PRESERVATION OF NUTRITIVE PROPERTIES OF TOMATO SAUCE BY HIGH PRESSURE CO_2 PASTEURIZATION

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Traditionally, heat processes have been used to ensure the safety of foods against pathogenic and spoilage microorganisms, and enzymatic activity. However, thermal energy inevitably leads to destruction of heat-sensitive nutrients, texture, colour and flavour. In the tomato processing industry retention of viscosity is one of the most important quality criteria; viscosity loss and cloud reduction are a direct consequence of the degradation of pectic substances by endogenous enzymes, in particular pectinmethylesterase (PME) and endo-polygalacturonase (PG); the enzymes activity may not be completely interrupted by classical heating processes.
High pressure CO_2 treatment could be an alternative to the heating of tomato products for preservation. The mild conditions of temperature, pressure and contact time allow the maintenance of fresh product characteristics destroying microbial contaminants, and inactivating endogenous enzymes. In this work, a study is presented to fill the lack of properly documented inactivation kinetics in tomato sauce by high pressure CO_2 technology, a key information in the high pressure processes for food preservation.
Experimental batch runs were performed to determine the inactivation kinetics of *Bacillus subtilis* bacteria at 35°C and 90 bar in tomato sauce, obtaining total inactivation after 75 minutes. Operating conditions were changed in order to understand their influence on bacteria inactivation, in the 7-45°C range of temperature and in the 75-150 bar range of pressure, at pre-selected treatment time.
It is concluded that high pressure CO_2 is effective to carry out the preservation of tomato sauce inoculated with bacteria at low temperature. Changes in colour, flavour and firmness of processed tomato sauce were not observed. No changes were also detected on treated samples which were maintained in the refrigerator for several days.

INTRODUCTION

The preservation of fresh aroma and flavour of fruit juices and vegetables is one of the most important goals of the modern food industry. Actually, the consumers' demand is oriented to natural and minimally processed products, with high quality and nutritional power. Thermal pasteurisation is typically used for shelf-life extension and inactivation of foodstuffs but it may have a detrimental effect on natural properties; in particular, the process temperature can

lead to vitamin and protein denaturation and the oxidation of organic components gives colour and taste changes.
High hydrostatic pressure treatment (HHP) is an alternative method for microorganisms inactivation investigated for long time, but it is often inadequate for some foods (for example, due to loss of nutritional values and texture, residual enzyme activities causing product browning). Moreover, this treatment requires very high process pressure (thousands of atmospheres), involving higher plant costs. Porretta and co-workers [1] have tested the ultra-high hydrostatic pressure treatment on tomato juice, obtaining an absolutely inedible food owing to a strong rancid taste, due to a remarkable increase in *n*-hexanal content, as well as an increased jelly-like translucent structure due to protein-tissue coagulation and compacting. On the contrary, consistency and retention of viscosity are fundamental characteristics in the tomato processing industry. As observed by others researchers [2,3], viscosity loss in tomato-based products is the direct consequence of the degradation of pectic substances by endogenous enzymes, pectinmethylesterase (PME) and endo-polygalacturonase (PG). The main cause for texture and viscosity loss is the breakdown of the pectin molecules induced by PG, whereas PME leads to cloud reduction in vegetables and fruits juices. Then, the inactivation must regard not only microorganisms but enzymes also [4,5].
High pressure carbon dioxide can be used as antimicrobial agent on foodstuffs because it is free from toxicity, it can be easily removed from treated products and its sterilizing power is ascertained by now. In previous works, published in open literature, there are no application of supercritical or high pressure CO_2 on tomato products, and this work checked the new application.

EXPERIMENTAL APPARATUS AND OPERATING PROCEDURE

Figure 1 presents the experimental set-up used for kinetic data determination.
Liquid CO_2 is sucked by pump P after under-cooling in CH; the function of the damper MM is to attenuate the pressure fluctuations and to set the maximum operating pressure. The CO_2 accumulator L allows the cell pressurization in short time, so this step does not influence the inactivation mechanism. The bath BT provides the freezing mixture, which flows in a brass coil, to the accumulator L. The reactor cell C is plunged in a thermostatic water bath V for temperature control; the freezing mixture can circulate in the coil submerged in V in order to attain lower temperatures. The CO_2 enters the cell at the bottom for better contact with the liquid treated; PC data acquisition continuously monitors the temperature and the pressure of the cell.
The cell is a cylindrical pressure vessel with screwed flanges and 73.6 mL of internal volume; batch experimental runs require total closure of vessel whereas in semi-continuous process a continuous CO_2 flow through the liquid phase is maintained. In this work only the first operating method was applied.
The tomato sauce was purchased in local supermarket; 15 mL of sauce were inoculated with *Bacillus subtilis* bacteria, obtaining an initial concentration of 10^7 CFU/mL, and loaded into the cell. When the desired temperature was reached, the cell was pressurized and valves V-2, V-3, V-4, V-5 remained closed throughout the experimental run.

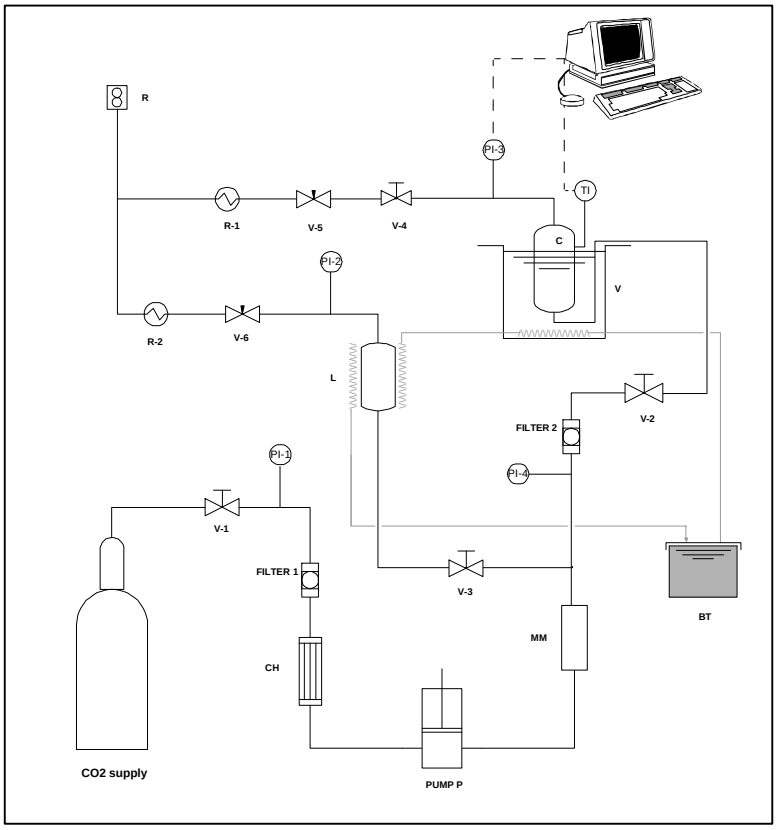

Figure 1. Schematic of batch and semi-continuous flow systems for microbial inactivation with high pressure CO_2. CH: cooling system; MM: damper and pressure regulator; L: CO_2 accumulator; V: thermostatic bath; C: reaction cell; V-1, V-2, V-3, V-4: on-off valves; V-5, V-6: regulation valves; R-1, R-2: resistance coils; TI: digital temperature indicator; PI-1, PI-2, PI-4: analogical pressure indicators; PI-3: digital pressure indicator; R: CO_2 meter.

The method of plate counting was adopted for inactivation degree determination and results were expressed as $(N_0/N)\%$ where N_0 indicates the *Colony Forming Units* of the control sample per mL (CFU/mL) and N those of the treated sample. The growth medium chosen for *Bacillus subtilis* was BHA (*Brain Heart Agar*), in Petri dishes.

RESULTS

Inactivation kinetics of Bacillus subtilis bacteria in tomato sauce.

In order to obtain the inactivation kinetics of *Bacillus subtilis* in tomato sauce, the sample was treated at 90 bar of pressure of CO_2 and at 35 °C, whereas the contact time was varied. Table 1 and Figure 2 shows the results obtained. Each experimental point is the arithmetic mean of two experimental runs at least, carried out at exactly the same operating conditions.

Table 1. Inactivation kinetic of *Bacillus subtilis* bacteria at 35 °C and 90 bar in tomato sauce in batch process.

Treatment time (min)	***Initial microbial load N_0 (CFU/mL)***	***Final microbial load N (CFU/mL)***	***Survival (N/N_0%)***
10	7.02E+06	1.32E+06	1.87E+01
15	1.79E+08	2.03E+05	1.13E-01
20	3.82E+06	1.29E+05	3.38E+00
30	2.14E+07	4.10E+02	1.92E-03
45	1.53E+07	6.68E+03	4.37E-02
60	3.55E+07	9.00E+02	2.54E-03
75	6.16E+06	0.00E+00	0.00E+00

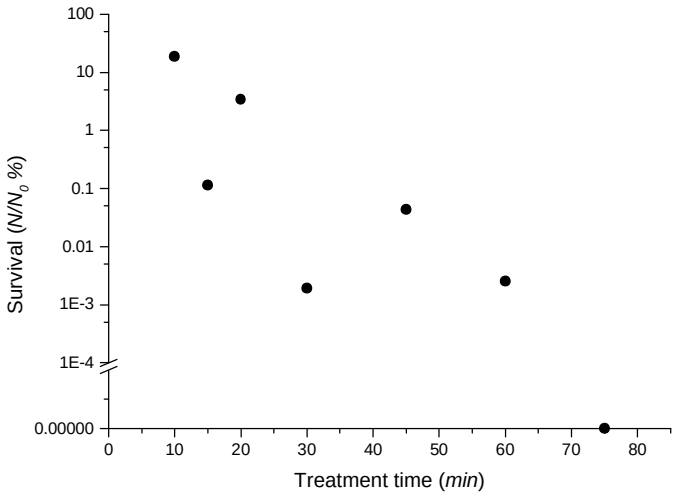

Figure 2. Inactivation of *Bacillus subtilis* in tomato sauce at 35 °C and 90 bar.

As reported in Table 1, the total inactivation of initial microbial suspension is attained after 75 minutes of treatment; in experimental runs carried out for 90 and 120 minutes this result have been confirmed. In according to qualitative observations, the treatment leads to a commercial tomato sauce without changes in colour, aroma texture and firmness. Moreover, these characteristics are preserved maintaining the treated sauce in domestic refrigerator for several days.

For determining the influence of operating variables on bacteria inactivation, the inoculated tomato sauce was subjected to different pressure and temperature, at selected treatment time equal to 45 minutes. Figure 3 represents the results obtained at 90 bar changing the temperature in the range from 7 to 45 °C.

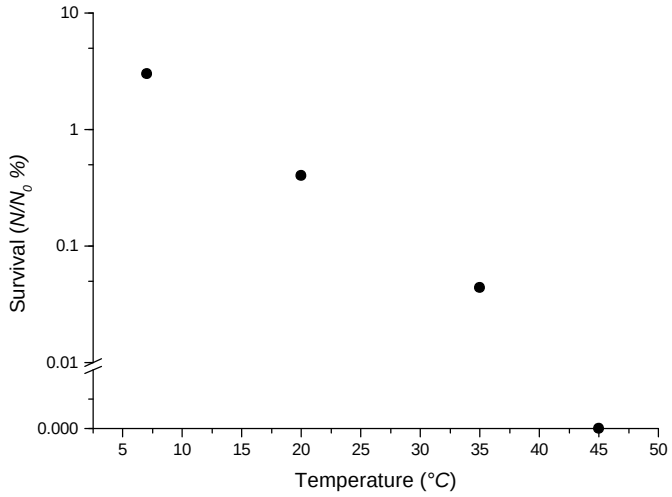

Figure 3. Batch inactivation of *Bacillus subtilis* in tomato sauce at 90 bar, 45 min and different temperature.

It is remarkable that *Bacillus subtilis* is totally inactivated at 90 bar, 45 °C and 45 min, which are relatively mild conditions for a complex substrate as tomato sauce.

Table 2. Batch inactivation of *Bacillus subtilis* in tomato sauce at 35 °C, 45 min and different pressure.

Pressure (bar)	***Initial microbial load N_0 (CFU/mL)***	***Final microbial load N (CFU/mL)***	***Survival (N/N_0%)***
75	1.05E+07	3.25E+01	3.09E-04
90	1.53E+07	6.68E+03	4.37E-02
120	9.06E+06	0.00E+00	0.00E+00
150	6.70E+06	0.00E+00	0.00E+00

The inactivation trend is unexpected when the pressure is changed in the range from 75 to 150 bar, at 35 °C, as reported in Table 2.

Enzymatic inactivation in fresh tomato pulp.

A high-consistency tomato product has almost complete absence of syneresis [1], namely absence of separation into pulp and serum; this phenomenon is due to the enzymatic activity of pectinmethylesterase (PME) and endo-polygalacturonase (PG). For qualitative study on enzymatic inactivation, fresh squeezed tomato pulp was treated with supercritical CO_2 (35 °C, 90 bar for 75 min) in batch process. The control and treated samples were maintained for

many days in refrigerator and at room temperature, in sterile and hermetic test tubes. In the control sample separation of serum was observed, whereas the tomato pulp subjected to high pressure CO_2 treatment remained completely unchanged. This means that also enzymes are most probably inactivated by CO_2.

CONCLUSIONS

High pressure CO_2 pasteurization of tomato sauce is effective in order to obtain its preservation. The very mild conditions of treatment allow maintaining the properties of fresh tomato products as taste, colour, firmness and consistency. This is due both microbial and enzymatic inactivation; however, further studies and analysis on CO_2-foodstuff interactions are necessary. The shelf-life extension of treated food is another important parameter in its high quality definition; the proposed pasteurization method is very promising in this direction.

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