Effect Of Supercritical CO_2 Pasteurization On Natural Microflora And Quality Attributes Of Fresh – Cut Coconut

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Supercritical carbon dioxide (SC-CO_2) treatment has been largely investigated as non – thermal technology for the pasteurization mainly of liquid foods. Recently, the research has been moving to the application of this technology also to solid food products. In this concern, the objective of the present study is the evaluation of the effectiveness of SC-CO_2 as non – thermal technology for the pasteurization of fresh cut fruits, in particular coconut. The inactivation kinetics of microflora naturally present in coconut was obtained after SC-CO_2 treatments carried out at 12.0 MPa in a multi – batch lab scale apparatus at different temperatures (from 35 up to 45°C) and treatment times (from 5 up to 60 minutes). Additionally, the texture of the samples was measured by means of a mechanical testing equipment while the color by an innovative on –line spectroscopic technique, in order to detect possible modifications of qualitative aspects of the treated samples.
The results showed that 15 minutes at 45°C and 12.0 MPa were sufficient to achieve 4 Log(CFU/g) reductions for total aerobic count, total coliform bacteria and yeasts and molds and that the treatment did not induce significant changes in color parameters (L^*, a^*, b^*) or in the elastic modulus of the sample.
The results highlight the possibility to apply the SC-CO_2 technology for the inactivation of natural microflora of fresh cut coconut. Based on the achieved experimental findings the process could be suggested as alternative technology for the pasteurization of ready – to eat products.

INTRODUCTION

The growing interest of consumers for high quality and "minimal processing" ready to eat products are forcing the manufacturers to develop innovative preservation techniques which are able to guarantee microbial safety without affecting food quality and causing alterations in taste and organoleptic features of the products.
Recently supercritical CO_2 (SC-CO_2) technology has been proposed to the food industry as a process able to induce a pasteurizing/sterilizing effect when applied both to solid and liquid matrixes. Compressed CO_2 is regarded by the FDA (Food and Drug Administration) as a GRAS (Generally Recognized As Safe) substance because it is relatively inert, nontoxic, nonflammable, recyclable and readily available in high purity, leaving no residues when removed from the product.
Due to these advantages SC-CO_2 process has been widely applied to liquid substrates with interesting results making the technology ready for a future industrial application. Published results demonstrate that the treatment is able to guarantee a better retention of natural flavor and nutrients of liquid foods compared to traditional thermal processes [1, 2, 3, 4].
Few papers concern the effect of SC-CO_2 on solid substrates [5, 6]: the application of the process is still at the beginning of its development due to the complexity of the matrix, which

can make CO_2 bactericidal action more difficult, and to the lack of information about the inactivation mechanism. As for liquids, the technology is attending to be promising also for solid products depending on the impact of pressurized CO_2 on the microbial and quality attributes of the treated products [7].
In this regard, the present work focuses on the applicability of SC-CO_2 as non –thermal technology for the pasteurization of fresh cut coconut. The objectives of the study are: 1. to optimize the process parameters (temperature and time) for microbial reduction of the natural microflora (total aerobic count, total coliform bacteria and yeasts and molds); 2. to monitor the color during as well as before and after the entire process thanks to an innovative spectroscopic technique; 3. to evaluate the effect of the treatment both on texture and color at the best microbial inactivation process conditions.

MATERIALS AND METHODS

Sample preparation

Coconut fruit (*Cocos nucifera*) was purchased from a local market. The edible part of the food was cleaned, washed with water and cut in 2 grams pieces with a surface of about 1 cm^2. The coconut pieces were loaded in a multi – batch apparatus to obtain the inactivation kinetics.

CO_2 multi – batch apparatus

Supercritical carbon dioxide treatment was carried out in the multi-batch apparatus shown in Figure 1. The system consists of 10 identical reactors with an internal volume of 15 ml connected in parallel, so that each experimental run provides a set of experimental data taken at identical process conditions but different treatment times.

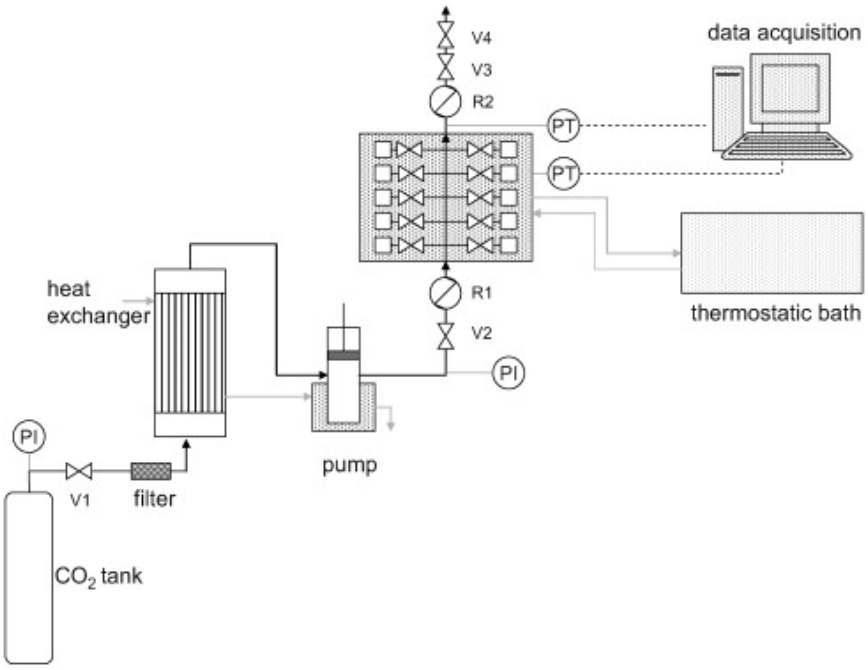

Figure 1 : Schematic of the multi-batch apparatus. V-1 to V-4: valves; R1, R2: Electrical resistance; PT: pressure transducer; PI: pressure manometer.

Each reactor is connected to an on-off valve that can be used to depressurize it independently from the others. The ten reactors are submerged in a single temperature-controlled water bath. Liquid CO_2 (Messer, Carbon dioxide 4.0, purity 99.990%) is fed into the reactors by a volumetric pump (LEWA, mod. LCD1/M910s). The apparatus is provided with a pressure transducer while one cover lid of the ten reactors is equipped with a fixed temperature probe (Pt 100 Ω). The operating parameters (temperature and pressure) are continuously recorded by a real time acquisition data system (NATIONAL INSTRUMENTS, field point FP-1000 RS 232/RS 485) and monitored by a specific software (LabVIEW ^{TM} 5.0).

Process conditions

The inactivation kinetics of the microflora naturally present in coconut were obtained with SC-CO_2 treatments performed at 12.0 MPa. Experimental results demonstrated that 12.0 MPa was the optimal pressure to induce a pasteurizing effect (data not shown). The effects of temperature from 35 up to 45°C and treatment time from 5 up to 60 minutes were studied.

Microbial analysis

Two grams of coconut were homogenized (1:3) with a phosphate buffer solution (PBS) in a Stomacher 400 (International P.B.I., Milano, Italy) at 230 rpm for 2 minutes. The homogenate was serially diluted in PBS for the enumeration of the natural microflora by plate count technique. Natural microflora was determined in terms of total aerobic count, total coliforms, and yeasts and molds. Depending on the expected counts the adequate decimal dilution was plated on Petri dishes containing Plate Count agar, Chromatic Coli/Coliform agar, and Yeast Glucose Chloramphenicol agar. The incubation temperatures and times were: 30°C for 48 h for aerobic count, 30°C for 24 h for total coliforms count, and 25°C for 4 days for yeasts and molds count. At the end of the incubation periods, the number of colonies was counted.
The inactivation level was determined by evaluating the Log(CFU/g) (logarithm of colony forming unit per gram of sample) of the microorganisms before and after the treatment. The results were means based on data from at least three experimental runs. Standard deviations were shown by error bars.

On – line color monitoring

The color appearance of the sample was measured on – line during as well as before and after the treatment with an innovative spectroscopic apparatus designed in the Department of Materials Engineering and Industrial Technology of the University of Trento. The principal element of the system was an optic probe (Ocean Optics Inc., Dunedin, FL) connected to a spectrophotometer (S2000 CCD, Ocean Optics Inc., Dunedin, FL) which transmitted the light from a halogen lamp to the sample and acquired the reflected light. The optical probe was fixed vertically on the cover lid of a CO_2 pressurized reactor with an internal volume of 310 ml. The color was continuously monitored during the supercritical CO_2 process by a specific software (Spectra Suite®, Ocean Optics, Dunedin, FL) providing L* (lightness), a* (redness) and b* (yellowness) color coordinates. Color measurements were performed in triplicate at the best inactivation conditions. Mean values and standard deviations were evaluated.

Texture measurement

An Instron Universal Testing Machine (Model 4502, Instron Corp., Canton, Mass, USA) was used to determine coconut texture. The samples were cut in cubes (12.0x12.0x12.0 mm) and 5 samples per treatment were used for the test. The firmness of the coconut was measured by driving a flat tip into the flesh at a speed of 1.3 mm/min. The amount of the maximum resistance to the compression (elastic modulus) of the samples was evaluated at the best microbial inactivation process conditions and expressed in kPa. The results were means based on data from five experimental runs. Standard deviations were shown by error bars.

RESULTS

Microbial inactivation kinetics

Figure 2 reports the effects of SC-CO_2 on the inhibition of aerobic microorganisms (a), total coliforms (b) and yeasts and molds (c). Kinetics obtained at 35°C show a low inactivation rate which takes 60 min to induce 3 Log(CFU/g) reductions of aerobic count and total coliforms and 2 Log(CFU/g) reductions of yeasts and molds.

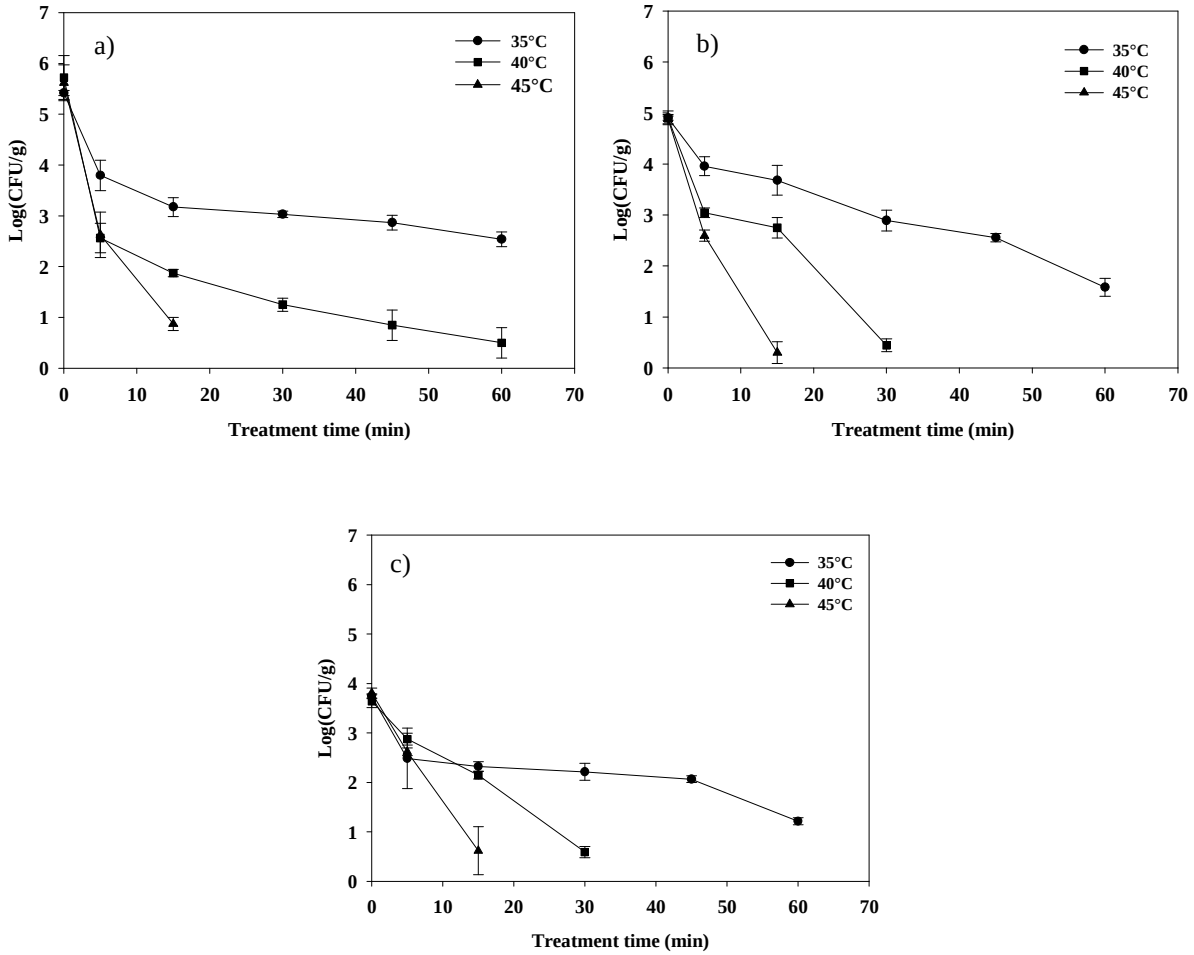

Figure 2 : Effect of SC-CO_2 treatment on: a) total aerobic count; b) total coliforms; c) yeasts and molds as a function of treatment time and temperature.

Increasing the temperature to 45°C, 15 min were sufficient to obtain 4 and 5 Log(CFU/g) reduction of aerobic count, and both total coliforms and yeasts and molds respectively. The results demonstrate that the temperature has a beneficial effect on the microbial inactivation rate: at low temperature longer treatment times are needed to obtain a high microbial reduction as demonstrated from the experimental data reported in Figure 2.
This fact is closely related to the CO_2 diffusivity in the liquid phase and to the fluidity of the cell membrane which both increase if the temperature is increased [8, 9, 10, 11].

On – line color measurements

The colorimetric results on – line acquired during the treatment performed at 12.0 MPa, 40°C, 30 min are reported in Table 1. The experimental data show no significant changes in a* and b* values. On the contrary, slight reductions in L* values are observed after the first minute of treatment highlighting a slowly lightness decrease of the sample during the process. The decrease of L* values is 3.72% and 7.58% during the on – line acquisition at 10 and 30 min of treatment time, respectively. Similar results are obtained during the treatment at 120 bar, 45°C and 15 minutes, the other process condition inducing a significant inactivation effect (data not shown).

Table 1 : On – line acquisition of color parameters during SC-CO_2 treatment performed at 12.0 MPa. 40°C, 30 min.

Treatment time (min)	L*	a*	b*
1	86.10±2.80	-0.73±0.51	-0.83±0.42
10	82.9±1.15	-0.63±0.67	-1.2±0.35
20	80.97±0.51	-0.61±0.69	-1.33±0.61
30	79.57±0.74	-0.67±0.81	-1.53±0.90

Color and texture measurements at the optimal process conditions

Table 2 reports the results of color parameters (L*, a*, b*) and texture (elastic modulus) for the control (untreated sample) and the sample after the treatment at 12.0 MPa, 40°C, and 30 min. Color results suggeste that while a* and b* are almost constant, L* slightly decreases after 30 minutes of treatment. However the overall color acceptability of the product is not affected by the treatment.

Table 2 : Measurements of color parameters for coconut before and after SC-CO_2 treatment at 12.0 MPa, 40°C and 30 min.

	L*	a*	b*	Elastic modulus (kPa)
Control	88.53±1.31	-0.2±0.66	-0.40±0.93	12.1±1.79
Treated sample	81.7±1.41	-0.53±1.18	-0.93±0.45	8.42±0.71

The experimental results of coconut texture demonstrate that the treatment induces a slight decrease of the elastic modulus of the sample (Table 2). This loss of consistency is justified

considering some liquids loss from the sample observed after the treatment. However the fruit still maintains its structure after the treatment. Similar experimental results for color and texture are obtained during the treatment at 120 bar, 45°C and 15 minutes (data not shown).

Published works reported that SC-CO_2 treatment has some limits when applied to fruits with a soft structure. Valverde et al. [12] demonstrated that pears treated with pressurized CO_2 lost their consistency and this loss was higher as pressure was increased. Haas et al. [13] reported similar findings for strawberries and melon treated with SC-CO_2. This negative aspect was related to the physical damage induced by the pressure which provoked a consistency loss manifested as a softer aspect and a loss of liquid from the product.

CONCLUSION

The present study clearly demonstrates the potential of the SC-CO_2 treatment for the pasteurization of fresh cut pieces of coconut.
The experimental findings show that: 1. temperature and treatment time influence the inactivation rate of natural microflora: high temperatures are required to decrease the treatment time and increase the microbial reduction; 2. color measurements during (on – line) and after the treatment reveal no significant changes of the color of the sample; 3. the SC-CO_2 process has great potential in the pasteurization of products where a firm texture is not essential, such as fruit cocktails, creams, juices, or fruits with a rigid structure.

ACKNOWLEDGMENTS

The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement no. 245280, also known under the acronym PRESERF.

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