Microbial inactivation and drying of strawberry slices by supercritical CO2

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Abstract

This work explores the feasibility to apply supercritical carbon dioxide ($scCO_2$) to dry and increase the microbial safety of strawberries. Strawberry slices were dried at 10 MPa and 40°C up to 6 h. The treatment was able to remove the 98% of the initial moisture content, corresponding to a weight loss of 90%. The method showed a limited inactivation power towards total mesophilic bacteria, while yeasts and moulds were under the detection limit of the technique (<10 log CFU/g). The process is efficient against different strains of pathogens (*E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes)*, which were inoculated on the sample surface. Results highlight the potential of $scCO_2$ drying as innovative food drying technology for the simultaneous drying and stabilization of the product without the use of high temperatures.

Keywords: strawberry; supercritical drying; carbon dioxide; microbial inactivation

1. Introduction

The increasing demand for a healthy diet calls for an evolution of the food processing chain, with special attention to fruit and vegetable.

Among these products, berries, and in particular strawberries (*Fragaria x ananassa*), are very attractive for the consumers thanks to their characteristic flavour, texture and colour, together with their high amount of both nutritive and non-nutritive compounds (e.g. antioxidants, organic acids, vitamins) [1]. Fresh strawberries are affected by quick spoilage, mainly caused by microorganisms, as bacteria, yeasts and moulds, and oxidative-enzymatic deterioration, leading to a limited shelf-life. Fresh or frozen berries also show a high risk of foodborne pathogens contamination as *E. coli, Listeria monocytogenes, Norovirus* and *Salmonella enterica* [2]. In particular, contamination and cross-contamination via equipment, water and food handlers can be risk factors for *Salmonella* and *Norovirus*. Indeed, outbreaks associated with strawberry and raspberry consumption have been correlated with the presence of these pathogens on the surfaces of fresh or frozen products [3]. Thus, technologies able to increase the shelf life and safety of strawberries are highly desired.

A valid alternative to increase the storage time is represented by food drying, which is an effective and well-known food preservation technique. However, current drying technology showed a limited inactivation capacity against microorganisms [4]. Thus, even if the products are stabilized because microbial growth is inhibited by lowering the moisture content [5], additional decontamination steps, like gamma radiation [6], are often adopted increasing the overall cost of the technology.

Hot-air drying is the most widely used drying method due to its low cost and high efficiency, but the high temperatures involved lead to inevitable side effects on the nutritive components of the products [7]. Improved performances are obtained by freeze-drying, but its high cost renders it suitable only for high-value food applications [8].

Innovative or combined drying technologies have been extensively studied to develop an efficient process able to improve safety, enhance food quality and reduce costs. These technologies, like

vacuum and microwave drying [9], adopt mild condition in order to minimize organoleptic changes, increasing the risk of microorganism survival [10]. In the case of strawberries, good results were achieved when treated with infrared combined with hot-air drying [11], showing faster drying kinetics and lower quality losses, but high temperatures were still adopted. A reduction of costs of vacuum freeze drying has been achieved by pre-treating samples with ultra-high pressure and/or ultrasounds [12], but an optimization of the treatment time should be performed.

In this regard, the use of supercritical carbon dioxide ($scCO_2$) has been recently investigated as an alternative drying process for food obtaining promising results in fruits (apples [11][12][13], mango and persimmon[16]), herbs (coriander [10], basil [17]), vegetables (red pepper [18], beetroot [19]) and poultry (chicken breast [20]). The process was also coupled with High Power Ultrasounds to achieve a faster drying in coriander [21] and chicken breast [20]. The avoidance of a vapour-liquid interface and the relatively low critical point of the carbon dioxide allows a better preservation of the original structure and the prevention of sensitive degradation reactions [22]. Noteworthy, supercritical carbon dioxide has widely demonstrated its antimicrobial effect in solid and liquid products [23]. To the best of our knowledge, only few researches have been addressed to demonstrate the inactivation capacity of supercritical CO_2 on fresh strawberries. Haas *et al*. [24] investigated the use of high pressure CO_2 to reduce the mesophilic load from strawberries, but the treatments resulted in gross tissue destruction of the products. Other studies in literature focused on strawberry juice [25] and puree [26]. Application of $scCO_2$ on small fruits like strawberries is still missing.

This work aims at investigating the use of supercritical CO_2 for the inactivation and drying of strawberry slices. The inactivation capacity was investigated on the natural present microorganisms (i.e. total mesophilic bacteria, yeasts and moulds) and inoculated pathogens (*E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp.) at different drying time.

2. Materials and methods

2.1. Sample preparation

Fresh strawberries (*Fragaria x ananassa*) were purchased from a local market in Padova (Italy), stored at 4°C and treated within 3 days after the purchase.

Unwashed strawberries were cut into small pieces before processing. For each test 2 ± 0.1 g of product were used. Every test has been repeated at least in duplicate.

2.2. Supercritical CO_2 drying

For the experimental tests, a semi-continuous lab-scale high-pressure vessel (Separex S.A.S., Champigneulles, France) were used. The vessel consists of a sapphire high-pressure visualization cell with an internal volume of 50 cm^3 designed to withstand up to 40 MPa and 100°C. The plant includes a CO_2 tank, a chiller reservoir (M418-BC MPM Instruments, Milan, Italy), and a thermostatic water bath (ME-Julabo, Seelbach, Germany) to regulate the temperature in the vessel. The depressurization of the plant is controlled by a micrometric valve installed on the vessel outlet line. Further details of the plant can be found in our previous work [27].

Before each treatment, the vessel was cleaned with ethanol and the samples were inserted inside a metal basket, which was cleaned with ethanol and sterilized with a Bunsen flame. For the microbial experiments at 0 min drying time, samples were placed in a sterile 15 mL falcon tube (without cap), which was then inserted in the high-pressure chamber. The insertion inside the falcon tube was necessary to avoid loss of sample due to the foaming after the process (see 3.1). The drying and inactivation treatment has been performed in a dynamic mode, with carbon dioxide continuously flowing inside the vessel. Firstly, the pressurization step starts with a quick pressurization to around 5 MPa, which is the carbon dioxide tank pressure. Then, the chamber was pressurized to 10 MPa in 10 min with a constant pressurization rate (0.5 MPa/min). The CO_2 flowrate during the process was

fixed at 23 cm^3/min (about 1.26 kg/h), which is the maximum value of the pump. After the desired treatment time, the vessel was depressurized in 20 min (constant rate 0.5 MPa/min) manually acting on the manometric valve. The internal temperature, instead, was kept at 40°C.

2.3. Drying performance

In order to address the drying efficiency of the method, the weight of each sample was measured before and after the treatment with a precision balance (Radwag, PS 6000 R2). The weight loss was calculated with Equation (1):

$$Weight\ loss = W_{loss} = \left(1 - \frac{W_{dry}}{W_{fresh}}\right) * 100\% \quad (1)$$

where W_{fresh} and W_{dry} are respectively the sample weight before and after the treatment.

Moreover, the moisture content of the samples was measured as claimed by de Brujin & Bórquez [28]. Both treated and untreated samples were inserted in an incubator (G-Cell 035, Fratelli Galli, Italy) at 70°C for 2-4 hours, until constant weight was reached. The moisture ratio, which is the rate of water content in the dried products with respect to the one of the fresh samples, was estimated as previously reported by Zhang *et al.* [12]:

$$Moisture\ ratio = MR = \left(\frac{W_{dry} - W_{sm}}{W_{fresh} - W_{sm}}\right) * 100\% \quad (2)$$

where W_{sm} is the solid matter weight, which is the sample weight after the complete dehydration.

2.4. Mesophilic bacteria, yeasts and moulds analysis

Fresh and treated samples were mixed with Phosphate Buffer Solution (PBS, Oxoid, United Kingdom) with a weight ratio of 1:9 using a vortex (Velp scientifica, Italy) for 90 s. Mesophilic bacteria, yeasts and moulds were quantified through the standard plate count technique by serially diluting (1:10 dilution) in PBS. Total mesophilic bacteria were included in Plate Count Agar medium (PCA, Sacco, Italy) by pouring on 1 mL of the decimal solution. Plates were incubated at 30°C for

72 h. Yeasts and moulds were quantified by spreading 0.1 mL of the decimal solution on Rose Bengala Agar (RBA, Sacco, Italy) and incubating the plates at ambient temperature for 120 h.

2.5. Bacterial strains

Three strains of *Escherichia coli* O157:H7 (NCTC12900, BRMSID 188, LFMFP 846), *Listeria monocytogenes* (LMG 23192, LMG 23194, LMG 26484) and *Salmonella enterica* (serovars Thompson RM1987 and Typhimurium SL1344, LFMFP 883) were used as target pathogenic bacteria. Each strain, firstly stored on glass beads at -75°C, were revived in 10 mL of Brain Heart Infusion (GBI, Oxoid, England) for 24h at 37°C. The strains were then streaked on Tryptic Soya Agar (TSA, Oxoid, England) and on an appropriate selective medium. After 24h, one colony was taken from the selective medium and TSA slants were inoculated and incubated for 24h at 37°C. These cultures were stored at 4°C for up to 1 month.

Table 1 reports information about the selective medium, the antibiotic resistance and the origin of the utilised strains. The strain source and other details are reported in our previous work [10].

Table 1. *Selective medium, antibiotic resistance, and origin of the utilised strains*

Strain	Selective medium	Antibiotic resistance	Origin
Escherichia coli O157:H7			
NCTC12900	Cefixime-Tellurite Sorbitol MacConkey Agar	Nalidixic acid (50 µg/mL)	Isolate from humans
BRMSID 188		Kanamycin (100 µg/mL)	Isolate from bovine
LFMFP 846		Nalidixic acid (50 µg/mL)	Isolate from beef carpaccio
Salmonella			
S. Thompson RM1987	Xylose Lysine Deoxycholate	Nalidixic acid (50 µg/mL)	Fresh coriander (cilantro)
S. Typhimurium SL 1344		Streptomycin (100 µg/mL)	[29]
S. Typhimurium LFMFP 883		Kanamycin (100 µg/mL)	Environmental isolate
Listeria monocytogenes			
LMG 23192	Agar Listeria Ottavani & Agosti	NA	Liver paste
LMG 23194		NA	Wijnendaele cheese
LMG 26484		NA	Isolate from tuna salad

2.6. Inoculation

For each test, one inoculation mixture composed of the three strains of a given microorganism was prepared. The strains, obtained in form of stock cultures by Ghent University, were prepared into two steps. Firstly, a loopful of the slant culture was placed in 10 mL of fresh Brain Heart Infusion broth (BHI, Fluka analytical) for 6 h at 37°C. Then, the working culture was obtained sub-culturing 100 µL of the prepared solution in 10 mL of BHI broth for 18 h at 37°C. 500 µL of the working culture of each strain was then transferred into a 2 mL Eppendorf tube, mixed for 15 s using a vortex (Velp scientifica, Usmate, Italy) and centrifuged for 10 min at 2900 rpm. The supernatant was discarded and substituted with 500 µL of Phosphate Buffered Solution (PBS, Sigma Aldrich). After another 15 s vortexing step, 500 µL of each strain of the same inoculum were mixed together and suspended in 500 µL of PBS, in order to obtain a final volume of 2 mL. The microbial load of each inoculation solution has been tested by plate count.

Fresh strawberry slices were inoculated inside a sterile Petri dish under a biosafety cabinet, by dropping 16 ± 4 µL of the inoculation broth per gram of fresh product to obtain an initial load of at least 5.5 ± 0.5 log CFU/g. After inoculation, the samples were left 30 min to dry in the bio-safety cabinet at 22°C, allowing the attachment of the inoculated microorganisms on the surface.

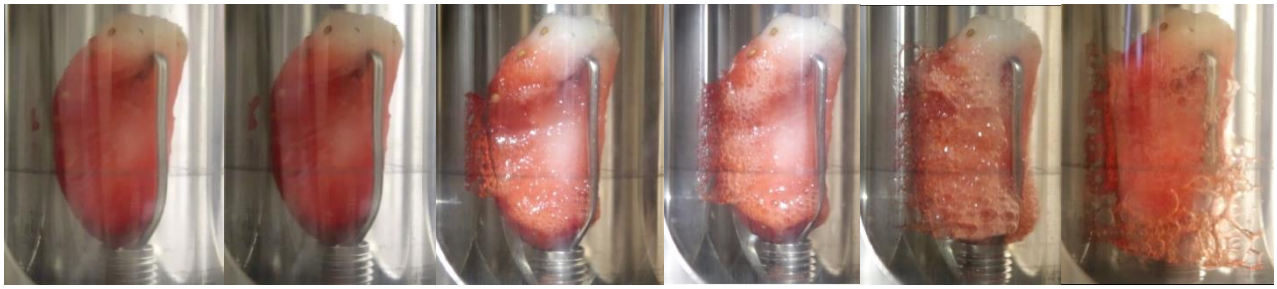
3. Results and discussion

3.1. Effect of supercritical CO_2 on fresh strawberries

Before investigating the drying effect of $scCO_2$ on strawberries, preliminary tests were performed in order to study the response of the product to the only pressurization and depressurization of the vessel. Indeed, high pressure treatments might have negative effects on the quality of some products as previously reported. Specifically, fruits, like honeydew melon, cucumber and also strawberries [30], were altered by the treatment, leading to a reduction of firmness and texture.

The sapphire windows of the high-pressure vessel allowed the visual observation of the sample during the depressurization phase. After noticing evident foaming after the depressurization, different depressurization rates were tested (data not shown) to understand their effect on the maintenance of the original structure and on the foaming formation.

Independently of the depressurization rate, the samples started foaming close to 4 MPa. The formation of the foam, together with a partial water dropping, lead to a weight loss of the samples up to 35%, with serious damage to the product. An example can be seen in Fig. 1, in which the effect of a 4-minute depressurization is reported.



(a) *(b)* *(c)* *(d)* *(e)* *(f)*

Fig. 1. *Effect of depressurization from 10 MPa to ambient pressure in 4 minutes on a strawberry slice. Pictures at different pressure during depressurization phase: (a) 10 MPa, (b) 5 MPa, (c) 3 MPa, (d) 2 MPa, (e) 1 MPa, (f) 0 MPa (ambient pressure).*

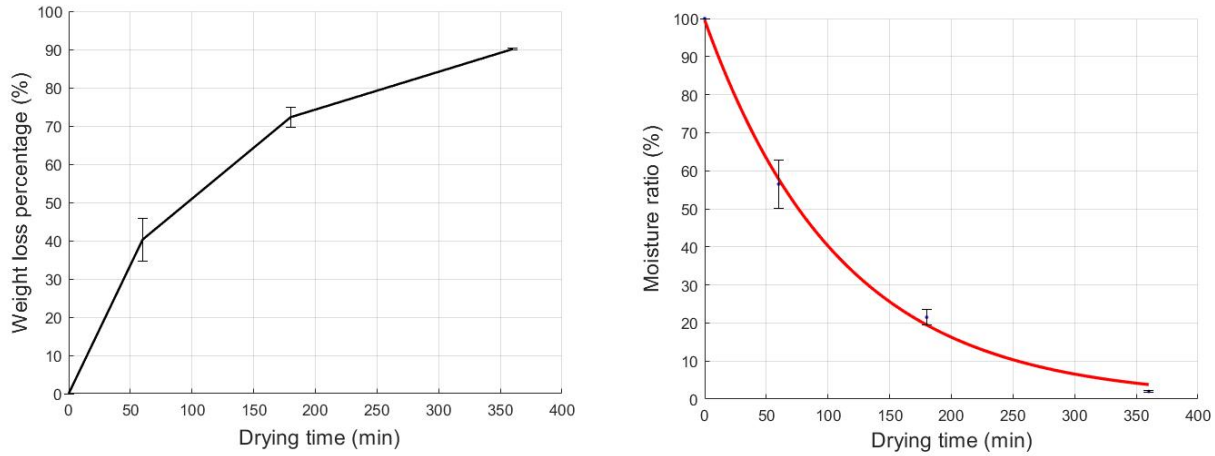
The tests confirmed the modification of the product structure making the $scCO_2$ pasteurization process not suitable for the treatment of small fresh fruits like strawberries.

After removing (even partially) the water content from the fresh sample with $scCO_2$ drying, the bubbling phenomenon was completely avoided. As a result, $scCO_2$ can be applied successfully in small fruits only as a drying process.

For the microbial analysis after only the pressurization and depressurization phase, the samples were inserted inside a 15 mL falcon tube (without cap) before being placed in the high-pressure vessel, avoiding the loss of product, which might have influenced the microbial count

3.2. Supercritical CO_2 drying

Fig. 2 shows the drying kinetics in terms of weight reduction (Fig 2a) and moisture ratio (Fig. 2b) measured after scCO_2 drying up to 6 hours.



(a) *(b)*

Fig. 2. *Drying kinetics of strawberry slices in terms of weight loss (a) and dry basis moisture content (b). In red is represented the fitting model from equation (3).*

After 6 hours of drying, strawberry slices lost 90.1 ± 0.3 % of their weight, which corresponds to 2.0 ± 0.0 % of moisture ratio. That means that the adopted drying procedure removed almost entirely (98%) the water content of the fresh samples.

The moisture ratio behaviour was efficiently fitted with an exponential model (R-squared = 99.83%), also shown in red colour in Fig. 2b, which is expressed by:

$$MR = 99.56 \exp(-0.00906 * t) \quad (3)$$

where *MR* is the molar ratio and *t* is the drying time in minutes.

The obtained result demonstrates the high efficiency of the water removal by $scCO_2$ drying treatment. Results achieved on vacuum-freeze drying allowed to obtain a moisture ratio of 8% after 20 h [12], which would be achieved in 4.6 h (=278.3 min) as predicted by the $scCO_2$ drying model.

3.3. Microbiological inactivation

In order to demonstrate the bactericidal effect of supercritical carbon dioxide, the inactivation of both natural flora and inoculated pathogens was addressed.

The initial load on the untreated fresh strawberries for mesophilic bacteria and yeasts & moulds was 3.02 ± 0.49 and 2.16 ± 0.29 log CFU/g, respectively. Strawberries from different suppliers have been tested for the naturally present microorganisms (data not shown) to choose a batch with a significant initial count. The inactivation has been measured after different drying time: 0 min, corresponding to pressurization and depressurization of the vessel only, and at 60, 180 and 360 min of drying.

Table 2 shows the inactivation kinetics of the mesophilic bacteria by $scCO_2$ treatment. Pressurization and depressurization phases had a slight positive effect on the microbial inactivation of mesophilic bacteria. However, the inactivation did not increase for any of the following drying time. Neither the 6-hour treatment, corresponding to complete water removal, leads to an increment of the microbial inactivation for the total mesophilic microorganisms. These findings suggest that only the most sensitive bacteria can be inactivated as previously observed for coriander [31]. In the case of chicken breast [20], an increase of the inactivation capacity was observed at higher drying time, demonstrating that the treatment is highly product dependent.

Table 2. *Inactivation on strawberry samples of mesophilic bacteria treated with $scCO_2$ drying (means ± standard deviations, in log CFU/g)*

Sample	Mesophilic bacteria load
Fresh sample	3.02 ± 0.49
0 h drying	2.48 ± 0.62
1 h drying	2.39 ± 0.39
3 h drying	2.36 ± 0.45
6 h drying	2.51 ± 0.43

Yeasts and moulds were completely inactivated (<10 CFU/g) after the pressurization and depressurization steps. A similar behaviour was previously observed for coriander [21,31] and chicken breast [20], demonstrating the high sensitivity of yeasts and moulds to $scCO_2$.

In order to further demonstrate the bactericidal effect of the supercritical CO_2 process, pathogenic bacteria were inoculated on the samples and treated at the minimum (0 min) and maximum (360 min) drying time previously tested. Three strains of three different pathogens (i.e. *E. coli, Salmonella* and *L. monocytogenes*) were studied.

Table 3 shows the initial load and the final count after the processes. The bactericidal effect of $scCO_2$ was significant against all the strains after the pressurization and depressurization phase. *Salmonella* and *Listeria* were the most sensitive to the process, reaching almost 2 log CFU/g reduction after the pressurization and depressurization step. *E. coli* O157:H7 were reduced less than 1 log CFU/g, demonstrating the higher resistance for short drying time. Comparing these results with the one achieved on apples [15], it is possible to confirm that $scCO_2$ inactivation is matrix dependent. The same pathogens inoculated on apple slices were completely inactivated after the pressurization and depressurization phase

After 6 hours of drying, all the pathogens were completely inactivated and below the detection limit of the technique (<10 CFU/g). Moreover, enrichment tests demonstrated the complete inactivation

on the samples (<1CFU/g). Similar results were also obtained for *Salmonella* on chicken breast [20], for which the complete inactivation was reached after only 45 min.

Table 3. *Inactivation of the inoculated pathogens on strawberry samples and treated with $scCO_2$ drying (means ± standard deviations, in log CFU/g) (U.D.: Under Detection)*

Microorganism	Initial count	Final count, after 0 min drying time	Final count, after 360 min drying time
Escherichia coli O157:H7			
BRMSID 188	5.31 ± 0.08	4.22 ± 0.32	U.D.
NCTC12900 & LFMFP 846	5.29 ± 0.19	4.29 ± 0.29	U.D.
Salmonella			
S. Thompson RM1987	5.56 ± 0.15	3.82 ± 0.04	U.D.
S. Typhimurium SL 1344	5.57 ± 0.28	3.86 ± 0.14	U.D.
S. Typhimurium LFMFP 884	5.28 ± 0.49	3.75 ± 0.03	U.D.
Listeria monocytogenes			
LMG 23192. LMG 23194 & LMG 26484	7.19 ± 0.39	5.23 ± 0.43	U.D.

4. Conclusions

This study investigated the possibility to use supercritical carbon dioxide to simultaneously dry and inactivate strawberries. The method allowed to remove 98% of the initial water content in 6 hours of treatment at 10 MPa and 40°C.

The microbiological analyses demonstrated that $scCO_2$ is able to efficiently inactivate yeasts and moulds by only pressurizing and depressurizing the samples, but mesophilic bacteria naturally present on strawberries are not significantly affected by the process, even after long drying treatments.

The bactericidal effect of the process has been confirmed by the complete inactivation of pathogenic bacteria after the 6-hours treatment.

Overall, this study demonstrated the efficacy of $scCO_2$ drying to inactivate microorganisms on strawberries. Nevertheless, additional studies should be performed in order to optimize the treatment time and efficiency, and to evaluate the quality aspects of the final product in comparison with other drying techniques.

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