SUPERCRITICAL PASTEURIZATION: MICROBIOLOGICAL AND SENSORY DIVERSITIES BETWEEN FRESH AND TREATED APPLE JUICES

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Recently low temperature technologies are receiving a great deal of attention as alternatives to thermal pasteurization in foodstuff stabilization. Pasteurization by means of supercritical (SC) fluids represents one of the most promising alternatives.
In this study the microbiological and sensory effects on natural apple juice of SC fluids treatment, both carbon dioxide (CO_2) and nitrous oxide (N_2O), was investigated and compared to the untreated product.
In a high pressure lab-scale equipment freshly squeezed apple juice was treated at 36°C and 100 bar for 10 minutes with both the gases. An almost complete microbial inactivation naturally present in the juice was achieved.
The effects of the treatment on the sensory quality were evaluated by means of:
1. Discriminative analysis performed by a panel of trained judges: triangle tests were used to compare CO_2-treated and N_2O-treated juice versus fresh juice. The judges could find moderate difference between N_2O/ CO_2 treated juice and the fresh one.
2. Basic chemical characterization (total solids, sugar, organic acids, polyphenols): no significant modification between N_2O/ CO_2 treated juice and the untreated one.

INTRODUCTION

Commercial pasteurization 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 of heating on the product quality. In particular, as far as high pressure processes are concerned, previous studies demonstrates the feasibility of both hydrostatic pressure and dense gases treatments as alternative techniques for pasteurization of different substrates and different kind of bacteria commonly present in foodstuff [1-3].
Little information, however, are 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 literature are scarce and conflicting and seem to depend on the food system investigated [4-7]. The literature on NO_2 appears even scarcer and less clear compared with the one on CO_2 [8,9].
The effect of stabilization treatments on perceivable quality is, however, of outmost importance because it is the key factor for the consumer product acceptance.
On the basis of these considerations the objective of the present paper is three-fold:
a) to confirm the efficiency of CO_2 pasteurization 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 by 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 soluble compounds, important for tastes related to sugar, organic acid and polyphenols content.

MATERIALS AND METHODS

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 until analysis.

For the kinetic studies (Trial B), in order to increase the total microbial count, the thawed juice was incubated for 1 day at 25 °C. The total initial microbial count was on the average of $5*10^2$ cfu/mL.

High pressure equipment

The trials were performed with the multi-batch pilot plant described in [10]. The vessels consist of two 310 mL cylinders (for the investigation on final product quality by sensory and chemical analysis/trial A) and of ten 15 mL cylinders (used only for the investigation on stabilization process by microbial analysis/trial B) provided with a magnetic system for stirring (VETROTECNICA, micro-stirrer, Velp, about 300 rpm).

Supercritical Pasteurization

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

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

Microbiological analysis

Before and after each treatment total microbial survivals were determined by standard plating techniques. Every sample was diluted (1:10) in peptonated water then plated in WL medium (composition: 4g yeast extract, 5g tryptone, 50g glucose, 0.55g H_2PO_4, 0.425g KCl, 0.125g $CaCl_2$, 0.125g $MgSO_4$, 0.0025g $FeCl_3$, 0.0025g $MnSO_4$, 20g Agar; 0.022g 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.

Sensory Analysis

We decided to investigate possible unspecific sensory differences in treated juice using the triangle test, an overall difference test that provide a sensitive measure of any sensory changes [11]. In this context, the selection and the training of the judges are necessary steps to warrant 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 in recognizing slight differences induced in fresh apple juices.

The products were compared according to standard triangle test procedure [12] and statistical analysis of data was based on binomial distribution with p=1/3 [13]. 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 test 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.

Chemical analysis of soluble compounds

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

Fructose, glucose and saccarose were measured by HPLC Waters LC Module I plus, detector Waters 410 Differential Refractometer (Meadows Instrumentation Inc., Chicago). 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 [14].

The total amount of polyphenols was measured as mg/kg of catechin according the optimized Folin-Ciocalteu method [15].

Statistical analysis

Analysis of Variance (ANOVA) was performed by means of the software STATISTICA 5.1 (StatSoft, Inc., Tulsa, OK, USA). Principal component analysis (PCA) was computed by the software The Unscrambler 8.5 (CAMO PROCESS AS, OSLO, Norway).

RESULTS AND DISCUSSION

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 [10].

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

As a consequence, the treatment time for all the experimental runs, carried out for the sensory analysis (Trial B), was set at 10 minutes, at 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 [10,16].

Table 1: Microbial inactivation data of apple juice by high pressure CO_2 and N_2O. Treatment conditions: 36°C – 100 bar – 75mL – 300rpm.

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

Sensory analysis

Table 2 reports 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" [13]. χ^2 test indicates that the results are compatible (99% confidence) with a constant probability in all sessions.

Table 2: Results of the triangle tests in the 6 replicates for the two control tests on modified juices (1st column for odour, 2nd column for taste) and for the two tests on supercritical CO_2 and N_2O treated juices (last two columns): number of correct responses of each comparison and the related p value *.

		Control ODOUR		Control TASTE		CO_2		N_2O	
SESSION	Total responses	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 to the α risk of wrongly conclude that a perceptible difference exists when it does not									

For the control-taste test, only in the last two sessions there is a significant difference between reference and acidified juice (>99.9). On average, the panel corresponds to a small difference above chance and again the χ^2 test indicate that the data are compatible with constant panel performance in the case of little difference between the products.
Regarding CO_2 treatment, 1st, 3rd and 5th comparisons indicate a clear difference (>99.9% significance). In this case the χ^2 value is not compatible with constants panel performances and product characteristics.
In the case of the N_2O treatment the 4th replicate indicates a clear difference (>99.9% significance) while the other two comparisons, 5th and 6th , show differences even though at a minor level of significance (98%). 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 high difference (control-odour) and a little difference (control-taste). For this reason, we believe that the data indicate a high variability of the processed juice and that, under a proper process control, it is possible to reduce the impact of supercritical pasteurization below the sensitivity of our panel.

Chemical analysis of soluble compounds

Table 3: Chemical characterisation of treated and reference apple juices: parameters related to sugars and acidic fraction.

Parameter	N°	CO_2 reference		CO_2 treated		N_2O reference		N_2O treated		
		mean	(sd)	mean	(sd)	mean	(sd)	mean	(sd)	p
Brix (°)	3	11,8	(0,0)	11,7	(0,1)	11,9	(0,1)	11,7	(0,1)	0,28
Fructose (g/kg)	3	64,2	(0,5)	64,3	(1,2)	66,0	(3,0)	66,0	(1,0)	0,42
Glucose (g/kg)	3	15,0	(0,4)	14,6	(0,5)	15,2	(0,7)	15,2	(0,1)	0,41
Saccarose (g/kg)	3	30,7	(1,0)	29,1	(1,3)	29,5	(1,9)	29,7	(0,2)	0,48
Total acidity (meq/kg)	3	11,6	(0,0)	11,6	(0,1)	11,7	(0,1)	11,6	(0,1)	0,56
Malic acid (g/kg)	6	4,5	(0,4)	4,4	(0,4)	4,4	(0,4)	4,4	(0,4)	4,5
Citric acid (g/kg)	6	3,3	(0,2)	3,2	(0,2)	3,2	(0,1)	3,2	(0,2)	3,3
Ascorbic acid (g/kg)	6	1,8	(0,2)	1,9	(0,1)	1,8	(0,2)	1,8	(0,2)	1,8
Polyphenols (mg/kg)	6	580	(143)	622	(242)	493	(114)	510	(128)	0,50

The results shown in Table 3 indicate no significant differences (ANOVA, $p<0.05$) induced by supercritical pasteurization (both N_2O and CO_2) for any of the parameter investigated in relation to sugars, acids and polyphenols.

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 minute 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 panel triangle test indicates a moderate effect of the treatment, both with 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 poliphenols concentration.
In conclusion, both high pressure carbon dioxide and nitrous oxide can be considered a promising alternative to thermal pasteurization of apple juice: these treatments induce just slight modifications on the sensorial quality of the product. It remains an open issue to verify whether the difference detected by the trained sensory panel is high enough to be detected also by naïve consumers that usually have a higher discriminant threshold.

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