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HIGH POWER ULTRASOUND COMBINED WITH SUPERCRITICAL CARBON DIOXIDE FOR THE DRYING OF HERBS

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Abstract

This work explores the use of supercritical carbon dioxide (scCO₂) drying in combination with High Power Ultrasound (HPU) to enhance both the dehydration kinetics and the microbial inactivation on coriander leaves. Indeed we compared the scCO₂ drying process alone and in combination with HPU at different powers (10, 40 and 80W), different drying times (up to 90 min) and two process temperatures (40 and 50°C). In 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 resulted under the detection limit. We identified 40W as the threshold HPU value to achieve a beneficial effect on mesophilic spores reduction. Besides, the use of HPU enhanced the water loss and lowered the water activity of the sample, compared to the one processed with scCO₂ alone. The appearance of the dried sample did not show significant differences after the two processes. Overall, scCO₂.HPU combined process resulted a promising technology to enhance both the dehydration and microbial inactivation kinetics compared to the use of scCO₂ alone.

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

1. Introduction

Among the food conservation methods, the drying is either one of the oldest and one of the most spread worldwide [1]. The low water activity of an exsiccated food product inhibits the growth of microorganisms and decreases the enzymatic deterioration during the 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 foods were registered worldwide [3]. Indeed, even if traditional drying methods are efficient to obtain low water activity, they do not provide a strong microbial reduction during the dehydration [4]. Pasteurization of the dried products is possible but its efficacy might be inhibited because the heat resistance of microorganisms increases upon dehydration [5]. Therefore, there is a

significant interest to develop technologies capable to dry and pasteurize food products simultaneously. Supercritical carbon dioxide (scCO₂) is considered an emerging technology that could assure both the extraction of water and a sufficient microbial inactivation. However, there is still no evidences in literature that demonstrate the antimicrobial effect during scCO₂ drying process. ScCO₂ 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, scCO₂ has deeply demonstrated to show antimicrobial effect both in solid and liquid products [12;13]. However, despite its potential, scCO₂ alone is not able to inactivate spores: previously studies demonstrated that multiple pressurization cycles [12], chemical additive [14] or other technologies [15;16] must be used in combination, to address this issue. Among novel techniques, it was demonstrated that High Power Ultrasound (HPU) combined with scCO₂ enhances the reduction kinetic of pathogens in both solid and liquid foodstuffs [17;18]. Interestingly, 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.

In this regards, the present work focuses on scCO₂ drying of herbs in combination with HPU, exploring the effects on dehydration capacity and microbial inactivation. Coriander leaves were used as case product and inactivation kinetic was studied in terms of mesophilic bacteria, mesophilic spores and yeasts and molds naturally present on the leaves. Experiments were carried out in lab scale semi-continuous reactor, comparing the effect of scCO₂ drying alone or in combination with HPU at different powers.

2. Materials and methods

2.1. Sample preparation

Coriander (*Coriandrum sativum*) was purchased from a local market in Padua (Italy), stored at 4°C and treated within 3 days after the purchase. The leaves were separated from the stems and selected for similar dimension and color. Only the leaves were used during these trials. In each experimental run 1 g ± 0.1 g of product was used as processed sample. It was placed inside a metallic net basket before the insertion into the reactors. The basket was previously sterilized with absolute ethanol (Sigma Aldrich, 99.8%) and then burned by mean of a Bunsen flame.

2.2. Combined process procedure

Experiments were run by mean of a combined high pressure apparatus with high power ultrasound previously described [21,22].

The experimental run consisted in three main phases (i) pressurization (20 min); (ii) drying (0-90 min) and (iii) depressurization (40 min). 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, CO₂ flow rate was set at 22.31 g/min up to 90 min. Experiments were carried out at 10 MPa and 40 or 50 °C. As the HPU power output was found to be a function of the loaded pressure, the amplitude of the ultrasound generator was 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. The ultrasounds were switched off during depressurization phase.

2.3. Microbial analysis

Natural flora was quantified in terms of mesophilic bacteria, mesophilic spores, and yeasts and molds before and after the process, by mean of the standard plate count techniques as previously reported [17]. Phosphate buffy Saline (PBS- Sigma Aldrich) was used for 1:10 serial dilution (weight ratio). Incubation condition and media used for the enumeration are summarized in Table S1 of the supplementary material. Mesophilic 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. Microbial quantification was referred to the mass of initial fresh product and expressed in CFU/g. Inactivation degree was calculated as the $\text{Log}(N/N_0)$, where N_0 (CFU/g) and N (CFU/g) respectively stand for the number of colony forming units per gram in the untreated and treated sample.

The quantification limit was set at 200 CFU/g for the pour plate, while 2000 CFU/g for the spread plates while the limit of detection was < 10 CFU/g for the pour plate and < 100 CFU/g for the spread plates. Statistical significance was evaluated with an ANOVA and post hoc Tukey HSD ($p < 0.05$).

2.4. Physical analysis

At the end of each experimental run, the basket containing the sample was removed from the reactor and placed in a sample tube for physical analysis in terms of weight reduction, water activity and total color difference.

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

$$\text{Weight reduction} = \left(1 - \frac{m}{m_0} \right) * 100 \quad (1)$$

where m and m_0 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.

3. Results and discussion

3.1. Microbiological analysis

Table 1 reports the microbial load naturally present on coriander leaves. As expected for a land-grown herb, the initial microbial load is reasonable high, making the coriander an optimal case product for the evaluation of the bactericidal capacity of the process.

Table 1 - Natural flora on the untreated fresh coriander leaves expressed as Log (CFU/g).

Total Mesophilic count	Mesophilic Spores	Yeasts and Molds
7.63 ± 0.60	3.64 ± 0.22	5.57 ± 0.45

Firstly, in order to evaluate the strength of the inactivation due to the combined process, and understand the effect of HPU powers on the inactivation, we focused on the pressurization and depressurization phases, setting the drying time at 0 minutes. The pressurization and depressurization phases are mandatory in every high-pressure process and they are strongly influenced by the volume of the reactor and the maximum flow rate of the pump. We chose intermediate values of pressurization and depressurization rate to better simulate the behaviour of a pilot plant, making our study more general as possible and industrial oriented. For these reasons pressurization was set at 0.20 MPa/min meaning that 20 minutes was 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 maintained above the supercritical phase for about 20 minutes during the pressurization and depressurization phases.

The results of inactivation at 0, 10, 40 and 80 W are shown in Figure 1. We demonstrated that the pressurization and depressurization phases positively contribute to the microorganism inactivation during scCO₂ drying, reducing up to 4 log inactivation for mesophilic bacteria and completely inactivating yeasts and molds under the detection limit for all the conditions (data not shown). However, as expected, mesophilic spores were not inactivated after the scCO₂ treatment. An increase of HPU power implied the enhancement of inactivation rates for both mesophilic bacteria and mesophilic spores, however, the increment of the inactivation was statistically significant only for mesophilic spores, starting at 40W. Using this power value, the final load of mesophilic spores turned out to be under the quantification limit and significantly lower than the scCO₂ process alone. The sensitivity of spores to HPU was also observed by Ordonez and Burgos [23] in which HPU were applied as pre-treatment for thermal pasteurization. The complete inactivation of yeast and molds was observed also in previous works with carrots [24] after 5 minutes of treatments, while 4 minutes was necessary with cooked ham [21]. The resistance of mesophilic bacteria was also observed on cooked ham [21] where it was not possible to reach a complete inactivation. Even with coriander, it can be explained by the presence of a distribution of sensitivities to the treatment among the bacteria population, which limits the maximum achievable inactivation, unless more severe processing conditions are applied [21]. For this reason we performed additional analysis at higher temperature. In general a temperature increase promotes the CO₂ diffusion within the cell membrane [25], increasing both the penetration of the CO₂ and the intracellular material extraction, which are considered two of the key steps of scCO₂ inactivation mechanism [26]. On the other hand CO₂ solubility in water decreases as temperature increases, limiting the contact with the microorganisms [17]; moreover high temperature induces loss and degradation of thermosensitive molecules that should be retained for a high quality product. Thus, even if temperatures higher than 50°C could have a positive effect on microbial inactivation [27], they were not taken into account in this work.

Regarding the operative conditions, since there was a threshold value for the inactivation of mesophilic spores, we chose 40W as the operative HPU power for all the further investigations of the combined process.

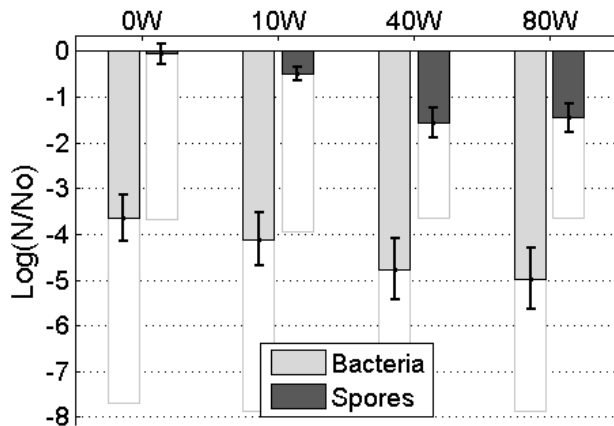


Figure 1 - Inactivation of mesophilic bacteria (grey) and mesophilic spores (white) as a function of HPU power. The bars in the background stand for the average of the initial microbial population ($-N_0$)

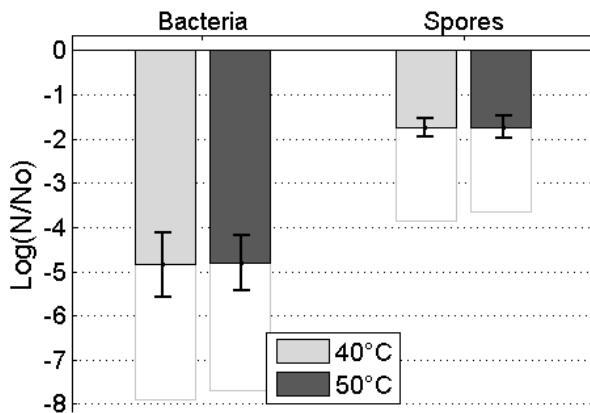


Figure 2 - Inactivation of mesophilic bacteria and mesophilic spores as a function of temperature 40 (light grey) and 50°C (dark grey). The bars in the background stand for the average of the initial microbial population ($-N_0$)

Figure 2 shows the effect of 40 and 50°C on the inactivation of mesophilic bacteria and spores for scCO₂-HPU combined process at 40W, after the pressurization and depressurization phase. 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. Following, we investigated the effect of the drying time on the microbial inactivation. It is well known that the microbial inactivation due to scCO₂ process is affected by the treating time both for solid and liquid matrices [28;13] and, regarding scCO₂-HPU process, that the inactivation of bacteria is much faster than the ones obtained with scCO₂ alone [17;24;30]. However, the microbial inactivation both for scCO₂ alone and in combination with HPU has never been investigated so far, as a function of drying time. In general, the reduction of water content, together with the prolongation of the drying process, significantly inhibits the viability of microorganisms while, at the same time, affects the inactivation capability of traditional pasteurization techniques, as they result less effective in a dried environment [31]. For these reasons contrasting effect on microorganism

inactivation during supercritical drying, worthy to be investigated, might happen. After 90 minutes of drying the water activity resulted (0.29 ± 0.03) below the maximum amount to inhibit the growth of bacteria [2]. Moreover we observed that longer drying times induced fragmentation of the sample, especially regarding the samples treated with the combined process, with a high risk of sample lost during the depressurization. For this reason, even if a complete dried product was not obtained, we chose 90 minutes as drying time for both the conditions. In Figure 3 the inactivation kinetics of mesophilic bacteria and mesophilic spores as function of drying time for the scCO_2 drying alone and in combination with HPU at 40 W are reported.

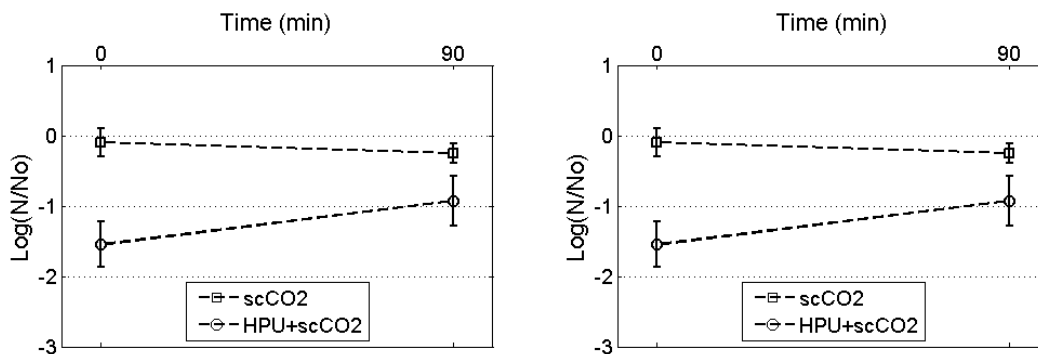


Figure 3 Inactivation of mesophilic bacteria (left) and mesophilic spores (right) as a function of drying time (0 and 90 minutes) at 40°C, 10 MPa. scCO_2 refers to the supercritical drying process alone, while HPU+ scCO_2 refers to the combined one at 40W

For both the treatment the drying time did not seem to significantly increase the inactivation degree, meaning that the main inactivation was reached after the pressurization and depressurization phase. This evidence is explained by the sensitivity of the natural microbial load at the scCO_2 treatment, suggesting the need of specific microorganism strains to confirm the results and hypothesis through a deeper analysis.

3.2. Physical analysis

Water loss and reduced water activity are considered the main indicators of the achievement of food drying: Figure 4 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. HPU enhanced the water extraction making the process faster and influencing the obtainment of a lower water activity. High-intensity acoustic waves may produce cavitation of water molecules within the solid matrix, which are helpful for the removal of firmly linked moisture [32]. Beneficial contributions introduced by the use of ultrasound to the internal and external mass transfer in solid-fluid systems, can be referred to the oscillating velocities and microstreaming at the interfaces, affecting the boundary layer and to alternative contractions and expansion (sponge effect) of the solid material in which US are travelling. The compressions and expansions can create micro-channels suitable for fluid movement [19]. For this reason a stronger effect in terms of speeding up the weight reduction kinetic due to ultrasound effect on internal

mass transfer, would probably have been observed in the case of more porous 3-D food material [19] instead of the flat 2-D coriander.

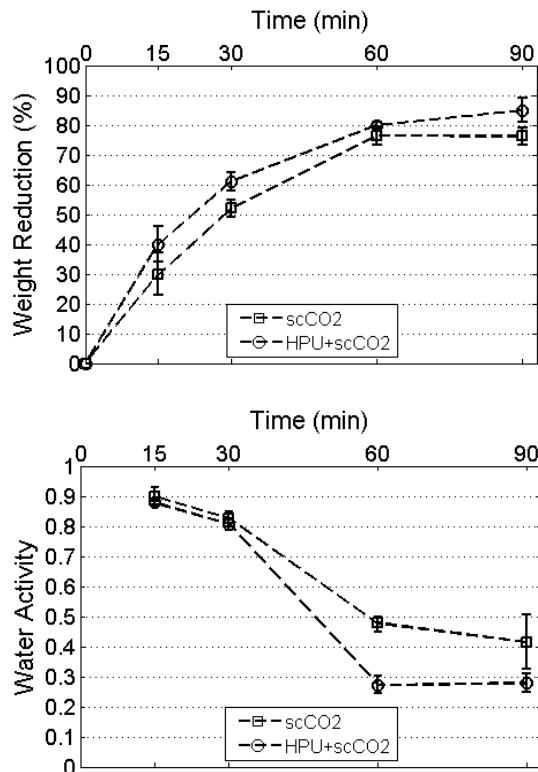


Figure 4 – Water activity (left) and weight reduction (right) kinetics at different drying times. scCO₂ refers to the supercritical drying process alone, while HPU+scCO₂ refers to the combined one at 40W.

As expected, the drying process induced significant modification of the product appearance compared to the fresh one: 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 5. However the sample fragility should not be an issue since most of the herbs are commercialized in small pieces once desiccated.

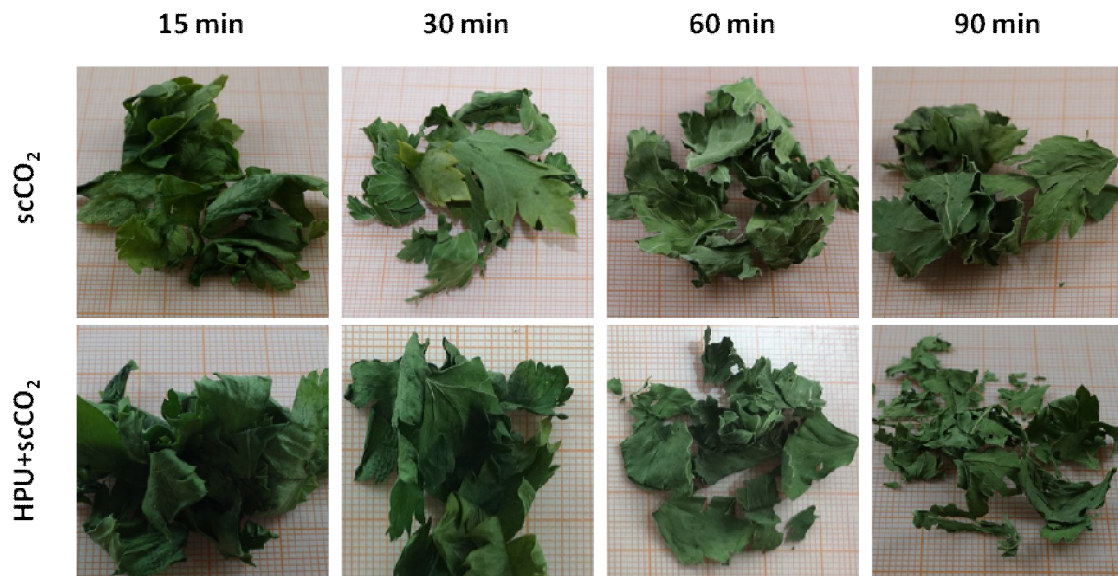


Figure 5 – Comparison of the coriander after the scCO₂ process alone (top) and in combination with HPU (bottom) at different drying times. Process conditions used were 40°C, 10 MPa, 90 min, with or without 40W HPU.

4. Conclusion

This work reported a feasibility study regarding the use of scCO₂ in combination with HPU to dry herbs. We demonstrated that coriander leaves can be dried in a shorter time in comparison with the single scCO₂ treatment, without a significant modification on the sample appearance. Furthermore we proved that CO₂ at supercritical conditions is able to inactivate microorganisms while drying the sample and that the inactivation is obtained mainly during the first phase of the process. Treatment time, in terms of drying time, does not significantly enhance the inactivation yield. Yeast and molds are the most sensitive microorganisms to the treatment, while mesophilic bacteria can be strongly reduced up to 4 log but they remain always above the quantification limit. Interestingly, the combined treatment shown a threshold at 40W that resulted significant for mesophilic spores inactivation. These results demonstrate that HPU+scCO₂ process has the potential to ensure a better inactivation of microorganisms compared to the scCO₂ treatment alone. Nevertheless additional studies are needed to demonstrate the synergic effect of HPU+scCO₂ combined process, specifically with pathogens, and in particular spore forming gram-positive bacteria, that might contaminate the food products. Moreover additional sensory analyses should be performed to evaluate the quality of the dried product also in comparison with the traditional air-drying technique.

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