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High Power Ultrasound Assisted High Pressure Carbon Dioxide Pasteurization of Fresh Cut Coconut

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The feasibility of a combined high pressure carbon dioxide and high power ultrasound (HPCD+HPU) treatment was tested for the pasteurization of fresh cut coconut.
Inactivation kinetics of the natural microbial flora were determined at 12 MPa and 10 W delivered every 2 min of treatment as a function of temperature (24 and 35 °C) and time (from 5 up to 30 min). *Salmonella enterica* was also spiked on fresh cut coconut and inactivation kinetics were obtained at the same process conditions as a function of temperatures (40 and 50 °C) and times (from 3 up to 30 min). Additionally, the quality attributes of the product were measured after HPCD+HPU and during a refrigerated shelf-life of 4 weeks.
The results revealed that the combined treatment increased microbial inactivation rates: *S. enterica* was reduced to undetectable levels at 12 MPa, 40 °C, 20 min while just 4 Log reductions were achieved when HPCD was performed alone. Inactivation of the natural microbial flora was obtained at milder conditions compared to the optimal ones identified for HPCD alone: 35 °C, 12 MPa for 30 min assured inactivation to undetectable level even of mesophilic microorganisms, the most resistant strain. Total acidity, and pH attributes were stable while slight differences were observed for color and texture. The results showed the feasibility and the potential of HPCD+HPU as an innovative non-thermal pasteurization technology of fresh cut fruits.

1. Introduction

Fresh-cut coconut, prepared to be eaten and served as a snack, is gaining a great appeal; however, the operations for its production such as cutting, and washing could fasten the microbial growth shortening its shelf-life in comparison with the fresh product. To avoid or retard microbial spoilage of the product reducing or minimally compromising its quality aspects, several treatments are currently under investigation such as: sodium chloride (Tatsumi et al., 1993), steam blanching (Howard et al., 1994), immersion in acid or basic solutions (Bolin, 1992), use of modified atmosphere packaging (Amanatidou et al., 2000).
Recently, high pressure carbon dioxide (HPCD) has been increasingly investigated as a novel non-thermal technology for the pasteurization of foodstuffs. It has been demonstrated that the treatment successfully inactivate microorganisms with no significant effects on food quality attributes (Ferrentino and Spilimbergo, 2011). However, although the experimental results demonstrated the efficiency of the process, long treatment times and temperatures were often needed to guarantee the safety and stability of some food products (García-González et al., 2007). Accordingly, the scientific interest towards combining HPCD with other low-temperature techniques is increasing (Ortuño et al., 2013). High Power Ultrasound (HPU) at low frequencies (20 to 100 W) has the potential to be used for the inactivation of bacterial populations. Ultrasound is known to have a significant effect on the velocity of the food industry processes involving heat and mass transfer. There are many potential applications in food processing, such as extraction, filtration, extrusion, freezing or crystallization. The application of ultrasound in food preservation processing is relatively recent. An effective microbial inactivation can be achieved by combining ultrasound with either heat (thermosonication), or pressure (manosonication) or with both (manothermosonication) increasing its efficiency with respect to the

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treatment time and energy consumption, compared to each individual treatment. However, the main drawback of the treatment is the transmission of the acoustic wave from the emitter's surface to the sample. The air, in fact, is a high attenuating medium that absorbs the acoustic energy preventing its transfer to the solids to be treated. In addition, the high impedance difference between the solid surface of the emitters and the air, and between the air and the solid samples, produces the reflection of a high proportion of the generated acoustic signal (García-Pérez et al., 2006). The use of a "dense fluid", such as CO_2 at high pressure, as a transmission medium can overcome this problem, allowing the application of the technology to solid products. Literature results demonstrated the efficiency of the simultaneous application of HPCD+HPU for microbial inactivation in liquid (Ortuño et al., 2012) and solid foods (Spilimbergo et al., 2014). The present research focuses on the possibility to apply a combined HPCD+HPU treatment for the pasteurization of fresh cut coconut. Inactivation of the natural microbial flora and *Salmonella enterica* spiked on the surface of the product were obtained aiming to understand the effect of the process parameters and to study the efficiency of the combined treatment. Furthermore, the microbial and quality (pH, total acidity, texture and color) stability of HPCD+HPU treated fresh cut coconut was also investigated during a refrigerated shelf-life of 4 weeks and compared to the fresh untreated product.

2. Materials and methods

2.1 Fresh cut coconut preparation

Coconut fruit (*Cocos nucifera*) was purchased from a local market. The edible part of the food was cleaned, washed with water, cut in 2 g pieces (with a surface of about 1 cm^2). After these operations, the natural microbial flora of the product was about 6.12±0.77 Log(CFU/g) of mesophilic microorganisms, 4.10±0.10 Log(CFU/g) of lactic acid bacteria, 5.16±0.70 Log(CFU/g) of total coliforms, and 3.90±0.43 Log(CFU/g) of yeasts and molds.

2.2 Sample contamination

A fresh colony of *Salmonella enterica* ATCC 14023 (DSMZ, Braunschweig, Germany) strain was used for fresh cut coconut contamination. The microbial culture was grown on solid Luria-Bertani (LB) agar medium (Sigma-Aldrich Co., Milan, Italy) at 37 °C for 16 h. One colony was picked up and inoculated into 10 mL of LB medium. Bacterial culture was incubated at 37 °C with constant shaking (200 rpm) to stationary phase (16 h). Cells were collected by centrifugation at 6,000 rpm for 10 min and re-suspended in 5 mL of phosphate buffered saline solution (PBS, Sigma-Aldrich Co., Milan, Italy) to reach a final concentration of about 10^9 CFU/mL. The 2 g pieces of fresh cut coconut were spiked with 50 µL of microbial suspension reaching a concentration of about 10^8 CFU/g. The samples were left 1 h in a sterile chamber at room temperature to let the microorganisms absorb on the surface and then were treated with HPCD, HPU, and combined HPCD+HPU processes.

2.3 HPCD apparatus

HPCD treatment was carried out in a multi-batch apparatus deeply described in a previous paper published (Ferrentino et al., 2012).

2.4 HPCD+HPU apparatus

The apparatus consisted in a sapphire high pressure visualization cell (Separex S.A.S., France) with an internal volume of 50 mL, designed to withstand up to 40 MPa and 100 °C. The plant included a CO_2 tank, kept at room temperature, a chiller reservoir, a HPLC pump, and a thermostatic bath to keep the inactivation vessel at the desired temperature. The apparatus was equipped with an ultrasound system (Aktive Arc Sarl, Switzerland) designed on purpose and embedded in the HPCD plant. The HPU system consisted in a transducer, a buster, a special retainer (M36x1.5), a sonotrode and a power generator unit (40 W and 30 KHz). For the experiments with HPU, the ultrasound unit was turned on (time zero) when the desired pressure and temperature were reached in the vessel. Pressure and temperature were kept constant during the experiment through the pump and the thermostatic bath, respectively. The treated samples were collected in individual sterile tubes for microbial analyses. The vessel was cleaned and disinfected with ethanol (96 % v/v) after each sampling.

2.5 Microbial analyses

The standard plate count method was used to determine the initial microbial load and the effectiveness of the treatments in reducing the number of the natural microbial flora (mesophilic microorganisms, total coliform bacteria, lactic acid bacteria, and yeasts and molds) and *S. enterica* spiked on the sample surface. After each treatment, fresh cut coconut were prepared and mixed with 4 mL of PBS in a sterile vial, which was stomached at 230 rpm for 2 min (Stomacher 400; International P.B.I., Milan, Italy). The homogenate was

serially diluted in PBS and plated in duplicate onto selective media containing Plate Count agar for the detection of mesophilic microorganisms, Chromatic Coli/Coliform agar for the detection of total coliforms, MRS (de Man, Rogosa and Sharpe) agar for the detection of lactic acid bacteria, Yeast Glucose Chloramphenicol agar for the detection of yeasts and molds, and Chromatic Salmonella for the detection of *S. enterica*. The incubation temperatures and times were: 30 °C for 48 h for mesophilic microorganisms, 30 °C for 24 h for total coliforms, 35 °C for 48 h for lactic acid bacteria, 25 °C for 4 days for yeasts and molds, and 37 °C for 24 h for *S. enterica*. At the end of the incubation periods, the number of colonies was counted. The degree of inactivation was determined by evaluating the $\text{Log}(N/N_0)$ versus time, where N_0(CFU/g) was the number of microorganisms initially present in the untreated sample and N(CFU/g) was the number of survivors after the treatment. The detection limit of the standard plate count method was of 30 CFU/g. The results were means based on data from at least three experimental runs. Standard deviations were shown by error bars.

2.6 Shelf-life study

Fresh cut coconut samples processed with HPCD+HPU combined treatment at the optimal conditions were stored at 4 °C for 4 weeks. During the shelf-life, the following parameters were monitored: the natural microbial flora content, *Salmonella enterica* load, pH, total acidity, color and texture. Same parameters were also monitored for the untreated sample stored at 4 °C for 4 weeks as a control.

2.7 Total acidity and pH

The sample was homogenized with 2 mL of distilled water and the pH was measured using a digital pH meter (Eutech Instruments, Nijkerk, The Netherlands), after calibration with commercial buffer solutions at pH 7.0 and 4.0 (Crison, Vetrotecnica s.r.l., PD, Italy). The measurements were performed in triplicate, and mean values and standard deviations were evaluated. Total acidity (TA) measurements were performed by titrating 2 mL of the homogenized sample with standardized NaOH (0.05 N) to the phenolphthalein end point (pH = 8.2 ± 0.1). The volume of NaOH was converted to g of lauric acid per mL of the homogenized sample and TA calculated based on the following formula:

$$\text{TA (lauric acid g/L)} = \frac{(\text{mL NaOH used}) \cdot (\text{Normality of NaOH}) \cdot (\text{Lauric acid molecular weight})}{\text{mL homogenized sample}} \quad (1)$$

2.8 Color

The color of the samples was measured with a spectroscopic apparatus (Ferrentino et al., 2012). The system consisted of a high-resolution miniature spectrometer (HR2000+; Ocean Optics Inc., Dunedin, FL, USA) to which a fiber optic reflection probe (Ocean Optics Inc., Dunedin, FL, USA) was connected. The probe transmitted the light from a halogen lamp to the sample by the illuminating fibers while the reflected light from the sample was acquired by the reading fiber and measured by the spectrometer (Apruzzese et al., 2000). After the calibration of the signal, the reflectance spectrum of the treated samples was acquired by a specific software (Spectra Suite®, Ocean Optics Inc., Dunedin, FL, USA) providing L* (lightness), a* (redness), and b* (yellowness) parameters. Color measurements were performed in triplicate, and mean values and standard deviations were evaluated. Total color difference (ΔE) were calculated between the untreated and HPCD+HPU treated samples from the numerical values of L^*, a^* and b^* according to Eq(2):

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad (2)$$

2.9 Texture

Texture measurements were performed with an Instron universal testing machine (Model 4502, Instron Corp., Canton, MA, USA). The compression force at 30 % strain was obtained by using a cylindrical flat probe (surface area of $7.1 \cdot 10^4$ mm^2). The coconut samples, 12 mm in height with a surface of 144 mm^2, were placed on the platform and vertically compressed at room temperature by a flat upper tip (surface area of $7.1 \cdot 10^4$ mm^2) with a 10 kN cell and a deformation rate of 1.3 mm/min. Load and displacement were continuously monitored during the tests, and the hardness (N) of the samples, defined as the peak force at 30 % strain, was measured in triplicate with evaluation of the mean values and standard deviations.

3. Results and discussion

3.1 Inactivation kinetics

3.1.1 Natural microbial flora

The inactivation kinetics of the natural microbial flora obtained at 12 MPa, 24 °C showed the high efficacy of the combined treatment: inactivation to undetectable level for lactic acid bacteria, coliforms, and yeasts and

molds was achieved after 30 min while 3 Log reductions were observed for mesophilic microorganisms (data not shown). Increasing the temperature to 35°C reduction to undetectable was obtained after 30 min for mesophilic microorganisms and lactic acid bacteria (Figure 1) while for coliforms and yeasts and mold, inactivation to undetectable level was achieved in 5 min. Ferrentino et al. (2012) showed that HPCD alone did not induce inactivation to undetectable level of mesophilic microorganisms and lactic acid bacteria of fresh cut coconut, even at higher process conditions (12 MPa, 40 °C, 30 min or 12 MPa, 45 °C, 15 min).

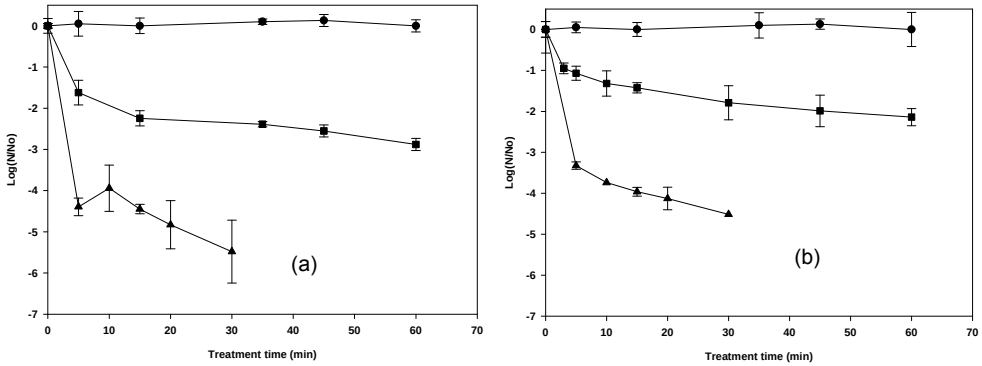

Figure 1: Inactivation kinetics of mesophilic microorganisms (a) and lactic acid bacteria (b) of fresh cut coconut obtained by HPU (●), HPCD (■) and HPCD+HPU (▲) at 35 °C, 12 MPa as a function of the treatment time

Besides the impact that thermal treatment itself has on the inactivation of microorganisms, the effect of the increase of temperature from 24 to 35 °C on microbial inactivation kinetics is closely related to CO_2 mass transfer properties. More specifically, a higher temperature increases the fluidity of cell membranes while the ultrasounds stimulate the diffusion of CO_2 to facilitate its penetration in the cells causing several metabolic alterations responsible for cellular death (Garcìa-Gonzalez et al., 2007).

3.1.2 *Salmonella enterica*

Figure 2 shows the inactivation kinetics of *S. enterica* spiked on fresh cut coconut performed at 12 MPa, 40 (a) and 50 °C (b). The results demonstrated that HPU had no effect on microbial inactivation while HPCD induced 3 and 4 Log reductions after 30 min at 40 and 50 °C, respectively. As regards the combined treatment, a higher efficiency could be noticed: inactivation to undetectable levels of *S. enterica* was achieved in 20 and 10 min at 40 and 50 °C, respectively.

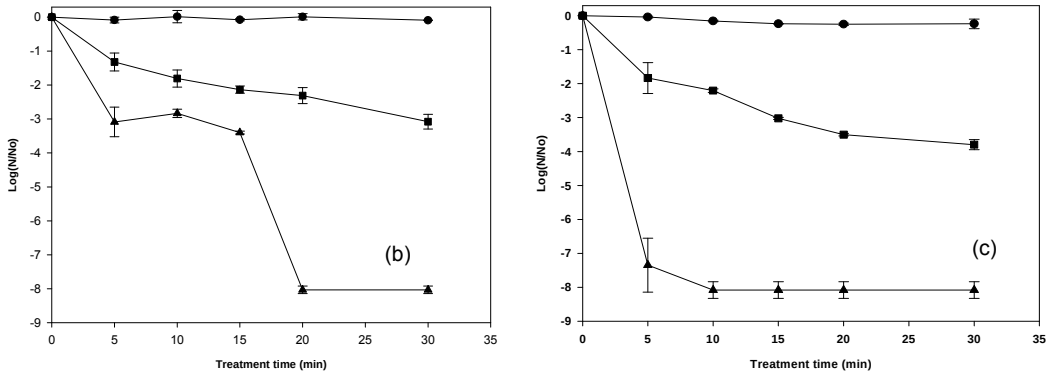

Figure 2: Inactivation kinetics of S. enterica spiked on fresh cut coconut treated by HPU (●), HPCD (■) and HPCD+HPU (▲) at 12 MPa as a function of treatment time: (a), 40 °C; (b) 50 °C

The results clearly showed the synergistic effect of HPCD+HPU combined treatment for the inactivation of *S. enterica* spiked on fresh cut coconut: a faster inactivation rate was found compared to HPCD alone, keeping

the other operative conditions constant. However, compared to the inactivation kinetics of the natural microbial flora, a higher temperature was needed to achieve inactivation to undetectable level of *S. enterica*.
Based on these results, fresh cut coconut was treated at 12 MPa, 40 °C, 20 min with 10 W of HPU delivered every 2 min of treatment to perform the shelf-life study. These conditions were chosen since they assured 8 Log reductions of *S. enterica* and inactivation to undetectable levels of the natural microbial flora.

3.2 Shelf-life study

3.2.1 Microbial stability

Both the natural microbial flora and *S. enterica* loads on the untreated and HPCD+HPU treated coconut was monitored for 4 weeks at 4 °C to check a possible regrowth of the microorganism during the shelf-life even under refrigerated conditions. As concerns the natural microbial flora, lactic acid bacteria and total coliforms significantly increased in the untreated samples while mesophilic microorganisms, yeasts and molds remained constant for all the storage time. HPCD+HPU treated samples showed a stable load of mesophilic microorganisms (about 1.5 Log(CFU/g)) while lactic acid bacteria, total coliforms and yeasts and molds were undetected after the treatment and during the entire shelf-life. It worth saying that the untreated samples were characterized by adverse sensory properties as the appearance of sour odour, similar to fermented products, indicating the spoilage microbial activity. As concerns the treated products spiked with *S. enterica*, a high microbial stability was observed: no colonies were detected on the treated samples during the entire shelf-life while the microbial concentration on the untreated samples stayed almost stable (about 7.7 Log(CFU/g)).

3.2.2 Quality stability

TA and pH of the treated products stayed stable after the treatment and during the whole shelf-life while the untreated ones significantly changed their acidity and pH after the third week, probably because of the development of spoilage microorganisms in the product (data not shown). Spilimbergo et al. (2014) reported similar results on dry cured ham surface processed with HPCD+HPU combined treatment: no significant differences in pH and TA were shown between the treated and untreated samples. Color parameters (L*, a*, and b*) and ΔE values for HPCD+HPU treated samples during 4 weeks of refrigerated shelf-life are reported in Table 1.

Table 1: Color parameters and □E values for HPCD+HPU treated samples during 4 weeks of shelf-life

Storage time (weeks)	L*	a*	b*	ΔE
0	77.84±1.68	2.87±0.47	5.40±0.66	2.31±0.91
1	75.31±0.85	2.75±0.10	4.25±0.10	3.15±0.43
2	75.10±1.13	1.35±0.36	3.75±0.10	3.12±1.10
3	74.95±0.64	1.15±0.10	3.70±0.28	3.75±1.06
4	74.05±0.78	1.55±0.36	3.30±0.14	4.60±0.25

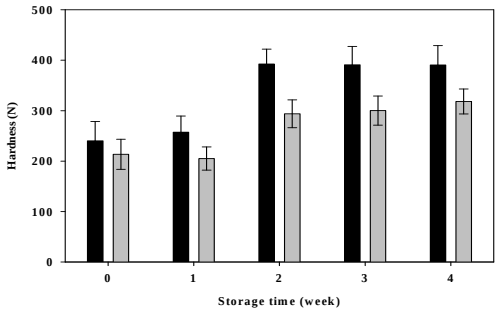

Figure 3: Texture measurements of untreated (■) and HPCD+HPU (▭) treated fresh cut coconut during refrigerated shelf-life

L* values remained stable for the all shelf-life while a significant decrease of b* and a* was observed after the second week of storage. ΔE values showed significant differences between the untreated and treated samples at the third and fourth week of shelf-life resulting equal to3.75±1.06 and 4.60±0.25, respectively. An absolute threshold value for human color discrimination has only been determined for few specific products (Martínez et al., 2001); nevertheless, a ΔE value of 4 is usually considered a clearly distinguishable color difference to the

average person. Figure 3 shows the texture behavior of the untreated and HPCD+HPU treated fresh cut coconut in terms of hardness after the process and during the shelf-life: no significant changes were observed after the first week, while, with the second week, both samples showed an increase in the hardness, which was statistically higher for the untreated one.

4. Conclusions

The results assessed the feasibility of HPCD+HPU combined treatment for the inactivation of the natural microbial flora and *S. enterica* spiked on fresh cut coconut highlighting the synergistic effect of the two techniques operated in parallel. Inactivation of the natural microbial flora was obtained at milder conditions compared to the optimal ones identified for HPCD alone: 35 °C, 12 MPa for 30 min and 10 W delivered every 2 min assured inactivation to undetectable level, even of mesophilic microorganisms, the most resistant strain. Inactivation to undetectable levels was achieved with the combined treatment for *S. enterica* at 12 MPa, 40 °C, 20 min with 10 W delivered every 2 min while just 4 Log reductions were achieved when HPCD was performed alone. The analyses performed to evaluate the quality stability of the product after the combined process and during the shelf-life study confirmed its feasibility and attractiveness. Some attributes of the product analyzed after the fourth week of storage were stable as the microbial content, pH and TA while slight differences were observed for color and texture.
In conclusion, the results demonstrated the potential of HPCD+HPU as an innovative non-thermal pasteurization technology for fresh cut fruits.

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