STERILIZATION OF PAPRIKA POWDER USING HIGH PRESSURE CO_2

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ABSTRACT

This work describes the use of supercritical CO_2 for the inactivation of the microbial contamination of paprika powder. Different operational parameters have been explored. First, we explored the effect of pressure between 60 and 300 bar. The increase in pressure did not significantly influence the inactivation effect. Inconveniently, at high pressures there was oleoresin extraction so the operation pressure was set at 60 bar for most of the study. Then, we studied the effect of temperature, which was increased from 20° C to 95° C, the addition of water up to 20% and the treatment time from 10 to 120 minutes. As expected, the more aggressive the conditions were, the more efficient the treatment was. By adding water, we discovered a significant increase in the effect of microbial inactivation, but only up to 10% addition. The method was effective in eliminating coliforms (*Escherichia coli* and others), the enterobacteria *Enterobacter cloacae*, the streptococcus *Enterococcus faecalis*, molds and yeasts (*Fusarium exosporium*, *Sacharomyces cerevisiae*) and *Staphylococcus aureus* which were inoculated to the paprika. No significant changes in the quality parameters: color and water content were produced by the contact with the CO_2. Nevertheless, complete sterilization was not possible due to a protective effect of the solid matrix or a poor contact with the CO_2.

INTRODUCTION

The more efficient and available techniques to inactivate micro organisms in food are the thermal treatments. Nevertheless, there is an increased interest in the development of other techniques, such as irradiation, treatment with ozone or the application of high hydrostatic pressures (HHP). Among, these alternatives, the use of dense CO_2 has progressively gained interest since the 80s. The use of pressurized CO_2 presents several advantages versus the other technologies. The CO_2 is inert, non toxic, accessible and cheap. At room conditions, it is a gas; so, it does not leave residues in the treated product. It is considered a GRAS solvent, which means that is tolerable for the use in food. It is used at near or supercritical conditions where it acquires very good mass transfer properties for the penetration into solid matrices. Its critical temperature is low, so the organic thermolabile products may be processed with no risk of denaturalization or decomposition.

Most of the research has been conducted using suspensions of pure cultures of different micro organisms and the method has been preferentially applied to liquid food products, such as juices, beer, wine, milk, etc [1, 2]. Little work has been conducted in solid food products; however, much more work is necessary in order to evaluate the efficiency of the method once the microbes are in their original environment.

In a previous investigation conducted by our team, it was demonstrated that it was possible to sterilize cocoa powder by high pressure CO_2 [3]. Here, the objective is to study the inactivation of the natural and artificially inoculated microbial contamination in paprika powder, using high pressure CO_2 as an alternative to traditional treatments. Additionally, the aim is to analyze the effect of the treatment on the quality of this foodstuff, measured in terms of color and humidity.

MATERIALS AND METHODS

Materials. Paprika powder with a water content ranging from 5 to 8% was used as raw material. Apart from the natural contamination, it was inoculated with several micro organisms obtained from the Spanish Type Culture Collection (CECT), including *Fusarium exosporium* (CECT 2154, EAN 350**)**, *Enterobacter cloacae* (CECT 194, ATCC 1304), *Saccharomyces cerevisiae* (CECT 1325, ATCC 13602), *Enterococcus faecalis* (CECT 481, ATCC 19433), *Eschirichia coli* (CECT 515, ATCC 11775) and *Staphylococcus aureus* (CECT 435, ATCC 25923). The count of this contamination is shown in Table 1. Liquid carbon dioxide at its vapor pressure (about 50 bar), 99.998% purity, was kindly given by Carburos Metálicos, S.A.

Table 1: Microbial contamination of the raw material.

Aerobic mesophilic count (cfu/g)	**Enterobacteria (cfu/g)**	**Coliforms (cfu/g)**	***E.coli* (cfu/g)**	***E. faecalis* (cfu/g)**	**Molds and yeasts (cfu/g)**	***S. aureus* (cfu/g)**
$11 \cdot 10^5$	$27 \cdot 10^4$	$38 \cdot 10^4$	$11 \cdot 10^3$	$84 \cdot 10^4$	$12 \cdot 10^3$	$59 \cdot 10^4$

Experimental apparatus. A semi continuous flow apparatus consisting on a CO_2 feeding line and a 50 mL capacity 316 ss vessel equipped with a heating jacket to control temperature was used. Pressure was given by a membrane pump while the flow rate was controlled by a metering valve placed at the end of the line. The details of the equipment are described in [3].

Experimental procedure. Distilled water was added to 12 grams of paprika powder to reach the required percentage humidity. The powder was then loaded to the vessel forming a fixed bed. Redistributors made of stainless steel mesh were introduced every 1.5 cm approx. to avoid channelling. A glass wool stopper prevented paprika particles entrainment. Once the bed was at the target temperature, the CO_2 was preheated and pumped in. The CO_2 entered the bed from the bottom. At the desired pressure, the valve was opened to allow the flow of CO_2 which was set at about 4 grams CO_2/ min. When the treatment was completed, the system was depressurized and the treated paprika was collected in sterile atmosphere. Then, it was sealed and kept refrigerated to conduct the microbial analysis.

Microbial analysis. Five grams of paprika powder were added to 45 mL peptone solution under sterile conditions. Serial dilutions were prepared (10^1,10^2,10^3,10^4 y10^5) and 1 ml of every dilution was cultured in Petri dishes containing 15 mL the specific medium for each type of microorganism. Mesophilic aerobic micro organisms count was analyzed in PCA, Scharlau at 30ºC for 24 hours. Enterobacteria analysis was made in VRBD, Scharlau at 37ºC for 24 hours. Molds and yeasts were determined in OGYEA, Scharlau at 25ºC for 5 days. Coliforms and *E. coli* were counted in Chromocult®, Merk at 37ºC for 24 hours. *E. faecalis* analysis was done in KAA, Sharlau at 37ºC for 48 hours. *Staphylococcus aureus*

was determined in Baird Parker, Scharlau at 37ºC for 48 hours. The results were expressed in colony forming units per gram (cfu/g) and the degree of inactivation was reported as the logarithm of the ratio of surviving cells after treatment with CO_2 (N) to the cell count in the control culture (N_0).

Quality analysis. The percentage of humidity and the color intensity (in ASTA units) were used to assess the quality of the treated paprika. The humidity was determined by difference in weight following the instructions given in [4]. The color analysis was carried out by measuring the absorbance of an acetone extract at 460 nm according to the standard method of the American Spice Trade Association, ASTA 20.1, 1997. The average standard deviation in the readings was 3 ASTA units

The assays and the analysis were conducted at least by duplicate which means that every point is the average of a minimum of four measurements. The standard deviation on the reported counts was typically within 1.0 log cycles.

RESULTS

As Table 1 shows, the initial contamination of the paprika consisted of aerobic mesophilic micro organisms plus other pathogen bacteria, moulds and yeasts that are typically present in this product and can cause its rapid deterioration or be harmful to humans. The aim of this work was to investigate if the application of high pressure CO_2 was useful in the elimination of this contamination while preserving the quality of the paprika. For that purpose, the impact of various parameters that affect the antimicrobial capacity of the CO_2 was explored. The study is first focussed on the total count of aerobic micro organisms. Then, the inactivation of the inoculated germs is briefly discussed. Finally, the impact of the treatment on the quality of the product is analyzed.

Effect of pressure

In liquid phase studies, the rate of microbial deactivation increased as pressure was raised [1, 2]. Conversely, in our research, the variation of the pressure from 60 to 300 bar was not beneficial at any temperature or any treatment time. As the pressure was increased, the degree of inactivation practically remained constant (results not shown). Similar results were previously obtained in the sterilization of cocoa powder [3]. However, the raise in pressure significantly modified the density (i.e. solvent capacity) and the diffusivity (i.e. mass transfer properties) of the CO_2. We think that in liquid samples, pressure was an important factor because a previous step of dissolution of the CO_2 before its contact with the cells was necessary. This is not the case in solid powdered samples, where the contact between the CO_2 and the micro organisms directly occurs on the surface of the powder particles. Therefore, it is more important to provide a good contact between the CO_2 and the microbial cells than to apply high pressures which on the other hand, would increase the costs of the process. These results also suggest that the effect of the CO_2 is more related to a chemical effect than to a physical hydrostatic action.

Effect of product initial moisture

Previous works on the microbial inactivation of cocoa and spices showed that carbon dioxide under pressure was microbiocidal only in the presence of some water [3, 5]. The

raw material came with some humidity but we added increasing quantities of water up to 20% by weight. Then, the samples were treated with CO_2 at 60 bar and 80ºC for 30 min. The results in terms of total aerobic count are shown in Figure 1. The inactivation degree progressively augmented up to 20% total humidity. Higher amounts were not beneficial. The reason for the need of water is not completely clear since in solid media, there is no need for CO_2 dissolution. Probably water provoked membrane cell swelling allowing an easier CO_2 penetration and attack [2].

Effect of temperature and treatment time

The impact of increasing temperatures and treatment times is shown in Table 2. As expected, the degree of inactivation improved as temperature was raised, because higher temperatures facilitates the diffusion of CO_2 and increases the fluidity of the cell membrane. As a result, the dense CO_2 may penetrate easier [2]. The increase in contact time also improved the efficiency. However, the achieved inactivation was not large even after two hour treatment at 95ºC. The contamination probably corresponded to highly thermo-resistant spores which are very difficult to eliminate. Although, Watanabe *et al*., 2003 proved that it is possible to achieve a 5 log order spore inactivation of *Geobacillus stearothermophilus* at 95ºC for 120 min [6]. This fact indicates the solid matrix might be exerting a protecting effect or the contact between the CO_2 and the spore cells is not sufficiently good. Perhaps, the humidity and the oleoresin made the particles stick one to each other preventing the CO_2 reach all the micro organisms' cells. We are investigating both possibilities.

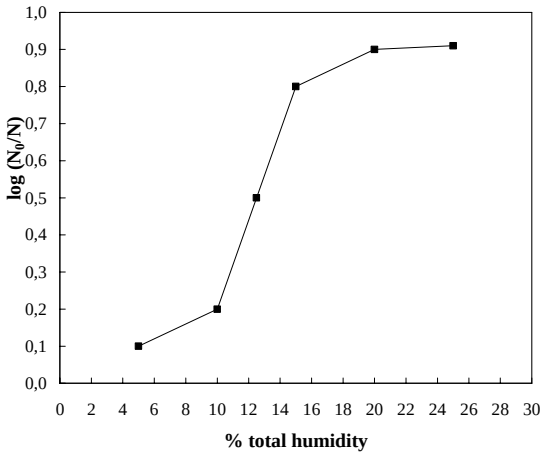

Figure 1: The influence of percentage humidity on the inactivation of mesophilic aerobic micro organisms in paprika powder treated with dense CO_2 at 60 bar and 80ºC during 30 min.

Temperature (ºC)	Treatment time (min)	Log (N_0/N)
60	10	0.1
70	10	0.2
80	10	0.6
60	30	0.2
80	30	0.8
95	30	1.2
80	60	0.9
95	60	1.3
95	120	1.7

Table 2: The impact of temperature and treatment time on the inactivation of mesophilic aerobic micro organisms in paprika powder treated with dense CO_2 at 60 bar.

Effect of the treatment on the inoculated micro organisms

Table 3 compiles the work done by other researchers in the inactivation on some of the same micro organisms that we inoculated into the paprika, but in liquid media. Usually at moderate pressures and temperatures lower than 40ºC, all these germs were inactivated in log ratios higher than six which is considered the minimum reduction to achieve

sterilization. We discovered that at 60ºC after 10% water addition and 10 min contact time, all of them were inactivated too. Nevertheless, we are studying if it is possible to achieve same result but at lower temperatures.

Table 3 : Results of the application of high pressure CO_2 on the inactivation of several micro organisms in batch operation.

Microorganism	Liquid medium	Process conditions			Log(N_0/N)	Ref.
		P (bar)	T (ºC)	t (min)		
S. cerevisiae	Nutrient broth	90	38	6	6.9	7
	Nutrient broth	68	35	6	7.0	8
E. faecalis	Physiological saline	61	35	18	7.5	9
E. coli	Nutrient broth	100	30	50	7.5	10
		75	30	65	7.5	
	Physiological saline	80	40	15	6.0	11
		100	35	30	8.0	
S. aureus	Nutrient broth	70	25	100	7.0	12
		80	25	60	7.0	

Quality of the paprika powder after the CO_2 treatment

The fundamental parameter to determine the category of paprika is the color intensity. Three paprika categories exist according to this factor: extra (106.6 ASTA units), first (77.9 ASTA units) and second (49.2 ASTA units). Table 4 shows the color measurements of selected samples before and after the treatment with high pressure CO_2. In general terms, a slight color reduction of about 6 ASTAs was observed in the treated samples together with some browning at the highest temperatures and longest treatment times. In most cases, this did not cause a reduction in the category of the paprika. The single experiment where there was a considerable color change occurred at the highest pressure because there was extraction of oleoresin and pigments.

The other factor used as quality standard for exportation is the water content that can not surpass 14% w/w. None of the samples violated this norm in spite of the water addition, as demonstrated in Table 4. During the process, the samples were partly dried because some of the water was dissolved in the CO_2 or simply evaporated due to the application of heat. Consequently, the final water content was lower at the longest treatment times and highest temperatures.

CONCLUSION

The aim of this research was to explore the use of high pressure CO_2 to sterilize paprika powder. The most conflictive pathogen bacteria, molds and yeasts were easily inactivated. Nevertheless, the aerobic total count was difficult to reduce probably because of the presence of thermo-resistant spores, the protective effect of the solid matrix and/or the lack of good contact between the CO_2 and the powder particles. The increase in pressure did not exert a significant impact; thus, it was kept at low values to avoid extraction of oleoresin and the loss of color. From a possible implementation at industrial scale, this fact is also important to diminish the operation costs. On the contrary, the inactivation

efficiency improved as temperature and treatment time increased. The addition of some water to the paprika powder before the treatment was highly beneficial. It might help membrane cell swelling, facilitating the penetration of the CO_2 and lastly, the cells death. About 20% total humidity was enough and part of it was removed during the procedure to comply with the levels marked by legislation for secure storage, manipulation and exportation. The treated product preserved its quality in terms of color keeping its category. Therefore, the method could be useful to achieve the microbial reduction stated by law for spices, at milder conditions than the traditional thermal treatment (e.g., temperatures higher than 120ºC for several minutes).

Table 4 : Quality analysis of selected paprika samples treated at different conditions.

Pressure (bar)	Temperature (ºC)	Treatment time (min)	Inicial humidity (% w/w)	Added humidity (% w/w)	Final humidity (% w/w)	Inicial colour (ASTA units)	Final colour (ASTA units)
Effect of temperature							
60	25	10	5.1	10	13.6	107	102
60	60	10	5.1	10	13.3	76	70
60	70	10	5.1	10	13.2	76	70
60	80	10	5.1	10	13.3	69	62
60	95	10	5.1	10	5.6	69	62
Effect of treatment time							
60	60	10	5.1	10	13.3	76	70
60	60	30	5.1	10	11.7	76	69
60	80	10	5.1	10	13.3	69	62
60	80	30	5.1	10	10.2	69	67
Effect of pressure							
60	80	30	8.1	15	14.1	69	63
80	80	30	8.1	15	NA	69	70
100	80	30	8.1	15	NA	69	63
300	80	30	8.1	15	10.6	69	45

ND: not analyzed

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