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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 80 W), different drying times (up to 90 min) and two process temperatures (40 and 50 °C). At the most effective condition tested (40 W; 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). 40 W 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.

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 [12,13,28,39]. 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 [17,18,29]. 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.

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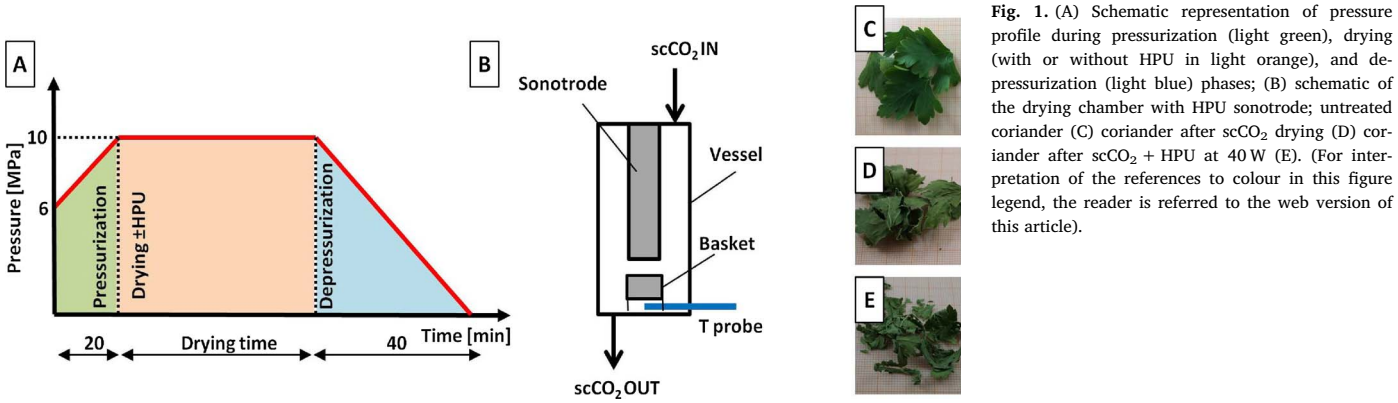

Fig. 1. (A) Schematic representation of pressure profile during pressurization (light green), drying (with or without HPU in light orange), and depressurization (light blue) phases; (B) schematic of the drying chamber with HPU sonotrode; untreated coriander (C) coriander after $scCO_2$ drying (D) coriander after $scCO_2$ + HPU at 40 W (E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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. 1C). 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. 1B). 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 Fig. S1 in the supporting information and a schematic representation of the drying chamber is shown in Fig. 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 Fig. 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 s each. Fig. 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 (Fig. 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 (Biotec 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 min 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 $Log(N/N_0)$, 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:

$$\text{Weight reduction} = W_{red} = (1 - m_{dry}/m_{fresh})*100 \quad (1)$$

where $m_{dry}$and m_{fresh} 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 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 Eq. (2):

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (2)$$

where L_0^* 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 min at 100 bar, 40 °C with supercritical