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

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A B S T R A C T

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.