Non-Thermal Pasteurization of Tomato Puree by Supercritical CO_2 And N_2O

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The use of supercritical fluid technology as an alternative non-thermal pasteurization technique for food is receiving more and more interest because the current pasteurization and sterilization systems usually leads to leakage of nutritional substances and change in food characteristics and organoleptic properties.
Supercritical carbon dioxide (CO_2) and nitrogen protoxide (N_2O) pasteurization of tomato paste was performed on a multi-batch apparatus at condition between 50-100 bar and 30-55°C and in order to study the inactivation kinetics.
The results showed that the pasteurization process with N_2O is pressure-dependent, while that one with CO_2 is temperature-dependent. In addition, the treatment with supercritical N_2O was more efficient than that with supercritical CO_2.
The differences of the effects of the two supercritical fluids on microorganisms were studied by Differential Scanning Calorimetric (DSC) on solid 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), in the range of 30-180°C and 1 – 50 bar. The thermograms showed that both the CO_2 and the N_2O had similar plasticizer effect as can be seen by the reduction of the melting.

KEYWORDS: tomato paste, supercritical fluids, CO_2, N_2O, pasteurization.

INTRODUCTION

The current pasteurization and sterilization systems of food, such as the thermal treatment [1], and the high hydrostatic pressure processing (up to 9000 bar) [2] may lead to leakage of vitamins, denaturation of proteins, and change in food characteristics. The main goal of this treatment is the elimination of the spoilage and pathogen microorganisms by a process which does not deteriorate the quality of food.
Among other techniques currently under development, as the use of membrane filtration and the processes exploiting oscillating magnetic fields, ultrasounds, microwaves and radio frequency, the interest towards pasteurization with supercritical fluids is growing more and more.
In fact, at moderate temperature and pressure, a dense gas such as carbon dioxide (CO_2) is able to inactivate significantly bacterial cells [3], moulds and yeasts [4] and, at suitable conditions, it can reduce the activity of enzymes [5]. Among others, Gunes *et al*. [6] investigated the yeast inactivation in grape must and observed that the process didn't cause degradation of taste and odour, while Ginneken *et al*. [7] had successfully pasteurized eggs. Parton *et al.* [8] published more data on the pasteurization of grape must and tomato puree by dense CO_2 treatment. Another supercritical fluid used for sterilization is nitrogen protoxide (N_2O), but only few works have been published on it [9,10]. In the review of Garcia-Gonzalez *et al.* [9], these authors concluded that the pasteurization effect of supercritical N_2O is found

to be lower than that of supercritical CO_2, in simpler medium such as distilled water, in synthetic medium and also in apple juice [10].
The aim of this work is to study the pasteurization of tomato paste by supercritical fluids. This is a complex system, not easy to treat [8]. In our study, a multi-batch apparatus has been applied to investigate the tomato puree inactivation by sc-CO_2 and sc-N_2O. The natural foodstuff was prepared in the laboratory. Experiments were carried out to determine the best operative conditions (pressure, temperature and treatment time) to pasteurize the sample. The pasteurization efficiency of supercritical CO_2 and N_2O was measured by studying the inactivation kinetics of typical microbial population of tomato puree [8]: bacteria, yeasts and moulds.

MATERIALS AND METHODS

Fresh tomatoes were bought in a grocer's shop at Piazzola sul Brenta (Padua, Italy) and the tomato puree was produced in our laboratory.
The CO_2 was supplied by Sapio srl (Monza, Italy), instead the N_2O by Air Liquide Srl (Padova, Italy).
Three kinds of medium (from Sigma-Aldrich, Milan, Italy) were used: a *Brain Heart Infusion Agar* (BHA) for bacteria, *Sabouraud* (SAB) for yeast and moulds and *Tomato Juice Agar* (TJA) for *Lactobacillus* sp..
DPPC (dipamitoyl-phosphatydilcoline %) was from Sigma-Aldrich

Pasteurization treatment: apparatus and procedure

In Figure 1 the set-up of the high pressure pasteurization apparatus is outlined. The plant is provided with a high pressure storage reservoir to reduce the pressurization time of the reactors system. Details of this apparatus are reported elsewhere [11,12].

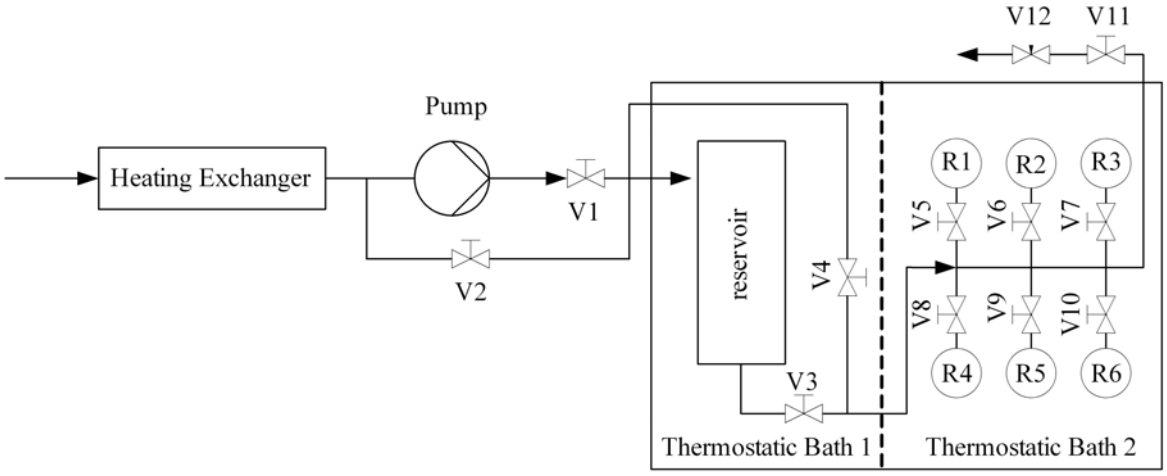

Figure 1. Block Diagram of sterilization by SCF process: R1-R6: high pressure reactors; V1-V11: on-off valves, V-12: regulating valve.

The plant is provided with two thermostatic water baths: one for the reservoir and the other for the reactors. In the case of the experiments with CO_2, the reservoir was loaded using a HPLC pump, so the temperature of the two baths was the same throughout the experimental time, while in the case of N_2O the fluid was cooled in a heating exchanger at 5°C and fed at the same temperature into the reservoir. Then the valves V1-V4 were closed and N_2O was heated until the pressure in the reservoir reached the desired value.

1.5 mL of well mixed tomato puree was loaded in each reactor under sterile conditions, which were inserted in the thermostatic bath and then connected to the high-pressure apparatus. After temperature equilibration, the six samples were subjected to the identical pressure of CO_2 or N_2O for different treatment times, opening the valves V3-V10: in this way, the pressurization rate by the supercritical fluid was fast. Each sample was treated for a different time, and then the proper reactor was depressurized and removed.
Different combinations of temperature and pressure were investigated. Each experiment was done at least twice, and the average values are reported as final results.

Microbiological procedures

A well mixed volume (100 μL) of non-diluted or 10-fold serially diluted tomato puree in sterile physiological saline solution (0.85% NaCl) was used and plated on suitable solid media. The total microbial load was expressed as *colony forming unit* per mL (CFU/mL). Inactivation was expressed as $logN_0/N$, where N_0 is the number of microorganisms contained in the sample at time *0*, and *N* is the number counted after a time of treatment *t*.

DSC measurements

DSC spectra were performed using a Q10P/PDSC (TA Instruments) equipped with a pressure cell system that allows operating at high temperature (up to 200°C) and under different pressure values (from 1 up to 50 bar) on about 5 mg of substances.
In all the runs, the heating and cooling rate were of 5°C/min.

RESULTS AND DISCUSSIONS

The efficiency of the pasteurization process was determined by the count of the number of viable cells in treated and untreated tomato paste. Each run was executed in duplicate; an error less than to 25% on an average can be applied to final microbial numbers.

Comparison between CO_2 and N_2O

The comparison of the kinetics of tomato puree bacteria inactivation due to pasteurization with supercritical CO_2 and N_2O at 100 bar and two temperatures, 33°C and 55°C, is represented in Figure 2a and 2b, respectively.

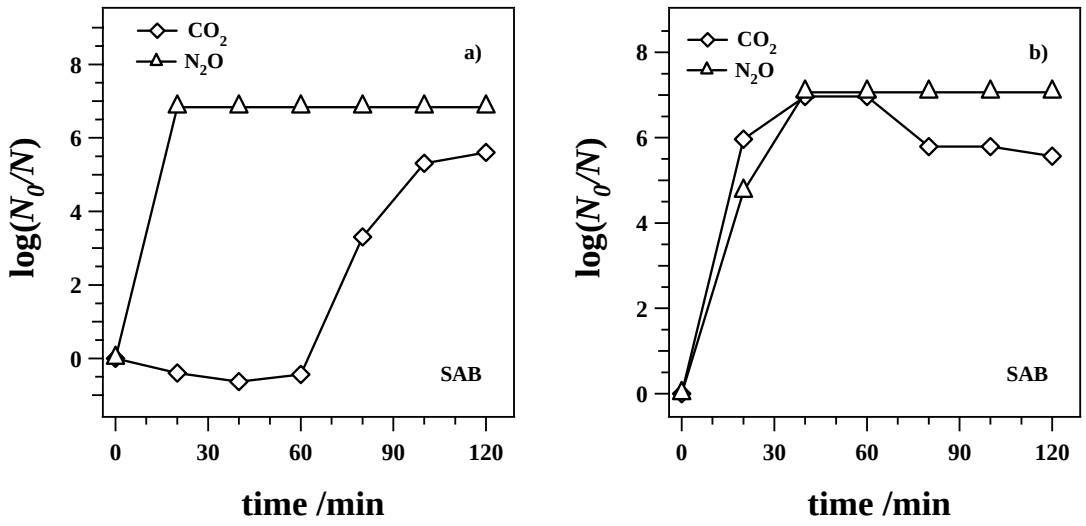

Figure 2. Inactivation kinetic curves for tomato paste treated at a) 33°C and 100 bar of CO_2 (♦) or N_2O (▲) pressure and b) 55°C.

The results of tomato paste pasteurization by sc-CO_2 (Figure 2a and 2b) showed that yeast and mould are inactivated by about 95% after 40 min of treatment with sc-CO_2 at 55°C, while at 33°C, around 90 min are needed. The inactivation kinetics is initially fast for the tomato puree treated at 55°C, while it is possible to observe a "latency" zone (phase lag) in the treatment at 33°C.
The sc-N_2O treatment at 33°C (or 55°C) and 100 bar lead to total inactivation of mould and yeast after 20 min and of bacteria after 60 min (Figure 2) of treatment, respectively.
The effect of the two gases is quite similar at the operative condition of 55°C and 100 bar (Figure 2b), though the process with sc-N_2O is faster. On the other hand, a real difference was obtained at a temperature of 33°C, as shown in Figure 2a. The treatment with N_2O shows behaviour similar to that at higher temperature, without a lag phase (evident in the treatment with CO_2).
The process with high pressure N_2O is strongly pressure-dependent (data not shown), while that with dense CO_2 is temperature-dependent. Similar results have been observed in the case of inactivation of bacteria (data not shown).
These differences cannot be due to the difference in the critical points of the two substances, because they are very similar (CO_2 T_c = 31.1°C, P_c = 73.8 bar; N_2O T_c = 36.4°C, P_c = 72.4 bar). In addition, both N_2O and CO_2 are relatively non-polar, have a low molecular weight, and a high solubility in lipids, so dense N_2O can quickly diffuse into phospholipids cell membranes similar to CO_2. The difference between the two substances is the acidification of water by CO_2, which produces carbonic acid, while N_2O does not acidify water [9]. Therefore, at present it is not clear yet why N_2O is effective in reducing microbial activity.

Differential Scanning Calorimetric on DPPC under pressure of CO_2 or N_2O

DSC measurements on DPPC were carried out under pressure of CO_2 and N_2O to study the different effect of the two gases on the microorganisms. For this scope, we have selected the DPPC as it is a typical membrane phospholipid.

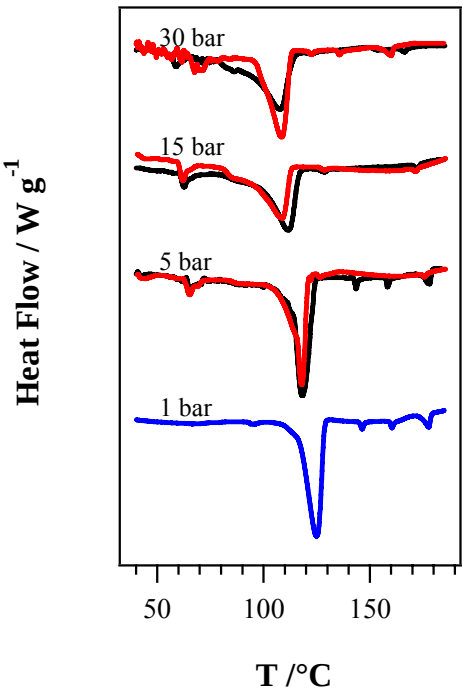

Figure 3. DSC of DPPC under pressure: a) CO_2 in black; b) N_2O in red.

The results are reported in Figure 3. It is possible to observe that an increasing of the gas pressure leads to a reduction on the transition temperature of the main peak (from 125°C to about 110°C) and a reduction on its area (transition heat). Another, the peak became more widening.
It is noteworthy the appearance of a peak at about 80°C when the measurements were carried out under pressure of CO_2 or N_2O. This peak demonstrates the strong interaction between DPPC and the two supercritical fluids considered in this work.
Clearly no relevant difference is observed between the behaviour of the two gases.

CONCLUSIONS

In this work, the pasteurization with supercritical fluids was efficiently applied to tomato puree so that an inactivation of about 6-7 log was obtained. The fluids used were carbon dioxide (CO_2) and nitrogen protoxide (N_2O). The effect of temperature, pressure and treatment time were studied to find out the best condition to pasteurize this substrate, and the inactivation kinetic was studied.
The efficiency of pasteurization process with sc-CO_2 increased at higher temperatures (at 33°C and 55°C) the 95% of yeast and mould were inactivated after 90 min and 40 min, respectively. The process with supercritical N_2O gave different results. The pasteurization was faster at 33°C than at 55°C. The best results were obtained with N_2O at 33°C and 100 bar: here, 99% of inactivation was achieved after 60 min of treatment.
It was found that the process with N_2O is pressure-dependent, while the one with CO_2 is temperature-dependent. In addition, the pasteurization with supercritical N_2O was more efficient than that with sc-CO_2 and it could be applied at milder conditions: 33°C and 100 bar, which permits to preserve all organoleptic properties of the substratum.
The DSC measurement carried out on DPPC under pressure of CO_2 and N_2O demonstrated their strong interaction with this phospholipid even though no substantial great difference between the two gases was found.

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