Effect of Supercritical Carbon Dioxide Pasteurization on Natural Microbiota, Texture, and Microstructure of Fresh-Cut Coconut

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Abstract: The objective of the present study was the evaluation of the effectiveness of supercritical carbon dioxide (SC-CO_2) as a nonthermal technology for the pasteurization of fresh-cut coconut, as an example of ready-to-eat and minimally processed food. First, the inactivation kinetics of microbiota on coconut were determined using SC-CO_2 treatments (pressures at 8 and 12 MPa, temperatures from 24 to 45 °C, treatment times from 5 to 60 min). Second, the effects of SC-CO_2 on the hardness and microstructure of fresh-cut coconut processed at the optimal conditions for microbial reduction were investigated. SC-CO_2 treatment of 15 min at 45 °C and 12 MPa induced 4 log CFU/g reductions of mesophilic microorganisms, lactic acid bacteria, total coliforms, and yeasts and molds. The hardness of coconut was not affected by the treatment but the samples developed an irregular and disorderly microstructure. Results suggested the potential of SC-CO_2 in preserving fresh-cut fruits and ready-to-eat products.

Keywords: fresh-cut coconut, mild pasteurization, ready-to-eat products, supercritical carbon dioxide

Practical Application: The effectiveness of SC-CO_2 as a nonthermal technology for the pasteurization of fresh-cut coconut was studied. The results demonstrated the possibility to apply the treatment as a method to induce the inactivation of the natural microorganisms, preventing the microbial spoilage and, at the same time, preserving the hardness of the product. The available data will give a valuable input to the fresh-cut fruits industry.

Introduction

The market sales of ready-to-eat (RTE) fresh fruits have been growing rapidly in recent decades as a result of changes in consumer attitudes demanding fresh, healthy, and safe foods. The International Fresh-Cut Produce Association defines fresh-cut fruits or vegetables as products trimmed, peeled, or cut into 100% usable product bagged or prepackaged to offer the consumer a food with high nutrition, convenience, and flavor although maintaining its freshness (Lamikanra 2002); however, it is well known that the steps for RTE preparation may promote physiological deteriorations, biochemical changes, and a favorable environment for proliferation of spoilage microorganisms, even when used with high carefulness during all the production steps (O'Beirne and Francis 2003). Coconut (*Cocos nucifera L.*) is one of the 10 most exploited trees in the world as primary source of food, drink, and shelter. The white meat (flesh) of the nut can be eaten either raw or shredded and dried as an ingredient in a wide variety of foods from cakes to beverages. After harvesting, fresh coconuts can be stored at temperatures ranging from 0 to 15 °C and relative humidity of 75% or less, for 1 to 2 mo. After deshelling, coconuts require protection from oxygen that causes rancidity of the lipid content. The white meat has a low acidity (about pH 6) and is susceptible to microbial spoilage (Sinigaglia and others 2003). Similar to other fruits that are consumed raw, coconut can be a source of pathogenic or spoilage bacteria (Strawn and others 2011). Therefore, new sanitizing methods to retard the spoilage and extend shelf-life are being used. These can be classified as: (1) chemical-based washing treatments [that is chlorine dioxide (Seymour 1999), organic acids such as lactic acid, citric acid, acetic acid, or tartaric acid (Uyttendaele and others 2004; Bari and others 2005), hydrogen peroxide (Juven and Pierson 1996), calcium-based solutions (Suutarinen and others 1999; Rico and others 2006), ozone (Kim and others 1999; Grass and others 2003), and electrolyzed water (Koseki and others 2001)]; (2) physical treatments (that is modified atmosphere packaging (Saltveit 1993; Sinigaglia and others 2006), irradiation (Foley and others 2004; Goularte and others 2004), ultraviolet light (Allende and Artes 2003), and high pressure processing (Palou and others 2000)].

Recently, carbon dioxide (CO_2) at high pressures (7.0 to 30 MPa) or in supercritical phase (above 31 °C, 7.3 MPa) has been investigated as a novel nonthermal technology for pasteurization or sterilization of foodstuffs (Spilimbergo and others 2002); it has been shown that this technology can be successful in the inactivation of microorganisms and enzymes. So far, research has been mainly focused on the applicability of the treatment to culture media or liquid foods with less attention on pasteurization of fresh-cut fruits and vegetables (Kuhne and Knorr 1990; Hong and Park 1999; Zhong and others 2008; Ferrentino and Spilimbergo 2011). Haas and others (1989) reported on the preservative action of SC-CO_2 in delaying surface molding of strawberries. Valverde and others (2010) achieved total inactivation (5 log reductions) of

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Saccharomyces cerevisiae spiked on pear pieces at 55 °C and relatively low pressure (<6 MPa) and exposure times (10 min).

To this extent, the objective of the present study was to evaluate the effectiveness of SC-CO_2 as a nonthermal method for preserving fresh-cut coconut. Specifically, the aims of this work were: (1) to evaluate the efficacy of the treatment in reducing the microbiota (total mesophilic count, lactic acid bacteria, total coliforms, and yeasts and molds) naturally occurring in fresh-cut coconut; (2) to optimize the process parameters (pressure, temperature, and time) to achieve a sufficient degree of inactivation (approximately 4 log reductions); and (3) to evaluate the effects of SC-CO_2 on texture and microstructure of fresh-cut coconut after the treatment at the optimal process conditions.

Materials and Methods

High-pressure CO_2 apparatus

Supercritical CO_2 treatments were carried out in a multibatch laboratory-scale apparatus (Figure 1). The system consisted of 10 identical reactors with an internal volume of 15 mL connected in parallel, so that each experimental run provided a set of experimental data taken at identical process conditions but different treatment times. Each reactor was connected to an on–off valve that could be used to depressurize it independently from the others. The 10 reactors were submerged in a single temperature-controlled water bath to keep a constant and uniform temperature. Liquid CO_2 (CO_2 4.0, purity 99.990%, Messer Group GmbH, Germany) was fed into the reactors by a volumetric pump (mod. LCD1/M910s, LEWA GmbH, Germany) after passing through a cooling bath containing a solution of water (50%) and antifreeze (50%) to reach low temperatures (approximately –3.0 °C). The apparatus was provided with a pressure transducer whereas 1 coverlid of the 10 reactors was equipped with a fixed temperature probe (Pt 100 Ω, Endress+Hauser, Milan, Italy). The operating parameters (temperature and pressure) were continuously recorded by a real time data acquisition system (field point FP-1000 RS 232/RS 485, National Instruments, Austin, Tex., U.S.A.) and monitored by a specific software (LabVIEW$^{T.M}$ 5.0). After each experimental run, the reactors were washed with water and sterilized in autoclave (121 °C, 15 min) to prevent possible contamination although CO_2 was flushed at 6 MPa through all tubes to ensure a good level of cleaning. More details of the pasteurization procedure could be found elsewhere (Ferrentino and others 2011).

Fresh-cut coconut preparation

Fresh coconuts (*C. nucifera*) were purchased from a local supermarket, deshelled, cleaned, washed, and manually cut into 2 g cubes. Samples were subjected to SC-CO_2 treatments performed at 8 or 12 MPa, temperatures ranging from 24 to 45 °C for treatment times from 5 to 60 min. After the treatment, the samples were collected in a sterile environment and microbiologically analyzed.

Microbiological analysis

The microbial count, before and after the treatment, was performed by the standard plate count method. The samples were prepared and mixed with 8 mL of phosphate buffer solution (PBS, 0.01 M, pH 7.4) in a 250 mL sterile bag and stomached at 230 rpm for 2 min (Stomacher 400, International P.B.I., Milano, Italy). The homogenate was serially diluted in PBS and plated in duplicate onto selective media: mesophilic and psychrophilic microorganisms, total coliforms, yeasts and molds, and lactic acid bacteria were plated onto plate count agar (Liofilchem, TE, Italy), chromatic coli/coliform agar (Liofilchem), yeast glucose chloramphenicol agar (Liofilchem), and MRS agar (de Man, Rogosa and Sharpe, Oxoid, Milano, Italy), respectively. The incubation temperatures and times were: 30 °C for 48 h and 4 °C and 7 d for mesophilic and psychrophilic microorganisms, respectively; 30 °C for 24 h for total coliforms; 25 °C for 4 d for yeasts and molds; and 35 °C for 48 h for lactic acid bacteria. At the end of the incubation periods, the number of colonies was counted and expressed as colony forming units (CFU) per g; in the results section the microbial concentrations were expressed as log N compared with time, where N (CFU/g) was the number of cells per g of

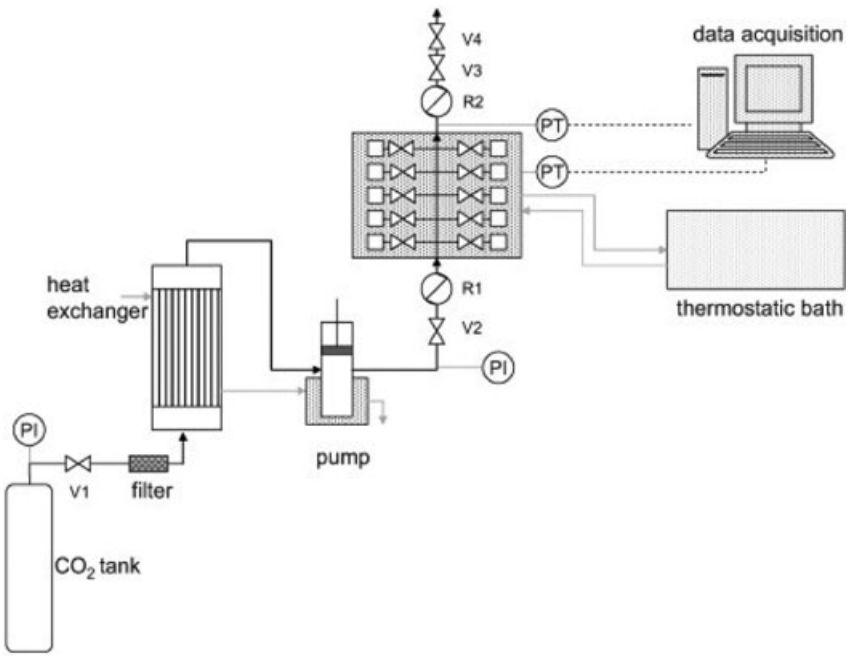

Figure 1–Schematic of the multibatch apparatus. V-1 to V-4 = valves; R1, R2 = electrical resistance; PT = pressure transducer; PI = pressure manometer.

sample. Three independent experiments were carried out for each single experimental condition and the results were calculated as mean value. Standard deviations were shown by error bars in the graphs.

Texture analysis

An Instron universal testing machine (Model 4502, Instron Corp., Canton, Mass., U.S.A.) equipped with a 10 kN cell was used to determine the texture of the samples at room temperature. The coconut pieces, 12 mm in height with a surface of 144 mm^2, were placed on the lower compression plate. Compression was performed by a flat upper plate tip (with a surface area of 7.1×10^4 mm^2) at a cross head speed of 1.3 mm/min. Load and displacement were continuously monitored during the tests and the hardness of the samples was conventionally defined as the peak force at 20% strain. Texture measurements were performed in triplicate and mean values and standard deviations were evaluated.

Environmental scanning electron microscopy (ESEM)

The microstructure of coconut tissue was observed using an environmental scanning electron microscope (Philips XL30, SEM, SEMTech Solutions, Inc., North Billerica, Mass., U.S.A.) with a tungsten filament, equipped with a Peltier cooling stage. Water vapor was used as the imaging gas, with constant pressure of approximately 6 Torr to control the dehydration process. The temperature was maintained at 11 °C during pumpdown and the duration of the whole experiment. The microscope operated at 15.0 kV. Photographs were obtained at 300X magnification.

Image analysis

Image analysis software LensEye (Balaban M. O., Gainesville, Fla., U.S.A.), developed in Visual Basic for Windows (Microsoft, Redmond, Wash., U.S.A.), was used to measure the nonuniformity of the micrographs intensity obtained from ESEM. The possibility to quantify this attribute allowed measuring the change of samples texture, where in this context "texture" was defined as how "varied" or "patchy" the surface looks (Rallabandi and Sett, 2005). The software identified every pixel in the image with intensity attributes lower than, or higher than a given threshold value. Once these pixels were identified and counted, their percentage and the defective areas (that is example dark spots) were estimated (Balaban 2008; Balaban and others 2008).

Table 1–Natural microbial contamination of fresh-cut coconut.

Mesophilic microorganisms	Total coliforms	Yeasts and moulds	Psychrophilic bacteria	*Lactobacillus* spp.
log CFU/g				
5.21 ± 0.21	4.90 ± 0.13	3.74 ± 0.05	ND	3.36 ± 0.05

Values are means ± standard deviations of 3 samples. ND = not detected.

Statistical analysis

Experimental data were analyzed by one-way analysis of variance and significant differences between means were calculated by the least significant difference test. Data were processed using the software Statistica for Windows (StatSoftTM, Tulsa, Okla., U.S.A.).

Results and Discussions

Microbial inactivation study

To study the applicability of SC-CO_2 treatment for the inactivation of the microbiota naturally occurring in fresh-cut coconut, the initial microbial contamination of the product was determined. From the results reported in Table 1, it was evident that the food was mainly contaminated by mesophilic bacteria (5.21 ± 0.21 log CFU/g), total coliforms (4.90 ± 0.13 log CFU/g), yeasts and molds (3.74 ± 0.05 log CFU/g), and lactic acid bacteria (3.36 ± 0.05 log CFU/g) although total psychrophilic microorganisms were not detected. The amount of mesophilic bacteria on fresh-cut coconut has also been determined by Sinigaglia and others (2003) who reported a contamination of approximately 4 log CFU/g, in agreement with our results. It is worth noting that the number and kind of microorganisms associated with fresh-cut products are highly variable: mesophilic bacteria typically detected from plate count studies range from 3 to 9 log CFU/g together with lactic acid bacteria and yeasts which cause spoilage of the product characterized by brown or black discoloration, production of off-odors, loss of texture, and soft rot (Jennylynd and Tipvanna 2010).

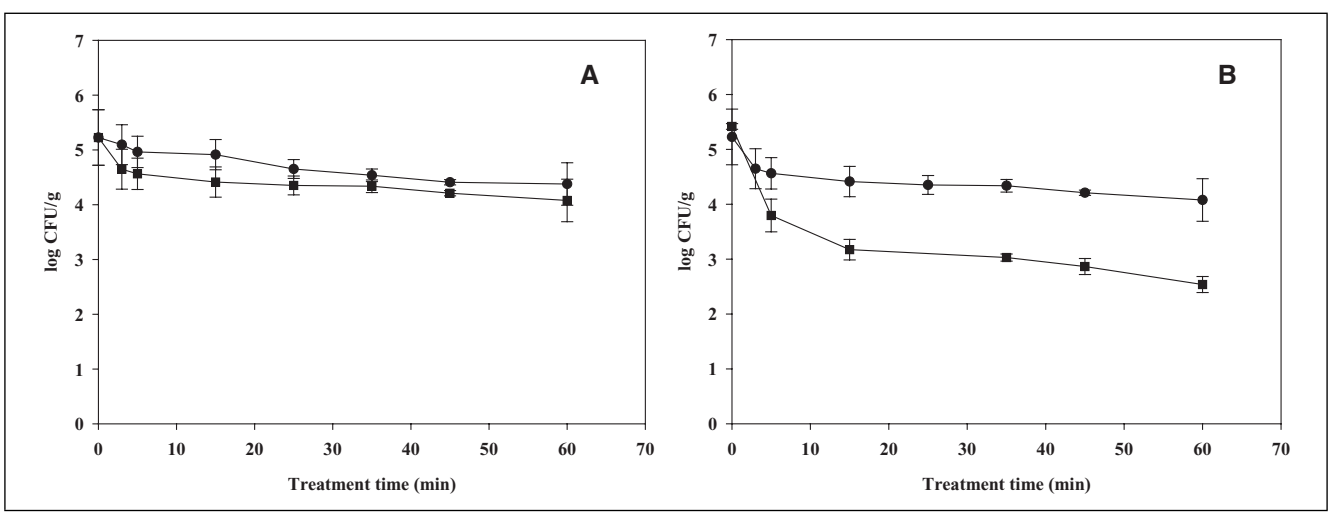

Figure 2–Effect of SC-CO_2 treatments on the reduction of mesophilic microorganisms. Experiments performed at ●, 8 MPa and ■, 12 MPa as a function of treatment time and temperature: 24 °C (a) and 35 °C (b).

Effect of pressure

Figure 2 showed the degree of inactivation of mesophilic microorganisms after SC-CO_2 treatment performed at 24 °C (Figure 2a) and 35 °C (Figure 2b) as a function of treatment time and pressure. The mesophilic survival slightly decreased as pressure increased; this effect was more evident at 35 °C compared to 24 °C: after 60 min of treatment 1.5 log and 2.5 log reductions were obtained at 8 MPa and 12 MPa, respectively whereas at 24 °C the inactivation degree almost remained constant at both pressures after the same treatment time. This behavior could be probably related to the different CO_2 phase which is subcritical at 24 °C and supercritical at 35 °C. Under supercritical conditions, an increase in pressure corresponds to a drastic increase in density and solvation power (Brunner 2005), which could result in a volumetric expansion of the double phospholipidic layer of the microbial membrane, and thus in the removal of vital constituents from cells or cell membranes leading to a faster microbial death (Garcia-Gonzalez and others 2007).

From Figure 2, it could be noted that an increase of treatment time, from 5 to 60 min at 24 °C (Figure 2a) and from 15 to 60 min at 35 °C (Figure 2b), did not induce a significant microbial reduction and the inactivation curve reached a plateau. The behavior could be explained considering the existence of a fraction in the microbial cells population more resistant to the treatment: once the most sensitive cells fraction was inactivated, the microbial reduction ended and the most resistant fractions survived, unless more intense processing conditions were applied.

Further experiments were performed increasing the pressure from 12 to 14 MPa at 35 °C, but no significant variations of the inactivation rates of mesophilic count on coconut were observed for all the treatment times tested (data not shown). Similar conclusions were drawn also in other studies for both liquid (Sims and Estigarribia 2003) and solid substrates (Valverde and others 2010). The results were justified considering that above 10 MPa, the solubility of CO_2 is a weak function of the pressure (Dodds and others 1956). Spilimbergo and others (2005) demonstrated that an increase of pressure from 10 to 30 MPa at 55 to 60 °C did not influence appreciably the solubility of CO_2 in water with no effect on the acidification of the external medium as well as on the CO_2 contact with the cells. From an economical point of view, it is important to keep the pressure at the minimum value to reduce both the operation and installation costs.

Effect of temperature

At a constant pressure of 12 MPa, Figure 3 reported the effects of increasing the temperature on the inhibition of mesophilic microorganisms (a), total coliforms (b), yeasts and molds (c), and lactic acid bacteria (d). Compared to higher temperatures, kinetics obtained at 35 °C showed a low inactivation rate: 60 min were needed to reduce mesophilic microorganisms from 5.41 ± 0.052 to 2.54 ± 0.15 log CFU/g, total coliforms from 4.90 ± 0.13 to

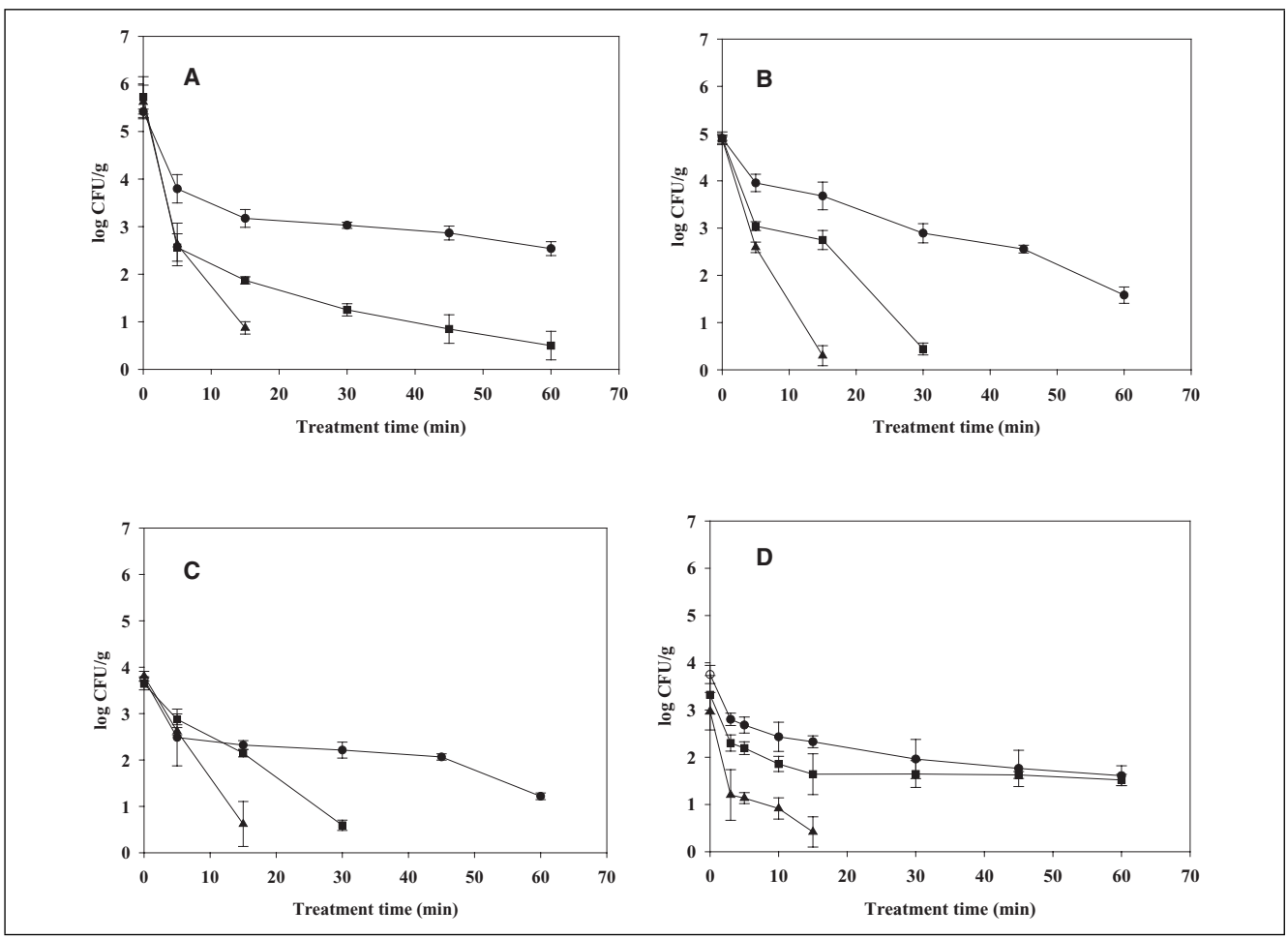

Figure 3–Effect of SC-CO_2 treatment at 12 MPa on: (a) mesophilic microorganisms; (b) total coliforms; (c) yeasts and molds; and (d) lactic bacteria as a function of treatment time and temperature (●, 35 °C; ■, 40 °C; ▲, 45 °C).

1.58 ± 0.17 log CFU/g, yeasts and molds from 3.74 ± 0.05 to 1.22 ± 0.07 log CFU/g, and lactic acid bacteria from 3.75 ± 0.19 to 1.61 ± 0.21 log CFU/g. When the temperature was increased to 45 °C, 15 min were sufficient to further reduce mesophilic microorganisms (0.87 ± 0.13 log CFU/g), total coliforms (0.30 ± 0.11 log CFU/g), yeasts and molds (0.62 ± 0.05 log CFU/g), and lactic acid bacteria (0.42 ± 0.32 log CFU/g).

According to several studies, the increase of temperature in the SC-CO_2 process has a beneficial effect on the inactivation rate of microbial species. The treatment temperature is closely related to the characteristics of CO_2 mass transfer: higher temperatures increase the CO_2 diffusivity and stimulate the fluidity of the cell membrane to make its penetration easier (Hong and others 1997; Erkmen 2001; Choi and others 2009). The beneficial effect of temperature on microbial inactivation has also been observed in other studies dealing with SC-CO_2 application to solid foods (Choi and others 2009; Jung and others 2009; Valverde and others 2010). Choi and others (2009) investigated the inhibition of foodborne pathogenic bacteria spread on pork marinated in soy sauce processed with SC-CO_2. It was demonstrated that the levels of *S. typhimurium* and *E. coli* O157:H7 were reduced more rapidly at 45 °C than at 40 °C. Jung and others (2009) applied the SC-CO_2 process for the decontamination of alfalfa sprouts from *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium*. Their published results confirmed that *E. coli* O157:H7 levels at 15 MPa were more rapidly reduced at 45 °C than at 35 °C or 40 °C for the same treatment time and that same effects were observed on *L. monocytogenes* and *S. typhimurium*. The results of the present study appeared to support these findings.

Indeed, when SC-CO_2 process was performed at 40 and 45 °C, significant increases were observed in the reduction of mesophilic microorganisms, total coliforms, and yeasts and molds for the tested treatment time range. A different behavior was observed for lactic acid bacteria: the inactivation curves at 40 and 35 °C were overlapped and did not significantly decrease with time after the first 10 min of treatment. The inactivation level remained almost constant (approximately 2 log reductions at 35 and 40 °C), and reached a plateau, even though the treatment was extended to 60 min, showing the so-called "tailing behavior" (Figure 3d). The different response to the treatment could be related to the fermentative metabolism of lactic acid bacteria and to their ability to grow under anaerobic conditions (Escalona and others 2005). At 45 °C, the tailing behavior disappeared probably because of the sensitizing effect of temperature on microorganisms.

Experimental results, showing the different inactivation kinetic of aerobic and anaerobic microorganisms when treated with CO_2, have been already published for liquid suspensions. Hong and Pyun (1999) achieved 5 log reductions of *Lactobacillus plantarum* after 50 min of treatment at 8 MPa and 30 °C while 5 min were needed by Spilimbergo and others (2005) to inactivate the same amount of *Bacillus subtilis*, a typical aerobic microorganism, at the same process conditions.

It is worth noting that in solid phase, CO_2 is directly in contact with the microorganism, and it does not need to solubilize into the liquid phase—the limiting step concerning the inactivation mechanism in liquid products—before performing its bactericidal action against microbes, and thus the required exposure time is essentially dependent on the specific resistance of the microbial target strain. As shown in several studies regarding SC-CO_2 pasteurization of liquid substrates, yeasts and molds and coliforms are more sensitive than mesophilic microorganisms and lactic acid bacteria to SC-CO_2 (Del Pozo-Insfran and others 2006; Liao and others 2010). Our study confirmed these data.

On the basis of the results obtained, the optimal process conditions chosen were 12 MPa, 40 °C, and 30 min, and 12 MPa, 45 °C, and 15 min. At 12 MPa, 40 °C, and 30 min, the microbial final counts achieved corresponded to 1.25 ± 0.13 log CFU/g for mesophilic microorganisms, 0.44 ± 0.13 log CFU/g for total coliforms, 0.59 ± 0.11 log CFU/g for yeasts and molds, and 1.65 ± 0.28 log CFU/g for lactic acid bacteria; whereas at 12 MPa, 45 °C, and 15 min, 0.87 ± 0.13 log CFU/g for mesophilic microorganisms, 0.30 ± 0.21 log CFU/g for total coliforms, 0.62 ± 0.49 log CFU/g for yeasts and molds, and 0.42 ± 0.32 log CFU/g for lactic acid bacteria. It must be highlighted that the above microbial reductions meet the guidelines for the microbiological safety and quality of RTE fruits (De Louvois and others 2000; Commission Regulation 2005) which considers a product to be satisfactory with less than 1.3 log CFU/g of total coliforms. No limits are reported for mesophilic microorganisms, yeasts and molds, and lactic acid bacteria; however, considering that these microorganisms are responsible for the spoilage of the

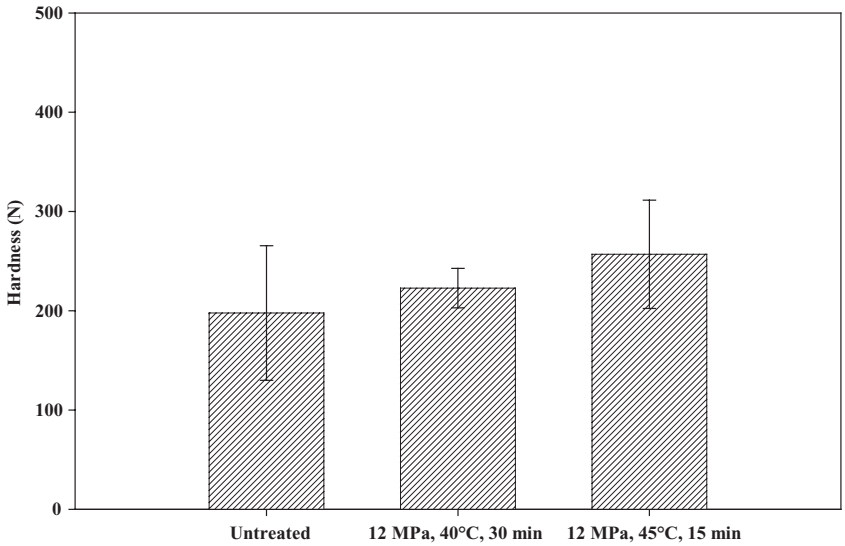

Figure 4–Hardness of fresh-cut coconut before (untreated) and after SC-CO_2 processes.

product, other criteria of acceptability must be taken into account such as appearance, smell, and texture.

Texture

As shown in Figure 4, no significant effects of SC-CO_2 treatment on the hardness were observed ($P < 0.05$): the fruit maintained the same consistency compared to the fresh untreated coconut. Published works reported that SC-CO_2 has some limits when applied to fruits with a soft structure. Valverde and others (2010) demonstrated that pears treated with pressurized CO_2 lost their consistency and this loss was higher as pressure was increased. Haas and others (1989) reported similar findings for strawberries and melon treated with SC-CO_2. This negative aspect was related to the physical damage induced by pressure resulting in a loss of consistency, a softer tissue, and a release of liquid from the product.

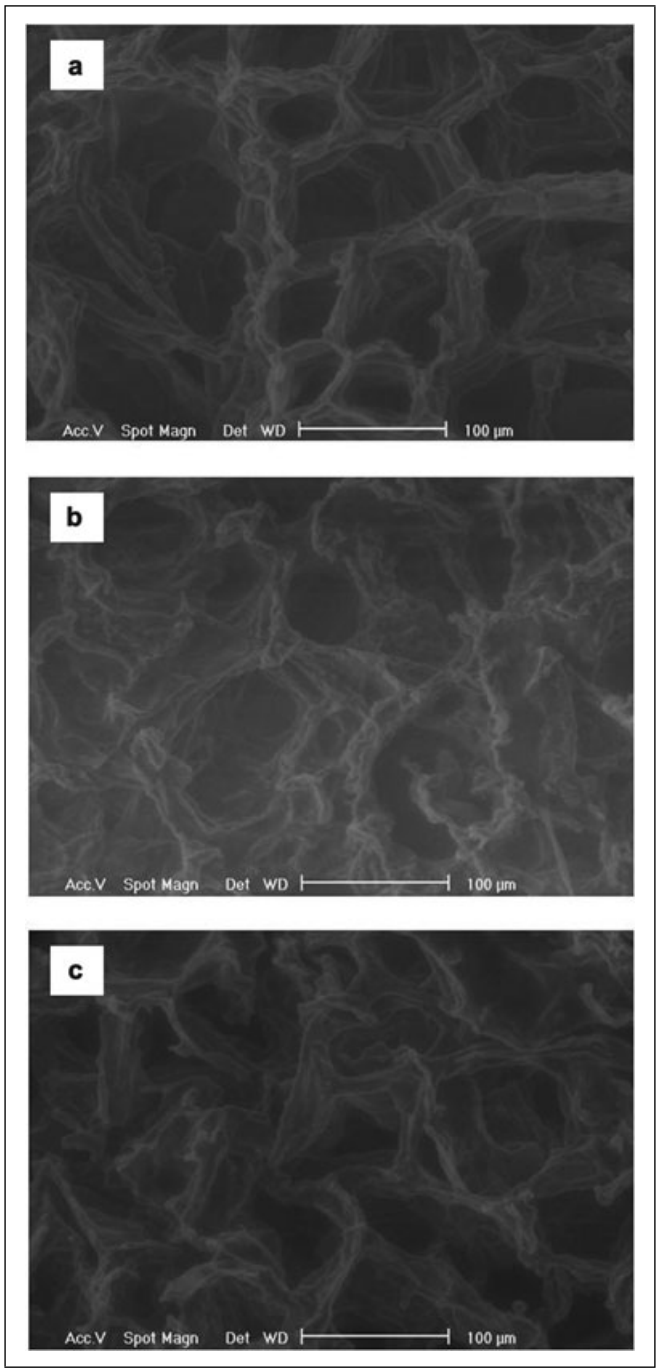

Figure 5–Effect of SC-CO_2 on the microstructure of fresh-cut coconut: (a) untreated, (b) treated at 12 MPa, 40 °C, 30 min, and (c) treated at 12 MPa, 45 °C, 15 min. Environmental scanning electron micrographs of cells tissue.

ESEM observations

The effects of SC-CO_2 treatment performed at 12 MPa, 40 °C, and 30 min and 12 MPa, 45 °C, and 15 min on the cell structure of coconut were shown in Figure 5b and c. For comparison, a micrograph of fresh-cut coconut was also shown (Figure 5a). In both treated coconut pieces, a disorder of the tissue was evident but no differences were detected between the two process conditions tested. The tissue was composed of a patchwork of groups of well-preserved cells and layers of collapsed, sunken cells. The analyses performed with the LensEye software confirmed the significant differences detected between the fresh and the SC-CO_2 treated coconut samples: a texture index equal to 1.56 ± 0.68 was evaluated for the fresh coconut although 0.56 ± 0.48 and 0.60 ± 0.12 were the values obtained for the coconut treated at 12 MPa, 40 °C, and 30 min and 12 MPa, 45 °C, and 15 min, respectively. The authors were unaware of any previous publication to quantify the effects of SC-CO_2 treatment on the microstructure of plant tissue.

Conclusions

The present study demonstrated the potential of the SC-CO_2 for the pasteurization of fresh-cut coconut. The treatment significantly reduced total mesophilic microorganisms, total coliforms, yeasts and molds, and lactic acid bacteria naturally detected on fresh coconut. Temperature and treatment time influenced the inactivation rate of all the microbial species: high temperatures were required to decrease the treatment time and increase the microbial reduction. The hardness of coconut pieces was maintained after the process, suggesting that the treatment had a great potential if applied to products with a firm texture or rigid structure. Some negative effects were observed on the microstructure of coconut tissue at both conditions chosen as optimal for microbial reduction. Further studies are needed to investigate the effect of SC-CO_2 on the quality, nutritional, and long-term stability of fresh-cut coconut as well as sensorial evaluations are necessary to verify the acceptability of the product by the consumers in view of a possible industrial application of the process.

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