HIGH POWER ULTRASOUND COMBINED WITH SUPERCRITICAL CARBON DIOXIDE FOR THE DRYING AND MICROBIAL INACTIVATION OF CORIANDER

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Abstract

This work explores the use of supercritical carbon dioxide ($scCO_2$) drying in combination with High Power Ultrasound (HPU) to enhance both the dehydration efficiency and the microbial inactivation on coriander leaves. $ScCO_2$ drying process alone was compared with a combined drying process (HPU+$scCO_2$) at different powers (10, 40 and 80W), different drying times (up to 90 min) and two process temperatures (40 and 50°C). At the most effective condition tested (40W; 10 MPa; 40 °C), mesophilic bacteria were reduced up to 4 Log, mesophilic spores up to 1 Log, while yeast and molds were never detected (< 2 log CFU/g). 40W was identified as the threshold HPU value to achieve a beneficial effect on mesophilic bacterial spores reduction. Besides, the use of HPU enhanced the water loss and lowered the water activity of the samples, compared to the ones processed with $scCO_2$ alone. The appearance and color of the dried samples did not show significant differences after the two processes. Overall, HPU+$scCO_2$ process resulted a promising technology to enhance both the dehydration and microbial inactivation efficiency compared to $scCO_2$ drying alone.

KEYWORDS: supercritical drying; carbon dioxide; high power ultrasound; microorganism inactivation; coriander

1. Introduction

Drying is one of the oldest and most spread worldwide food conservation methods [1]. The low water activity of desiccate food products inhibits the growth of microorganisms and decreases the enzymatic deterioration during storage [2]. However the risk of developing food borne illness is still very high, especially once the product is rehydrated. It is worth pointing out that, between 2007 and 2012, 7315 cases of bacterial infection and 63 deaths due to contaminated low-water activity food were registered worldwide [3]. Indeed, even if traditional drying methods reduce the water activity, they do not provide a strong microbial reduction during dehydration [4]. Microbial heat resistance increases upon dehydration, therefore pasteurization of dried products is inhibited [5]. Irradiation is often added to spices as decontaminant process [37], however it leads to an increase of the total production cost and may hinder the consumer's acceptance. [38] Therefore, there is a significant interest to develop technologies capable to dry and pasteurize food products simultaneously. Supercritical carbon dioxide ($scCO_2$) is considered an emerging technology that could assure both the extraction of water and a sufficient microbial inactivation. $ScCO_2$ has been already studied as alternative technique to dry basil [6], carrots [7], mango and persimmon [8], aerogel [9], and also biological matrices as decellularized tissues [10;11], showing promising results on the retention of original structure and quality attributes. Regarding pasteurization, $scCO_2$ has deeply demonstrated antimicrobial effect in solid and liquid products [12;13;36]. However, despite its potential, $scCO_2$ alone is not able to inactivate spores [34]: previously studies demonstrated that multiple pressurization cycles [12], chemical additive [14], acid environment [35] or other technologies [15;16] must be used in combination to address this issue. Among novel techniques, High Power Ultrasound (HPU) combined with $scCO_2$ enhanced the reduction kinetic of pathogens in both solid and liquid foodstuffs [17;18]. HPU increases the mass transfer in conventional hot air drying processes [19] and in supercritical extraction [20], making it promising also for other supercritical applications such as precipitation [31;32].

The present work focused on $scCO_2$ drying of coriander in combination with HPU, exploring the efficiency on dehydration and microbial inactivation. The study compared results obtained applying $scCO_2$ drying alone and in combination with HPU at different powers.

2. *Materials and methods*

2.1 Sample preparation

Coriander (*Coriandrum sativum L.*) was purchased from a local market in Padua (Italy), stored at 4°C and treated within 3 days after the purchase. Experiments were performed with coriander leaves, selected for similar dimension and color (Fig 1D). 1± 0.1 g of product was used for each experiment. Coriander leaves were placed inside a metallic net basket before the insertion into the reactor (Fig 1C). The basket was previously sterilized with absolute ethanol (Sigma Aldrich, 99.8%) and burned with Bunsen flame.

2.2 Combined process procedure

Experiments were run by mean of a high pressure apparatus with High Power Ultrasound combined system previously described [21,22]. A picture of the entire apparatus is reported in Figure S1 in the supporting information and a schematic representation of the drying chamber is shown in Figure 1B. The HPU system included a transducer (40 KHz), a buster, and a power generator unit. The experimental run consisted in three main phases: (i) pressurization (20 min), (ii) drying (0-90 min) and (iii) depressurization (40 min) as shown in Figure 1A. Pressurization was set at 0.20 MPa/min up to 10 MPa, while the depressurization was set at 0.25 MPa/min. When drying occurred, CO_2 flow rate was set at the maximum flow rate of the pump (23 mL /min) up to 90 min. Experiments were carried out at 10 MPa and 40 or 50 °C; 40°C was chosen to ensure supercritical conditions while temperatures higher than 50°C were not taken into account to minimize the overheating during the HPU [17] that might degrade the sensitive sample. As the HPU power output was found to be a function of the loaded pressure, the amplitude of the ultrasound generator was manually modulated during pressurization and drying phases to produce a constant applied power. Four different outlet powers values were tested (0, 10±3, 40±5 and 80±10 W). In order to avoid the sample overheating, experiments were carried out in cycles by intervals of 10 seconds each. Figure S2 in the supplementary shows the maintenance of an average constant temperature during the 90 min time of drying. The ultrasounds were not applied during de depressurization phase. Temperature was measured with a thermocouple placed at the bottom of the reactor (Figure 1B).

2.3 Microbial analysis

Mesophilic bacteria, mesophilic bacterial spores, yeasts and molds were quantified before and after the process, by mean of the standard plate count techniques as previously reported [17]. Phosphate Buffered Saline (PBS- Sigma Aldrich) was used for 1:10 serial dilution (weight ratio). Mesophilic bacteria and spores were cultured using total plate count agar (Microbial Diagnostici, Catania, Italy) at 30°C within pour plate, while yeasts and molds were cultured with DRBC agar (Bitec S.r.l., Grosseto, Italy) supplemented with chloramphenicol at 22°C within spread plate. Mesophilic bacterial spores were germinated placing the first dilution tubes for 10 minutes in a thermostatic bath at 80°C before plating. Each experimental condition was run at least in triplicate and all the samples were plated in duplicate; the results were calculated as mean value. Standard deviations are shown by error bars in the graphs. The enumeration was referred to the mass of initial fresh product and expressed in CFU/g. Inactivation degree was calculated as the $\mathrm{Log}(N/N_0)$, where N_0 was the number of initial microorganism in the fresh sample and N the number of viable microorganism after the process, in colony forming units per gram (CFU/g) of fresh product.

The limit of quantification was 200 CFU/g for the mesophilic bacteria and mesophilic bacterial spores, 2000 CFU/g for the yeast and molds, while the limit of detection was < 10 CFU/g and < 100 CFU/g respectively. Statistical significance was evaluated with an ANOVA and post hoc Tukey HSD ($p < 0.05$).

2.4 Water content

The weight was measured before and after the process and the mass loss was calculated as:

Weight reduction = = 1 − / ∗ 100 (1)

where and indicates the mass of the sample after and before the process, respectively. Water activity was measured with Hygropalm HP-23-A (Rotronic AG, CH) at the end of the process. Data are expressed as mean value of at least two repetitions for each time point.

2.5 Color analysis

Color was measured within a high-resolution miniature spectrometer (HR2000+, Ocean Optics Inc., Dunedin, FL). The signal was acquired by a specific software (Spectra Suite®, Ocean Optics Inc., Dunedin, FL, USA) and the CIE (L*a*b*) values were obtained. A custom support was used to keep the sample always at the distance of 1 cm from the fiber. The measurements were performed at least in triplicate, calculating mean values and standard deviations. Total color difference (ΔE) was calculated between the untreated and dried samples (with and without HPU) using equation (2):

$$\Delta E = \sqrt{(L^* - L^*) + (a^* - a^*) + (b^* - b^*)} \quad (2)$$

where L^* refers to the fresh sample, while L^* to the treated one (same for a* and b*). ΔE was calculated also between different time point during shelf life study.

2.6 Accelerated shelf life

Coriander were dried for 90 minutes at 100 bar, 40°C with supercritical CO_2 alone and in combination with HPU at 40W, as previously described. Dried samples were stored at 30°C up to 10 days in aluminum food packaging bag filled with N_2 atmosphere. During the shelf life, the following parameters were monitored at day 5 (T1) and day 10 (T2): color, water activity and total weight. The values were compared to the sample after the drying procedure (T0).

3 Results and discussion

3.1 Microbiological analysis

The initial load on the untreated fresh coriander for mesophilic bacteria, mesophilic bacterial spores, yeasts and molds was 7.63 ± 0.60, 3.64 ± 0.22 and 5.57± 0.45 log CFU/g of fresh sample respectively. The initial microbial load was reasonably high, making the coriander an optimal case product for the evaluation of the bactericidal efficiency of the process.

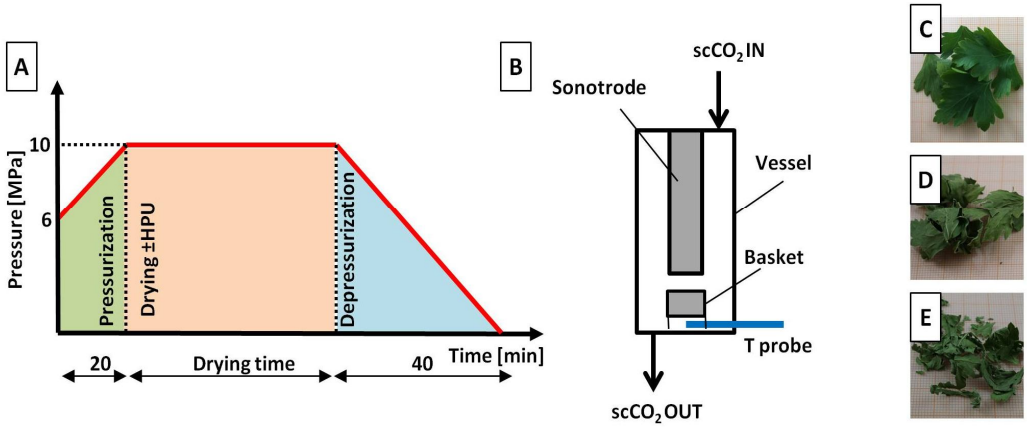

Figure 1. (A) Schematic representation of pressure profile during pressurization (light green), drying (with or without HPU in light orange), and depressurization (light blue) phases; (B) schematic of the drying chamber with HPU sonotrode; untreated coriander (C) coriander after $scCO_2$ drying (D) coriander after $scCO_2$+HPU at 40W (E).

Supercritical drying process consists in three main phases (Figure 1A): pressurization of the vessels; $scCO_2$ drying (with or without HPU); depressurization down to atmospheric pressure. Each phase is characterized by a specific operative time.

Firstly, the study was focused only on the pressurization and depressurization phase: no drying (drying time = 0 min) occurred, meaning that as soon as the reactor reached the operative pressure it was depressurized.

Pressurization and depressurization phases are mandatory in every high-pressure process and their length strongly depends on the ratio between the vessel volume and the maximum flow rate of the pump. Intermediate values of pressurization and depressurization rates were chosen to better simulate the behaviour of a pilot plant, making our study as general as possible and industrial oriented. For these reasons pressurization was set at 0.20 MPa/min meaning that 20 minutes were needed to increase the pressure from 6 (initial pressure of the tank) to 10 MPa (operative pressure), while 40 minutes were needed to reach the ambient pressure at the end of the process. Within these rates, the samples were already maintained in contact with CO_2 at supercritical phase for about 20 minutes during the pressurization and depressurization phases. Results of inactivation at 0 ($scCO_2$ alone), 10, 40 and 80 W are shown in Figure 2. Pressurization and depressurization phases had a positive effect on the microbial inactivation during $scCO_2$ drying: mesophilic bacteria were reduced down to 4 log CFU/g while yeasts and molds were not detectable (<2 log CFU/g) after every condition

tested (data not shown). However, mesophilic bacterial spores were not inactivated after the $scCO_2$ treatment. The enhancement of inactivation rates for both mesophilic bacteria and mesophilic bacterial spores were achieved pairing the $scCO_2$ process with HPU: for mesophilic bacterial spores the increment of the inactivation was significant between the two processes, starting at 40W. At this HPU power mesophilic bacterial spores were under the quantification threshold value. 40W was therefore chosen as HPU power for all the further experimental runs. The sensitivity of spores to HPU was also observed by Ordonez and Burgos [23] where HPU was applied as pre-treatment for thermal pasteurization. The complete inactivation of yeast and molds was observed also after 5 min of treatments with carrots [24], while 4 min were necessary with cooked ham [21]. A similar resistance of mesophilic bacteria was also observed for cooked ham [21]. Among the bacteria population, a sensitivity distribution to the process must subsist, which limited the maximum achievable inactivation, unless more severe processing conditions (e.g. higher temperatures) were applied [21]. High temperatures promote the CO_2 diffusion within the cell membrane [25], increasing both the penetration of the CO_2 and the intracellular material extraction, which are considered two of the key steps of $scCO_2$ inactivation mechanism [26, 32]. On the other hand CO_2 solubility in water decreases as temperature increases [40], limiting the contact with the microorganisms [17]. Moreover high temperatures could induce the degradation of thermosensitive molecules that must be present in a high-quality dried product. Thus, even if temperatures higher than 50°C could enhance the microbial inactivation [21,22,27], they were not taken into account in this first evaluation.

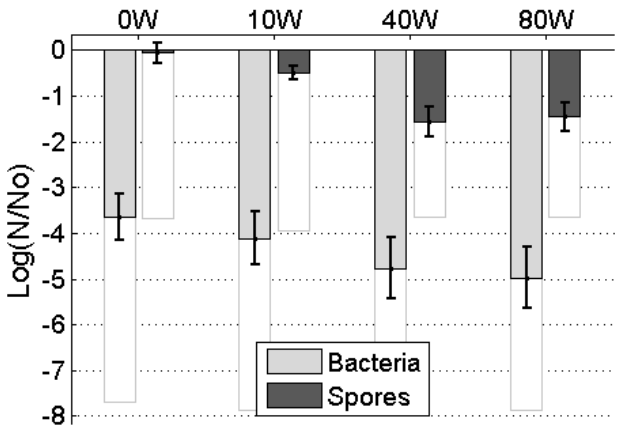

Figure 2 - Inactivation of mesophilic bacteria (light grey) and mesophilic bacterial spores (dark grey) as a function of HPU power. The bars in the background stand for the average of the initial microbial population ($-N_0$)

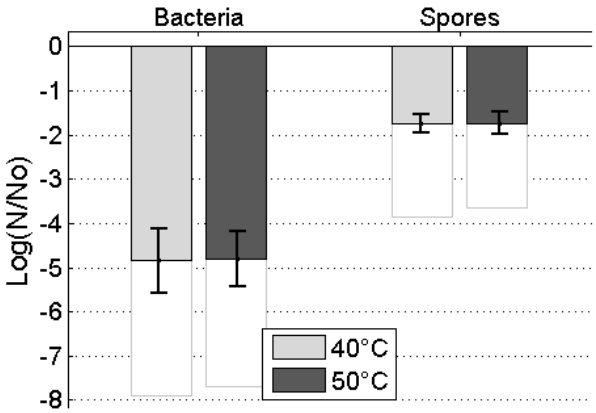

Figure 3 - Inactivation of mesophilic bacteria and mesophilic bacterial spores as a function of temperature 40 (light grey) and 50°C (dark grey). The bars in the background stand for the average of the initial microbial population ($-N_0$)

Figure 2 shows the effect of 40 and 50°C on the inactivation of mesophilic bacteria and spores after HPU+scCO_2 process at 40W, after the pressurization and depressurization steps. These data confirmed that the most sensitive bacteria and spores population were already inactivated at 40°C and an increase up to 50°C does not significant influence the inactivation rate. Temperature control experiments (data not shown) showed also a limited reduction of temperature sensitive bacteria. For all these reasons the effect of the drying time on the microbial inactivation after scCO_2 process was investigated only at

40°C. The water content reduction significantly inhibits the viability of microorganisms and it can affect the inactivation capability of traditional pasteurization techniques. [5]. For these reasons the microbial inactivation during supercritical drying is worthy to be studied and so far it has never been investigated for HPU+scCO$_2$ drying. Fresh coriander has a total moisture content of 88% [36] and it was completely dried after 90 minutes of HPU+scCO$_2$ process with a final water activity of 0.29±0.03. ScCO$_2$ drying alone did not allow a similar value after the same treatment time (water activity =0.42±0.09); however in both cases the final value was below the maximum amount to inhibit the bacterial growth [2]. Moreover we observed that 90 min of drying times induced fragmentation of the sample after scCO$_2$+HPU process (Figure 1E) with a high risk to lose small amount of sample during the depressurization. For this reason, even if a complete dried product was not obtained after scCO$_2$ drying alone (Figure 1D), 90 min was chosen as drying time for the investigation. In Figure 4 the inactivation of mesophilic bacteria and mesophilic spores after 0 and 90 min of scCO$_2$ drying alone and after HPU+ scCO$_2$ process at 40 W are reported.

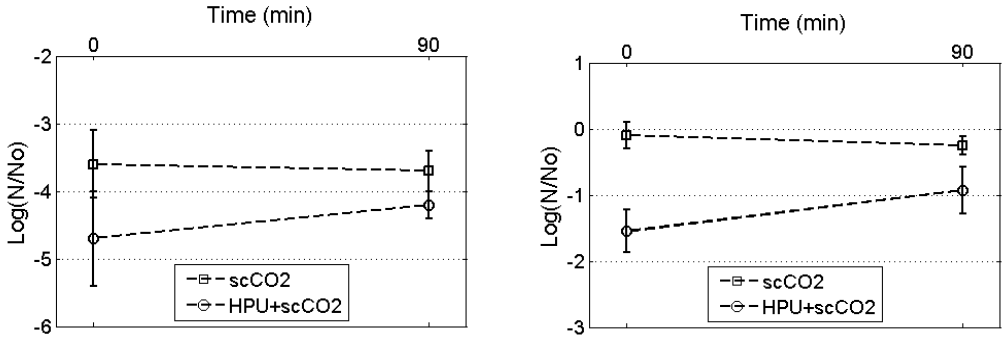

Figure 4 Inactivation of mesophilic bacteria (left) and mesophilic bacterial spores (right) after 0 and 90 min of drying at 40°C, 10 MPa. scCO$_2$ refers to the supercritical drying process alone, while HPU+scCO$_2$ refers to the combined one at 40W

An additional drying time did not significantly increase the microbial inactivation degree meaning that the main inactivation was already reached after the pressurization and depressurization phases. This evidence confirms the high sensitivity of the natural microbiota on the surface of coriander toward scCO$_2$ treatment.

Further analysis should be performed to investigate the effect of the drying process on specific microorganisms, especially pathogens, known to be resistant to the traditional thermal pasteurization.

3.2 Physical analysis

Figure 5 shows the weight reduction and water activity up to 90 min of drying, measured after processing the sample with $scCO_2$ alone or in combination with HPU at 40W. Water loss and reduced water activity are considered the main indicators of the achievement of food drying. The total water removal was achieved in 90 minutes for the combined process while, after the same drying time, $scCO_2$ drying alone achieved less than 80% of water reduction. The pressurization and depressurization phases already induced the removal of a small quantity of water, probably due to the swelling of the sample. Drying times lower than 15 minutes did not show big differences between the two processes in terms of weight loss and water activity. For higher drying times the difference between the 2 treatments became more evident: HPU enhanced the water extraction making the process faster and influencing the obtainment of lower water activities. High-intensity acoustic waves may produce cavitation of water molecules within the solid matrix, which are helpful for the removal of firmly linked moisture [30]. The use of ultrasound generates alternative contractions and expansions of the solid material: those phenomena can create micro-channels suitable for fluid movement [19]. For this reason, a stronger effect in terms of speeding up the weight reduction kinetics, would probably have been observed in the case of thicker geometry food material [19] since coriander leaves are characterized by a high surface to volume ratio. Trials with different food matrices should be performed to confirm this hypothesis.

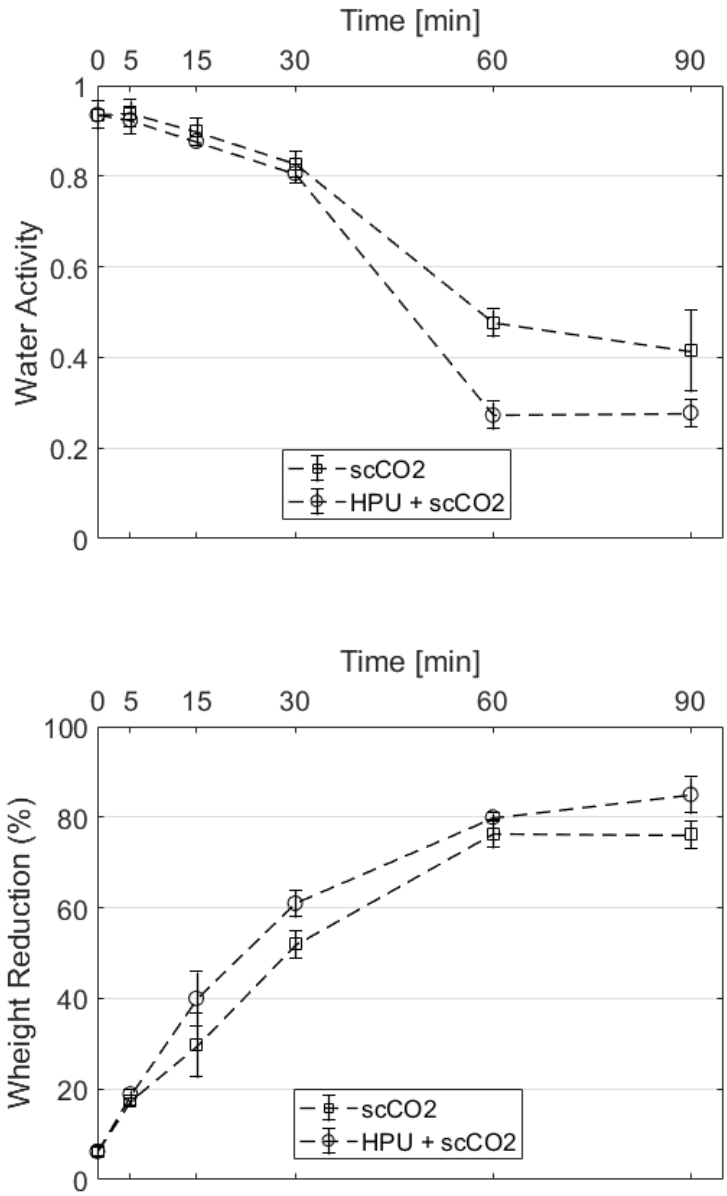

Figure 5 – Water activity (left) and weight reduction (right) kinetics at different drying times. $scCO_2$ refers to the supercritical drying process alone, while HPU+$scCO_2$ refers to the combined one at 40W.

Color parameters (L*, a*, and b*) and ΔE values for fresh (untreated) and dried coriander obtained with $scCO_2$ and HPU+$scCO_2$ drying process at 90 minutes, 40°C and 10MPa are reported in Table 1.

Table 1. $L^*a^*b^*$ values for fresh and treated coriander with $scCO_2$ and $scCO_2$+HPU (40W) drying after 90 minutes of drying at 40°C and 10MPa. ΔE refers to the color change with the respect to fresh coriander.

	L^*	a^*	b^*	ΔE
Fresh coriander	53.2 ± 6.1	-16.6 ± 2.4	44.1 ± 7.4	
$scCO_2$ dehydrated coriander	69.6 ± 5.3	-5.5 ± 2.8	37.3 ± 2.4	20.9 ± 7.1
HPU+ $scCO_2$ dehydrated coriander	66.3 ± 4.9	-2.1 ±1.6	33.4 ± 2.5	22.3 ± 6.33

As expected, the drying process induced significant modification of the product appearance compared to the fresh one. However the parameters of the two drying processes are similar as the ΔE referred to the fresh product. As the water is extracted the leaves shrink, moreover the mechanical effect of HPU lead to leaves fragmentation which was more evident for longer drying times, as shown in Figure S3 in the supporting informations. However, the sample fragility should not be an issue since most of the herbs are commercialized in small pieces once desiccated. As proof of concept for the stability of the processed sample, an accelerate shelf life up to 10 days was performed at 30°C. Water loss was constant for the sample during the time of storage (data not shown). Results for the water activity and color analysis are reported in Figure S4 and Table S1 respectively in the supplementary informations. The water activity shown an increment during time, but it remained under the minimum value for microbial growth [2], therefore we did not analyze the microbial content. Color analysis shown the maintenance of the original color of the dried sample after the process. Longer analysis should be performed to understand better the stability of the physical properties during storage, comparing also different packaging materials.

4 Conclusion

This work reported a feasibility study regarding the use of $scCO_2$ in combination with HPU to dry coriander. We demonstrated that coriander leaves was dried in a shorter time in comparison with the single $scCO_2$ drying treatment without a significant modification in the sample appearance, even during the storage time. Furthermore, we

proved that $scCO_2$ drying is able to inactivate microorganisms: the inactivation was achieved mainly in the pressurization phase while the drying time did not significant enhance the inactivation degree. Yeast and molds are the most sensitive microorganisms to the treatment, mesophilic bacteria can be strongly reduced down to 4 Log, however, they remain always above the quantification limit. HPU+$scCO_2$ process showed a threshold value of 40W that resulted significant for the inactivation of mesophilic bacterial spores. These results demonstrate that HPU+$scCO_2$ drying has the potential to ensure a better inactivation of microorganisms compared to $scCO_2$ treatment alone. Nevertheless additional studies are needed to demonstrate the synergic effect of HPU+$scCO_2$ combined process, specifically with pathogens that might contaminate the food products. Additional sensory and chemical analyses should be performed to evaluate the quality of the dried product in comparison with other traditional drying techniques. The cost analysis of the combined technology should also be performed to reinforce the industrial relevance of the study.

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