Microbial inactivation and drying by supercritical carbon dioxide

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1. Introduction

Food drying is an effective and well-known preservation technique that can be applied to a great variety of fresh foodstuff to increase their shelf life and safety. Among the numerous drying techniques, the use of supercritical carbon dioxide ($scCO_2$) has been recently investigated and promising results have been obtained for different fruits (apples[1,2], mango and persimmon[3]), herbs (coriander[4], basil[5]), vegetables (red pepper[6], beetroot[7]) and poultry (chicken breast[8]). The process was also coupled with High Power Ultrasounds to achieve faster drying in coriander[4] and chicken breast[8].
Strawberries (*Fragaria x ananassa*) are one of the most consumed and appreciated fruit 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)[9]. However, fresh strawberries are affected by quick spoilage due to the action of different microorganisms and oxidative-enzymatic deterioration, leading to limited shelf life. Fresh or frozen strawberries also show a high risk of foodborne pathogens like *E.coli*, *Listeria monocytogenes* and *Salmonella enterica*[10,11].
This work aims at investigating the use of supercritical CO_2 for the drying and microbial inactivation of strawberry slices as a case study.

2. Materials and Methods

Preliminary trials were addressed by using a semi-continuous drying plant[12]. The plant consists of a high visualization cell with an internal volume of 50 cm^3, a CO_2 tank, a chiller reservoir and a thermostatic water bath to regulate the temperature in the vessel. Temperature, pressure and CO_2 flow rate were fixed at 40°C, 10 MPa, and 1.26 kg/h, respectively. The chamber was pressurized in about 10 min and depressurized in 20 min. Drying performances were monitored for a drying time of up to 6 h by calculating the weight loss and the moisture ratio of the strawberry slices, by means of the following equations:

$$Weight\ loss = \left(1 - \frac{W_{dry}}{W_{fresh}}\right) * 100\% \quad \text{and} \quad Moisture\ ratio = \left(\frac{W_{dry} - W_{sm}}{W_{fresh} - W_{sm}}\right) * 100\% \qquad (1)$$

where W_{fresh}, W_{dry} and W_{sm} respectively represent the weight of the fresh sample, the dried sample and the solid matter (obtained as described in [13]).
Mesophilic bacteria, yeasts, and moulds were quantified through the standard plate count technique. Target pathogenic bacteria, *E. coli*, *Salmonella* spp. and *Listeria monocytogenes* were inoculated on the fresh samples (around 5.30, 5.47 and 7.19 Log CFU/g, respectively) and quantified after the drying procedure. Microorganism enumeration techniques are reported in our previous work [1].

3. Results and discussion

Drying kinetics

Table 1 shows the drying kinetics in terms of weight reduction and moisture ratio. After 6 h of drying, strawberry slices lost around 90% of their initial weight, which corresponds to 2% of moisture content.

The moisture ratio profile was efficiently fitted with an exponential model (R-squared=99.83%), expressed by $MR = 99.56 \exp(-0.00906 * t)$, where MR is the moisture ratio and t is the drying time.

Table 1: Weight reduction and moisture ratio of strawberry slices during scCO2 drying (40°C, 10 MPa, up to 6 h)

Drying time	Weight reduction (%)	Moisture ratio (%)
0	-	100.0 ± 0.0
1	40.3 ± 5.7	56.5 ± 6.4
3	72.3 ± 2.6	21.5 ± 2.1
6	90.1 ± 0.3	2.0 ± 0.0

Microbial inactivation

Table 2 reports the microbiological inactivation data of both natural flora (mesophilic bacteria and yeasts & moulds) and inoculated pathogenic bacteria (*E. coli, Listeria monocytogenes* and *Salmonella* spp.) after 0 h (only pressurization and depressurization) and 6 h of drying. The obtained results for the mesophilic bacteria inactivation suggest that they are not very affected by the process up to 6 h. Anyway, if the weight loss is considered, the final count can be expressed as 1.51 ± 0.43 log CFU per g of fresh sample, with respect of 3.02 ± 0.49 log CFU/g, which is the initial load. Yeasts and moulds are instead completely inactivated after the pressurization and depressurization steps. Regarding the inoculated pathogenic bacteria, after 0 h of treatment, the inactivation was already significant, especially for *Listeria monocytogenes* which seems to be the more sensitive to the treatment. After 6 h of drying, all the analyzed bacteria were under-detected (< 10 CFU/g). Enrichment tests also demonstrated complete inactivation (< 1 CFU/g).

Table 2: Inactivation on strawberry samples of mesophilic bacteria, yeasts & moulds and inoculated pathogenic bacteria treated with $scCO_2$ drying (means ± standard deviation, in log CFU/g) (U.D.: Under Detection, < 10 CFU/g)

Microorganism	Initial count	Final count, after 0 min drying time	Final count, after 360 min drying time
Mesophilic bacteria	3.02 ± 0.49	2.48 ± 0.62	2.51 ± 0.43
Yeasts and moulds	2.16 ± 0.29	U.D.	U.D.
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 use of $scCO_2$ for the drying and microbial inactivation of strawberries. Results demonstrated the drying efficiency of the method, being able to remove up to 98.0% of the initial water content of the fresh sample. Microbial inactivation studies also showed the safety of the final product, especially regarding yeasts and moulds and pathogenic bacteria (*E. coli*, *Salmonella* spp. and *Listeria monocytogenes*). A design of experiment should be performed to optimize the method in terms of temperature, pressure, treatment time and CO_2 flow rate, and to evaluate the quality aspects of the dried products with respect to other techniques. Performances could be improved by using a drying plant with recirculation and regeneration of carbon dioxide, allowing higher flow rates, and hopefully lower treatment times, while still maintaining a contained cost. The use of High Power Ultrasounds should be also tested to study a possible synergic effect.

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