001	8	0.223
2	17	14	<0.001	9	0.075	9	0.075	7	0.326
3	18	14	<0.001	8	0.223	14	<0.001	7	0.391
4	18	15	<0.001	9	0.108	5	0.769	12	0.004
5	21	18	<0.001	13	0.007	15	<0.001	12	0.021
6	19	17	<0.001	12	0.007	8	0.279	11	0.024

p measures the probability associated with the risk of wrongly concluding that a perceptible difference exists when it does not.

Table 3
Chemical characterisation of reference and treated apple juices (CO_2 and N_2O treatment conditions: 75 ml juice, 100 bar, 36 °C, 300 rpm, 10 min): mean values (for the number of samples reported in the 2nd column), standard deviations (SD) and ANOVA *p*-values (*p*) for the parameters related to sugars and acidic fraction.

Parameter	No sample	CO_2 Reference		CO_2 Treated		N_2O Reference		N_2O Treated		
		Mean value	(SD)	Mean value	(SD)	Mean value	(SD)	Mean value	(SD)	p
Brix (°)	3	11.8	(0.0)	11.7	(0.1)	11.9	(0.1)	11.7	(0.1)	0.276
Fructose (g/kg)	3	64.2	(0.5)	64.3	(1.2)	66.0	(3.0)	66.0	(1.0)	0.422
Glucose (g/kg)	3	15.0	(0.4)	14.6	(0.5)	15.2	(0.7)	15.2	(0.1)	0.405
Sucrose (g/kg)	3	30.7	(1.0)	29.1	(1.3)	29.5	(1.9)	29.7	(0.2)	0.480
Total acidity (meq/kg)	3	11.6	(0.0)	11.6	(0.1)	11.7	(0.1)	11.6	(0.1)	0.561
Malic acid (g/kg)	6	4.5	(0.4)	4.4	(0.4)	4.4	(0.4)	4.4	(0.4)	0.985
Citric acid (g/kg)	6	3.3	(0.2)	3.2	(0.2)	3.2	(0.1)	3.2	(0.2)	0.666
Ascorbic acid (g/kg)	6	1.8	(0.2)	1.9	(0.1)	1.8	(0.2)	1.8	(0.2)	0.493
Polyphenols (mg/kg)	6	580	(143)	622	(242)	493	(114)	510	(128)	0.504

and CO_2) for any of the parameters investigated in relation to sugars, acids and polyphenols.

3.4. Head space analysis by GC–MS

Volatile compounds concentrations in treated and reference juices obtained by SPME/GC–MS are listed in Table 4. For each compound, the results of the repeatability analysis, including SPME extraction, are expressed as the relative standard deviation (CV%) of the analyses of five samples of the reference apple juice: the measured CVs are always below 10% and, for half of the investigated compounds, below 5%.

Both treatments reduce the concentrations of many volatile compounds: overall depletions of 35% for carbon dioxide and 26% for nitrous oxide were observed. The highest variation was observed for some esters (acetates) and aldehydes. Hexyl acetate, butyl acetate, amyl acetate and isoamyl acetate, with an intense sweet and fruity odour, are the most abundant esters of apple, as already reported for different cultivars (Echeverría, Fuentes, Graell, Lara, & López, 2004; Komthong, Igura, & Shimoda, 2007; Harker et al., 2002; Lo Scalzo, Testoni, & Genna, 2001). The concentrations of all these compounds show a significant reduction in treated juices: 69% and 67% for hexyl acetate, 65% and 53% for pentyl acetate, 63% and 45% for isopentyl acetate, 67% and 42% for isobutyl acetate and 42% and 29% for butyl acetate, respectively, in the case of CO_2 and N_2O.

The most abundant aldehydes are *n*-hexanal and (*E*)-2-hexenal. These compounds are related to grass/tallow/leaf odour and originate from fatty acids by β-oxidative enzymes or lipoxygenases (Echeverría et al., 2004; Song & Bangerth, 2003). These enzymatic reactions, caused by cell disruption, can occur during apple juice production (Takeoka, Flath, Mon, Teranishi, & Guentert, 1990). The reductions observed in the treated juices compared to the reference are 77% and 66%, in the case of (*E*)-2-hexenal, and 37% and 24%, in the case of *n*-hexanal, for CO_2 and N_2O, respectively.

For most of the esters and aldehydes, the observed reduction seems more intense for the N_2O. Some alcohols, e.g., *n*-hexanol, (*Z*)-3-hexenol and (*E*)-2-hexenol, are not affected by the treatment, while other compounds, e.g. *n*-pentanal and (*E*)-2-octenal, show different behaviour (ANOVA, *p* value <0.05).

The results can be summarised by principal component analysis (Fig. 1), showing a clear separation, in the first component, between the treated and the reference juices and no separation between the two different treatments. The loadings plot (Fig. 1, left panel) indicates an increase of *n*-pentanal, 1-octen-3-one, 1-octen-3-ol and (*E*)-2-octenal, with the treatment, while *n*-hexanal,

Table 4
Chemical characterisation of reference and treated apple juices (CO_2 and N_2O treatment conditions: 75 ml juice, 100 bar, 36 °C, 300 rpm, 10 min): mean values (of 6 samples), standard deviations (SD) and ANOVA *p*-values (*p*) for the volatile compounds quantified by SPME–GC–MS (concentration in μg/l).

Compounds	Repeatability[a]		CO_2 Reference		CO_2 Treated		N_2O Reference		N_2O Treated		
	RT (min)	CV%	Mean value	(SD)	Mean value	(SD)	Mean value	(SD)	Mean value	(SD)	*p*
n-Pentanal	12.75	4.1	1.3 a	(0.1)	1.9 b	(0.4)	1.2 a	(0.1)	1.9 b	(0.3)	<0.001
Isobutyl acetate	13.76	4.5	0.8 b	(0.4)	0.3 a	(0.3)	0.9 b	(0.1)	0.5 ab	(0.1)	0.001
1-Penten-3-one	14.15	5.8	0.7	(0.4)	0.3	(0.3)	0.7	(0.5)	0.5	(0.4)	0.412
Toluene	14.96	6.7	8.1	(2.8)	7.6	(0.9)	7.9	(3.6)	6.9	(1.9)	0.855
Butyl acetate	15.95	4.8	31.0 b	(3.5)	17.9 a	(4.1)	29.5 b	(4.3)	21.1 a	(1.8)	<0.001
n-Hexanal	16.49	1.7	85.6 b	(11.1)	54.3 a	(8.1)	80.3 b	(8.7)	61.9 a	(4.3)	<0.001
Isoamyl acetate	17.94	9.7	18.7 b	(2.3)	6.9 a	(2.1)	17.4 b	(2.6)	9.6 a	(1.4)	<0.001
2-Methyl- 4-pentenal	18.88	6.2	8.7 bc	(2.5)	4.2 a	(0.2)	9.6 s	(4.1)	4.7 ab	(1.1)	0.002
Amyl acetate	20.13	2.1	7.1 b	(1.3)	2.5 a	(0.9)	6.7 b	(0.9)	3.1 a	(0.4)	<0.001
(*E*)-2-Hexenal	22.44	8.1	52.2 b	(21.7)	12.2 a	(3.1)	43.6 b	(9.8)	14.9 a	(5.1)	<0.001
Hexyl acetate	24.44	8.6	20.8 b	(3.4)	6.4 a	(1.3)	19.3 b	(2.9)	6.4 a	(1.4)	<0.001
1-Octen-3-one	25.88	3.9	1.8 a	(0.4)	4.2 b	(1.7)	1.9 a	(0.7)	3.2 ab	(1.1)	0.002
(*Z*)-3-Hexenyl acetate	26.38	4.8	9.7 b	(1.3)	4.3 a	(1.4)	9.1 b	(1.5)	4.7 a	(1.0)	<0.001
n-Hexanol	27.91	1.3	64.0	(3.1)	59.7	(5.3)	61.6	(5.5)	62.4	(4.3)	0.403
(*Z*)-3-Hexenol	29.29	5.8	7.1	(0.7)	7.6	(0.9)	6.2	(3.0)	7.6	(1.0)	0.639
(*E*)-2-Hexenol	30.14	3.1	35.7	(2.0)	35.1	(3.2)	34.6	(4.5)	36.1	(2.0)	0.721
(*E*)-2-Octenal	31.57	3.6	2.1	(1.0)	3.2	(2.0)	2.4	(1.0)	3.2	(1.0)	0.335
1-Octen-3-ol	31.83	1.8	0.7	(0.3)	1.3	(0.8)	1.1	(0.4)	1.3	(0.4)	0.154
Exobornyl acetate	38.19	8.6	0.1	(0.1)	2.8	(6.4)	0.4	(0.6)	0.2	(0.2)	0.621
β-Damascenone	46.9	6.9	0.4	(0.2)	0.5	(0.2)	0.5	(0.3)	0.6	(0.3)	0.625

[a] Relative standard deviation values (CV%) from analysing five samples; values with different letters are significantly different by Tukey's HSD test ($p < 0.05$).

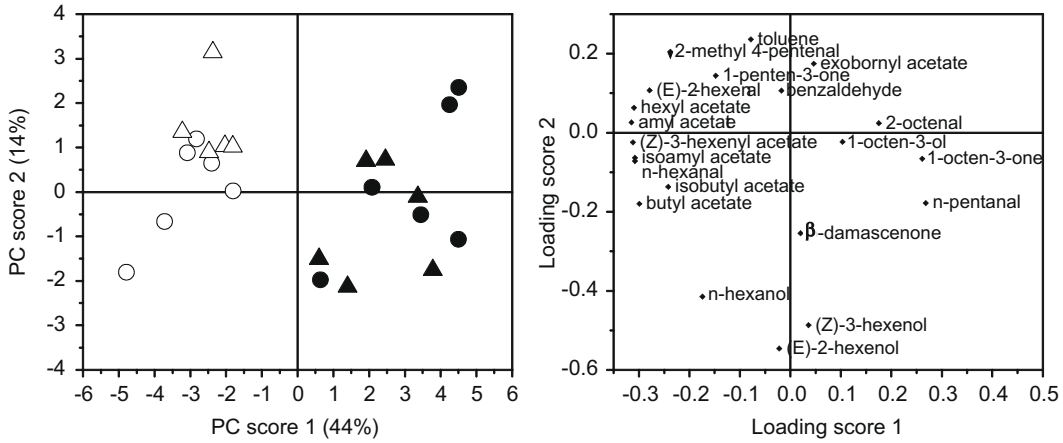

Fig. 1. PCA on SPME GC–MS data. Effect of CO_2 and N_2O pasteurisation (75 ml, 100 bar, 36 °C, 300 rpm, 10 min) on volatile compounds. Left panel: plot of the first two principal components calculated on standardized results. Triangles indicate N_2O treatment and circles indicate CO_2 treatment. Open symbols indicate reference juices while solid symbols indicate the treated ones. Right panel: loadings plot referred to the first two principal components reported in the left panel.

(*E*)-2-hexenal, 2-methyl-4-pentenal, butyl acetate, isobutyl acetate, amyl acetate, isoamyl acetate and hexyl acetate, (*Z*)-3-hexenyl acetate are negatively correlated with the first component and thus decrease (ANOVA, p value <0.05) after the pasteurisation process.

The depletion of volatile compounds, especially of odour-active ones, characterized by low threshold values (such as the C6-aldehydes or acetates), could be responsible for odour modifications induced by high pressure treatment and these modifications can explain the results of triangle tests. For the two most abundant aldehydes in the apple juice, *n*-hexanal and (*E*)-2-hexenal, with odours of grass/tallow/leaf, we observed the largest differences in concentration between reference and treated juice in those replicates where the triangle tests showed significant differences (1st, 3rd and 5th replicates for CO_2 treatment and 4th, 5th and 6th replicates in the case of N_2O treatment).

3.5. Headspace analysis by PTR–MS

The averaged volatile concentration, measured by PTR–MS in the headspace of the untreated juices, was about 6.3 ppm_V (±10%). The highest signal was recorded at m/z 45 (protonated acetaldehyde), corresponding to 3.3 ppm_V (±15%) of acetaldehyde, followed by the signal at m/z 83 (sum of *n*-hexanal, (*Z*)-3-hexenol and (*E*)-2-hexenol), corresponding to 0.8 ppm_V (±14%).

The high pressure treatments, in general, caused a depletion of total volatile compounds initially present in the fresh apple juices (Fig. 2), the average reductions being, respectively, 38% and 31% for carbon dioxide and nitrous oxide. The highest reduction was observed for (*E*)-2-hexenal: less than 15% of the initial concentration (0.19 ppm_V). Differently from GC results, no increase was observed for any of the monitored masses after pasteurisation treatment. A possible explanation could be the saturation of the SPME fibre coating by the most abundant compounds. The reduction of concentration induced by pasteurisation treatments can favour the adsorption, on the SPME fibre, of compounds with less affinity (showing an apparent increase). No significant differences ($p < 0.05$) between the two treatments were detected, indicating similar effects of both gases employed. The PCA measurements shown in Fig. 3 summarize the results of the 240 masses monitored.

These measurements are in good agreement with GC/MS analysis: semi-static PTR–MS analysis provides (more easily and quickly) the same qualitative information as is obtained by SPME/GC–MS. PTR–MS can be successfully exploited for on-line monitoring of the pasteurisation process: the effect of treatment

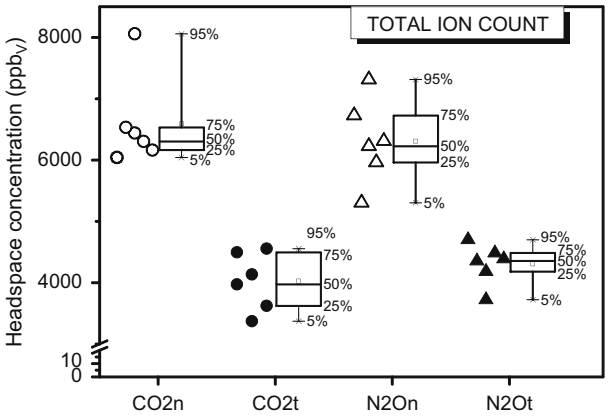

Fig. 2. BoxPlot of PTR–MS data (data of 6 replicates). Effect of CO_2 and N_2O pasteurisation (75 ml juice, 100 bar, 36 °C, 300 rpm, 10 min) on head space composition. Triangles indicate N_2O treatment and circles indicate CO_2 treatment. Open symbols indicate reference juices while solid symbols indicate the treated ones.

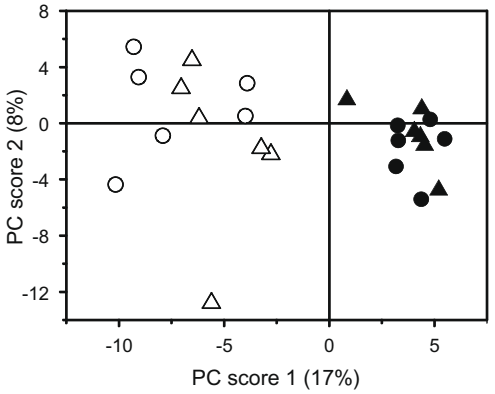

Fig. 3. PCA on PTR–MS data. Effect of CO_2 and N_2O pasteurisation (75 ml juice, 100 bar, 36 °C, 300 rpm, 10 min) on head space composition. Plot of the first two principal components calculated on standardized data. Triangles indicate N_2O treatment and circles indicate CO_2 treatment. Open symbols indicate reference juices while solid symbols indicate the treated ones.

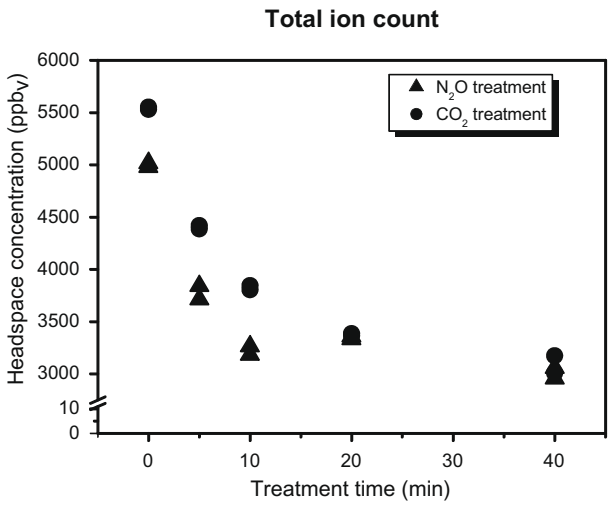

Fig. 4. PTR–MS data. Effect of CO_2 and N_2O pasteurisation (5 ml juice, 100 bar, 36 °C, 300 rpm) on headspace total concentration as a function of treatment time (two replicates). Triangles indicate N_2O treatment and circles indicate CO_2 treatment.

time on juice headspace (trial B) monitored by PTR–MS is shown in Fig. 4. It is clear that the volatile reduction kinetics follow the same trend as the kinetic microbial inactivation (Table 1). 10 min treatment at 100 bar and 36 °C causes, on average, a depletion of roughly one third of the total headspace concentration (Fig. 4) of the reference juice.

4. Conclusions

The effect of supercritical CO_2 and N_2O on the microbial and sensorial quality of fresh apple juice has been investigated.

The efficiency of CO_2 and N_2O process in inactivating microorganisms naturally present in fresh apple juice has been confirmed: a 10 min treatment at 100 bar and 36 °C is sufficient to assure the inactivation of all the microorganisms initially present in the sample.

Sensory analysis, by means of a panel triangle test, indicates a moderate effect of the treatment, with both CO_2 and N_2O, on the perceptible juice quality.

Instrumental analysis indicates no significant differences for any parameter investigated regarding basic composition (sugars and acids contents) and polyphenol concentration. However, headspace analysis, performed by both GC/MS and PTR–MS, indicates that CO_2 and N_2O treatments induce a reduction in the concentrations of many volatile compounds: the highest variation was observed for some esters (acetates) and aldehydes, characterized by low threshold values, responsible for the changes in the odour/flavour of treated juices.

PTR–MS is confirmed as a rapid and non-invasive characterisation tool that provides information, in quantitative and qualitative agreement with GC/MS analysis,

In conclusion, both high pressure carbon dioxide and nitrous oxide can be considered, at least from a microbiological point of view, as promising alternatives to thermal pasteurisation for apple juice. These treatments preserve the chemical characteristics of apple juice but induce overall volatile compound depletion in the juice head space. However it remains an open issue to verify whether the differences detected by the trained sensory panel are high enough to be detected also by naïve consumers who usually have a higher discriminant threshold.

For the sake of completeness, it is worth noting the high dispersion of the data obtained (Trials A and B), as regards both the reference and the treated juice. The reason can be partially found in the variability of the juice samples used in the different experimental runs. These probably contain different percentages of dry matter and particulates that can affect the head space composition (Biasioli et al., 2003a) and cause experimental uncertainty related to the apparatus. In this regard, further research is needed to optimize the process, in order to reduce the loss of volatile compounds and enhance process stability.

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